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Greater Agronomic Water Use Efficiency in Wheat and Rice Using Carbon Isotope Discrimination

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IAEA-TECDOC-1671

GREATER AGRONOMIC WATER USE EFFICIENCY IN WHEAT AND RICE USING CARBON ISOTOPE DISCRIMINATION

Prepared by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2012

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FOREWORD

Wheat and rice are the two most important cereal crops worldwide. The potential yield of a cereal crop may be constrained by many factors, both biotic and abiotic, which may be related to the physical, chemical and biological properties of the soil itself (edaphic factors) or to the environmental conditions pertaining during the growth of the crop. Foremost among the abiotic factors is the availability of water, which is governed by climatic conditions and the ability of the soil to store available water. Together with drought, soil salinity is becoming a major stress factor limiting cereal yields globally. Soil salinization is accelerated by anthropogenic activities, including various forms of land and water mismanagement, which allow salts in geological strata to rise to the surface soil or seawater to encroach on low-lying coastal paddy soils.

National programmes in crop improvement generally focus on local production problems through selection of stress resistant germplasm and traditional breeding methods, aided by modern molecular techniques. Traditional approaches are labour intensive and time consuming, with grain yield being the final arbiter of success or failure. Thus any technique that can predict yield well in advance of harvest has the potential to save considerable time, effort and money. The objective of this coordinated research project (CRP) was to evaluate carbon isotope discrimination (CID, or $\Delta^{13}C$) as a selection tool for yield and biomass of wheat under drought stress and rice under salt stress.

This CRP was implemented following the recommendations of a consultants meeting of international experts. The research network included 11 contract holders from Algeria, Australia, Bangladesh, China (2), India, Morocco, Pakistan, Philippines, Syrian Arab Republic and Yemen, and two agreement holders from Mexico and the United States of America. The CRP was conducted in collaboration with national agricultural research systems (NARS) in Africa and Asia, and with two Consultative Groups on International Agricultural Research (CGIAR) Centers: The International Rice Research Institute (IRRI) and the International Maize and Wheat Improvement Center (CIMMYT). The CRP was supported by in-house research and the provision of $^{13}C/^{12}C$ isotope ratio analysis of plant samples at natural abundance ($\delta^{13}C$) at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. Upstream research on CID was carried out at the IAEA prior to the commencement of the CRP and through an individual research contract.

The IAEA wishes to acknowledge P.M. Chalk and all the CRP participants for their valuable contributions. The IAEA officer responsible for this publication was L.K. Heng of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY

The studies conducted within this CRP concern three major cereal crops, namely bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. var. durum) and rice (*Oryza sativa* L.). Upstream research on carbon isotope discrimination in these cereals carried out at the IAEA [1, 2, 3] or funded through research contracts [4, 5, 6] provided a firm foundation for the studies reported in this IAEA-TECDOC. Studies were conducted across a wide geographic area in both northern and southern hemispheres under a range of environmental and edaphic conditions. The primary experimental variable was genotype (plant species) and within each genotype a broad range of phenotypes (cultivars/lines) were included to provide variation in morphological, physiological or phenological characteristics. The primary plant parameters measured were grain yield (GY) and carbon isotope discrimination, referred to as CID, $\Delta^{13}C$ or simply Δ.

The $\Delta^{13}C$ technique is based on the discrimination of the heavier stable isotope of carbon (^{13}C) in favour of the lighter and more abundant isotope (^{12}C) during the physical diffusion of CO_2 through the leaf stomata, and subsequent enzymatic decarboxylation. Thus CID provides a time integrated index of photosynthetic activity and is related to water use or transpiration efficiency. Relationships between yield and $\Delta^{13}C$ were studied across a range of soils having different water holding capacities, under a range of natural or imposed water regimes. Other nuclear techniques were used to study partitioning of photosynthate between biomass and grain ($\delta^{18}O$) and to measure temporal changes in stored soil water (soil moisture neutron probe).

Experiments were of two types, i.e. studies were conducted in the glasshouse or phytotron where some control could be exercised over experimental conditions (e.g. temperature, water regime, salinity, pest control) or in the field where much less control was possible, e.g. control of water through irrigation or the use of rainout shelters. Multiple field sites were used over several seasons, to cover natural differences in the soils' water holding characteristics (stored soil moisture) and incipient rainfall. Partial, full or no irrigation was used to artificially manipulate the water regime.

1. VARIATION IN $\Delta^{13}C$ WITHIN GENOTYPE

Two stable isotopes of carbon occur naturally, the more abundant ^{12}C (98.9 atom %), and the less abundant ^{13}C (1.1 atom %). During photosynthesis (the assimilation of atmospheric CO_2 into plant tissue), physico-biochemical processes discriminate against the heavier ^{13}C isotope in favour of the lighter ^{12}C species, which forms the basis of the $\Delta^{13}C$ technique.

$$\Delta^{13}C \text{ (per mil or‰)} = [(\delta_a - \delta_p)/(1 + \delta_p)] \cdot 1000$$

where $\delta_p = \delta^{13}C$ of the plant sample and $\delta_a = \delta^{13}C$ of atmospheric $CO_2 = -8‰$,

and $$\delta^{13}C \text{ (‰)} = [(R_{sample}/R_{reference}) - 1] \cdot 1000$$

where $R = {}^{13}C/{}^{12}C$ ratio, measured with a precise isotope ratio mass spectrometer. The primary reference standard for $\delta^{13}C$ measurements is fossil carbonate (Pee Dee Belemnite), where $R_{PDB} \times 10^6 = 11237.2 \pm 9.0$.

Many variables are known to affect Δ within a given genotype, including phenotype, plant organ, age of plant organ, environmental conditions (especially water regime) and edaphic factors (e.g. soil salinity), while very little is known in regard to other soil-related factors, e.g. plant nutrition. Thus, it is important to specify as precisely as possible the measurement parameters of Δ.

The variation in Δ measured within the CRP for the three genotypes is summarized below (Tables 1, 2 and 3):

1.1. Rice

TABLE 1. VARIATION IN $\Delta^{13}C$ (‰) AMONG RICE PHENOTYPES

Member State	No. of lines	Plant organ	Non-saline		Saline		Total range
			Variation	Range	Variation	Range	
Philippines	79 RILs‡	Seedling	22.7 – 23.8	1.1	21.0 – 22.8	1.8	23.8 –
Bangladesh	23		20.8 – 22.0	1.2	19.6 – 20.8	1.2	19.1 =
	mutants/cultivars	Flag leaf†	20.5 – 22.3	1.8	19.1 – 21.6	2.5	4.7

† Reproductive stage
‡ Recombinant inbred lines

• Average saline Δ values < non-saline Δ values.

1.2. Bread wheat

TABLE 2. VARIATION IN $\Delta^{13}C$ (‰) AMONG BREAD WHEAT PHENOTYPES

Member State	No. of lines	Plant organ	Average values or range †			Range	Total range
			RSMS	PAWS	WW		
India	16	Seedling	20.5	20.5	21.4	0.9	21.8 –
		Flag leaf	21.8	20.3	20.2	1.6	17.0 = 4.8
		Grain	17.0	18.6	19.5	2.5	
Pakistan	111	Grain	nd	nd	18.5 – 23.0		4.5
	8	Flag leaf	18.6 – 20.1	18.6 – 20.7 ‡	19.5 – 20.9	2.3	18.6 –
		Grain	18.2 – 19.6	18.6 – 19.7 ‡	19.5 – 20.2	1.2	20.9 = 2.3
Morocco#	20	Grain	13.6 – 15.0	16.7 – 17.4	17.1 – 17.8		4.2

†RSMS: residual soil moisture stress; PAWS: post-anthesis water stress; WW: well watered; nd: not determined.
‡Includes moderate water (MW) and low water (LW) regimes.
#RSMS = severe/high water stress; PAWS = irrigated with some stress; WW = little or no water stress.

• Grain Δ < seedling or flag leaf Δ
• RSMS grain Δ < WW grain Δ

1.3. Durum wheat

TABLE 3. VARIATION IN $\Delta^{13}C$ (‰) AMONG DURUM WHEAT PHENOTYPES

Member State	No. of lines	Season	Plant organ	Average value or range †			Range	Total range
				RSMS	PAWS	WW		
India	14	2003-04	Seedling	21.0	20.9	21.7	0.8	22.5 –
			Flag leaf	22.0	22.5	20.6	1.9	16.8
			Grain	16.8	18.7	19.4	2.6	= 5.7
	20 semi-dwarf	2004-05	Flag leaf	18.2	20.1	20.1	1.9	20.1 –
			Grain	15.8	17.1	16.6	1.3	15.8
		2005-06	Flag leaf	17.7	18.8	18.8	1.1	= 4.3
			Grain	16.2	16.5	17.6	1.4	
		Range		4.2	6.0	5.1		

†RSMS, residual soil moisture stress; PAWS, post-anthesis water stress; WW, well watered

• Grain Δ was always less than seedling or flag leaf Δ.
• RSMS grain Δ < WW grain Δ

Data from several Member States show that considerable genetic variation in Δ exists within large populations of rice and wheat. The extreme range of Δ was 4.7‰ for rice, 4.8‰ for bread wheat and 5.7‰ for durum wheat when sampled across a range of phenotypes, plant organs and abiotic stress factors (salinity for rice, water for wheat). However, for a given plant organ, especially within a small population, variation in Δ is likely to be relatively small (≤2.6‰, Tables 1, 2 and 3).

2. RELATIONSHIPS BETWEEN Δ AND GRAIN YIELD

2.1. Rice

2.1.1. Saline environments

The hypothesis was that selection for high CID would be positively correlated with tolerance to salinity. Pot experiments conducted at International Rice Research Institute (IRRI) (Philippines) and in Bangladesh confirmed that leaf CID was positively correlated with salinity tolerance measured at the seedling stage. Furthermore, both flag leaf and grain Δ were positively correlated with salinity tolerance measured at the reproductive stage. However, grain yield was not significantly correlated with flag leaf Δ under saline conditions.

2.1.2. Non-saline environments

The hypothesis was that selection for high CID would be positively correlated with yield. However, for the lines tested at IRRI and in Bangladesh, as well as inbred rice in China, there was no correlation between CID and yield. Conversely, for hybrids in China, preliminary data indicate a negative correlation between leaf Δ and grain yield.

2.2. Wheat

2.2.1. Post-anthesis drought stress environments

Originally, the environments were described as winter rainfall/Mediterranean rainfed environments, e.g. Morocco, Algeria and the Syrian Arab Republic. Based on the CRP

experience of the last 5 years, irrigated environments in India, China and Pakistan with high evaporative demand can also be included. Well watered temperate environments were not sampled in this CRP.

The hypothesis was based on earlier observations reported in the literature for studies in Mediterranean countries, that selection for high grain CID would give a grain yield advantage. The CRP confirmed the hypothesis. The correlations between grain CID and yield were significant and positive, and with few exceptions were consistent across sites and years. Substantial genetic variation in grain CID was observed, and heritability of grain CID was greater than heritability of yield.

2.2.2. Pre-anthesis drought stress environments

This category includes rainfed (non-irrigated) environments where growth depends on residual stored soil moisture. The hypothesis was that selection for low leaf CID would give a grain yield advantage, based on earlier studies conducted in Australia. This hypothesis was tested in Pakistan, Yemen, India and China. Very rarely did the participants observe negative correlations between leaf CID and yield. The hypothesis was therefore refuted in these environments. When grain CID was measured, positive correlations were observed in environments with high air temperature during grain filling (Pakistan, India and China). Substantial genetic variation in grain CID was observed, and heritability of grain CID was greater than heritability of yield.

3. RELATIONSHIPS BETWEEN Δ AND WATER USE EFFICIENCY IN WHEAT

Water use efficiency (WUE) has been defined as the ratio of total or above ground biomass to total water consumed (evaporation from the soil surface + growing season transpiration + runoff + deep drainage). Agronomic WUE is the ratio of harvested grain to total water consumed. Transpiration efficiency (TE) is the amount of biomass produced per unit of water transpired. Intuitively, one may assume that selection should be for greater WUE, but theory and prior research show that WUE or TE may correlate negatively with yield or Δ. Both positive and negative correlations were obtained between grain WUE and leaf and grain Δ in the CRP, depending on water regime and the plant population under study. Further targeted research is therefore required before this question can be satisfactorily resolved.

4. RELATIONSHIP BETWEEN Δ AND MINERAL NUTRITION

A significant negative linear relationship between Δ and nitrogen (N) fertilizer rate was demonstrated for hybrid rice over the range of 0 – 250 kg N ha^{-1}. Δ also decreased in rice with increasing rates of phosphorus (P) addition from 0 to 60 kg P ha^{-1} under non-saline conditions, but this effect was not observed under salt stress. Δ also decreased in wheat with increasing rates of P fertilizer from 0 to 40 kg P ha^{-1}, and the effect was more marked in spikes than in shoots. These results underline the importance of maintaining optimum mineral nutrition in plant breeding programs.

5. QUANTITATIVE TRAIT LOCI (QTL_S)

5.1. Rice

Three major QTLs for CID were identified in saline environments. These co-localized with previously identified QTLs for salinity tolerance. Nine QTLs were identified for CID in rice under non-saline irrigated conditions. One major QTL co-localized with QTL for panicle size and panicle number. Three genetically-analysed populations are available for mapping new QTLs associated with CID. QTLs associating CID with salinity tolerance are being used in marker-assisted breeding for salinity-tolerance.

5.2. Wheat

5.2.1. Durum

In studies of recombinant inbred lines (RILs) in India, a major QTL for CID was detected on chromosome 4B. Under limited irrigation, a major QTL for canopy temperature depression (CTD) was identified on chromosome 2A, while another QTL was identified on chromosome 6A under full irrigation. For ash content, a major QTL was detected on chromosome 5A under limited irrigation, whereas another QTL was detected on chromosome 6A under full irrigation.

5.2.2. Bread

CID was found to be linked to a molecular marker on chromosome 2A, and to a thousand kernel weight marker on the same chromosome. A poor QTL associated with grain yield was detected at chromosome 5A, similar to the one detected at CIMMYT in Obregon (Mexico) and northern Australia.

6. ASH CONTENT AS A SURROGATE FOR Δ

The expense of measuring $\delta^{13}C$ is a major disincentive to the adoption of CID by breeders. The significant positive correlation found between grain ash and grain Δ is a potential solution to the problem, provided a cheap and rapid method for determining ash content can be developed. A promising methodology using Near Infra Red Spectrometry was developed in India.

7. CONCLUSIONS

The studies carried out within the framework of the CRP fall within the major FAO/IAEA programme on crop improvement in harsh environments, and clearly show the value and strength of an interdisciplinary research approach. The expertise in plant breeding and genetics of the majority of participants was complemented by expertise in agronomy and soil science contributed by other participants and IAEA staff at HQ and Seibersdorf.

While success was achieved in demonstrating statistically significant relationships between Δ and salinity tolerance in rice, participants were unable to demonstrate significant relationships between Δ and yield in either saline or non-saline environments. Therefore the opportunity to use Δ as a selection criterion in rice appears to be limited at the present time. Greater progress may be possible by identifying phenotypes which are effective at either excluding salt at the roots or sequestering salt in vacuoles in the leaves.

On the other hand, grain CID of wheat is a tool that can be used now by breeders in environments having post-anthesis drought stress, due to the strong and consistent positive relationships with yield shown in the present CRP. In addition, breeders can use grain CID in rainfed environments that rely on stored soil moisture and experience pre-anthesis water stress, based on the strong positive correlations with yield that were consistently obtained. However, a more-comprehensive classification of environments in wheat growing regions is needed so that the applicability of the technique in a wider range of target regions can be determined.

Information gathered in the CRP on segregating populations was limited by the duration of the project. To be confident in taking up CID as a breeding tool, plant breeders will need much more information on key issues. The segregating populations generated in the CRP provide a strong platform for future work, building on project outcomes and developing CID as a powerful tool in breeding for greater agronomic water use efficiency. It is essential that the genetic association between yield and CID in segregating populations is tested more comprehensively. Breeders will require more precise information on key genetic parameters of CID such as narrow-sense heritability.

Cheaper alternatives to CID are needed, as $\delta^{13}C$ analysis by isotope ratio mass spectrometry is expensive and analytically demanding. There is an exciting opportunity to exploit an observed correlation between grain ash and grain CID, but more work is required to confirm the wider utility of measuring grain ash.

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CARBON ISOTOPE DISCRIMINATION IN RICE UNDER SALT AFFECTED CONDITIONS IN BANGLADESH

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Abstract

Carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) has been suggested as a practical screening tool for salinity tolerance of rice. 75 rice genotypes (60 landraces and 15 advanced mutants/varieties) were screened for salinity tolerance at the seedling stage using the IRRI standard protocol. Two local varieties, Bawoi Jhak and Dhol Kochuri, the Indian cultivar Pokkali and two advanced mutants, RD-2586 and THDB were salt tolerant, and seven varieties/mutants were moderately tolerant. Genetic variability in seedling CID (ΔS) was evident under saline and non-saline conditions, and ΔS was negatively correlated with salinity tolerance based on standard evaluation scoring (SES). Significant positive correlations between flag leaf CID (ΔF) sampled before panicle initiation and total dry matter (DM), number of filled grains and grain yield plant^{-1} under salt stress were obtained, but no relationships were observed under non-saline conditions. Contrasting parental genotypes based on high /low Δ and also salt tolerance were crossed among high Δ x high Δ, high Δ x low Δ and low Δ x high Δ. Out of 36 crosses, two were selected (Binadhan-5 x Horkuch and Mut-1-1 x Pokkali) based on phenotypic performance, but the latter F_3[1] population was lost due to unfavourable weather. A total of 38 F_3 lines of the cross Binadhan-5 x Horkuch were selected and used to screen for salinity tolerance at the seedling and reproductive stages. Five lines were found to be salt tolerant and 17 were moderately tolerant at the seedling stage based on SES. Five lines had significantly lower % reduction in number of filled grains plant^{-1}, total dry matter and grain yield plant^{-1} under salt stress, and these lines were salt tolerant at the reproductive stage. Thus, CID may be an additional tool for selection of salt tolerant rice genotypes.

1. INTRODUCTION

Salinity is a current threat to food security, especially in countries where irrigation is an essential input to agricultural production. The global extent of primary salt-affected soils is about 955 M ha, while secondary salinization affects some 77 M ha, with 50% of these in irrigated areas [1]. More than 54 M ha of rice soils in Asia are now affected by salinity. Of these, 9.5 M ha can be managed by large scale irrigation and drainage schemes and by chemical treatment, but the scale of the problem renders these solutions too costly [2]. In Bangladesh about 2.8 M ha are salt-affected. Use of salt tolerant varieties is considered to be the most economical and effective way of increasing crop production on saline lands. Attempts to improve the salt tolerance of crops through conventional breeding programmes have met with very limited success, due to the complex interactions of genetic and physiological traits associated with salinity.

[1] F_3 stands for Filial 3, the third filial generation of seeds/plants offspring resulting from a cross mating of distinctly different parental types.

The rice plant is one of the most suitable crops for saline soils, although it is considered moderately sensitive to salinity [3], particularly during the early seedling, flowering and reproductive stages. The understanding of the physiological basis of salinity tolerance was advanced by the discovery that less Na^+ and more K^+ were absorbed to maintain a high Na^+: K^+ ratio in the shoot [4], and a major quantitative trait locus (QTL) was mapped on chromosome 1. Marker technology can facilitate and speed the development of salt tolerant varieties, but this approach is expensive and will need effort and time. Even evaluation of farmers' fields is not a reliable technique because of soil and climatic heterogeneities. Given these constraints, alternative techniques such as CID can potentially be a more practical screening tool, if it can be shown to be correlated with salinity tolerance and yield. This technique could complement other strategies such as molecular markers to integrate all of the traits involved in salinity tolerance in popular rice varieties in Bangladesh.

The objectives of the present investigation were (i) to evaluate CID as a selection tool for identifying higher yielding genotypes of irrigated rice under both saline and non-saline conditions (ii) to develop a set of elite isomorphic lines by cross breeding using mutants/lines that contrast in yield potential, Δ and/or tolerance to salt stress, and (iii) to evaluate the developed lines.

2. MATERIALS AND METHODS

2.1. Screening, sampling and analytical procedures

2.1.1. Screening for salinity tolerance at the seedling stage

Rice genotypes were screened for salinity tolerance in the Bangladesh Institute of Nuclear Agriculture (BINA) glasshouse using the nutrient solution described by Yoshida et al. [5] and the International Rice Research Institute (IRRI) standard protocol [6]. Rice seeds were kept at 48 ^oC for one week to break the dormancy. They were surface sterilized, incubated on filter papers soaked with tap water in petri dishes and kept in the dark at 30 ^oC for two days. Two treatments were imposed with three replications (i) saline and (ii) non-saline. The radicles of the pre-germinated rice seeds were carefully inserted into nylon mesh in each hole of a styrofoam float, and then placed in the tap water. The tap water was replaced with the nutrient solution after three days. Sodium chloride (NaCl) was added until an electrical conductivity (EC) of 12 dS m^{-1} was reached. The solution was renewed every eight days, and the pH was monitored everyday and maintained at 5.0. The initial visual symptom evaluation was conducted after two weeks and final scoring at four weeks after the initial salinization. The scoring was based on a modified IRRI standard evaluation scoring (SES) system for the visual symptoms of salinity injury in rice. Leaf samples were collected after two weeks from all replicates and treatments for $\delta^{13}C$ analysis.

2.1.2. Screening for salinity tolerance at the reproductive stage

Perforated pots with fertilized soil were placed in a water tray. Seeds were sown in the pots and watered with tap water. Twenty-one days after sowing, tap water was replaced with saline water at EC 6 dS m^{-1} for the saline treatment, and the other trays (non-saline) were replaced with fresh tap water. Flag leaves were collected for $\delta^{13}C$ analysis before the panicle initiation stage.

2.1.3. Carbon-13

Leaves were dried in a convection oven at 70 ^{o}C for three days and ground in a mill to a fine powder. $\delta^{13}C$ (‰) analysis was carried out at IRRI using an isotope ratio mass spectrometer, and $\Delta^{13}C$ (‰) was calculated according to the formula given by Hubick et al. [7], using a value of –8‰ for the isotopic composition of atmospheric CO_2 relative to the PDB standard.

2.2. Experimental

2.2.1. Year 1

75 rice genotypes were screened for salinity tolerance at the seedling stage (Table 1). Of these, 60 (N^{o} 1-60) were local varieties (Bangladeshi landraces) which were obtained from the IRRI Gene Bank, and 15 (N^{o} 61-75) were advanced mutants developed at BINA from Bangladeshi high yielding varieties.

2.2.2. Year 2

From Year 1, 23 rice mutants/cultivars were selected with high and low Δ. Greenhouse experiments were then conducted for salinity screening at both seedling and reproductive stages as previously described. Leaf samples (seedling and flag leaves) were collected for $\delta^{13}C$ analysis. Plant height, root length, total dry matter (DM), yield and yield components, SES scores and phenology were measured and analyzed statistically.

Based on high /low Δ and salt tolerance, several crosses were made and F_2 progenies were grown in the glasshouse. The F_3 progenies were evaluated for salt tolerance at the seedling stage in the following year.

2.2.3. Year 3

Crosses were made in two different seasons (boro season = December-April, and aman season = July-November) i.e., in two batches (first batch and second batch). Two batches were required because some crosses were unsuccessful due to photo-sensitiveness of the cultivars/mutants. In Bangladesh, the boro season is featured by low temperature especially during flowering, while moderate temperatures exist during the whole aman season. Therefore, the aman season is the most favourable for crossing in rice. Crosses were made in three combinations (high Δ x high Δ, high Δ x low Δ and low Δ x low Δ).

2.2.3.1. First batch

Crosses between Binadhan-5 (low Δ) x Horkuch (high Δ), Baradhan (high Δ) x PNR-519 (high Δ), Mut-1-1 (high Δ) x Pokkali (high Δ), Binadhan-5 (low Δ) x Bawoi Jhak (high Δ), Binadhan-5 (low Δ) x PNR-519 (high Δ) and Mut 1-1 (high Δ) x Charnock (DA6) (low Δ) were made in the 2005 boro season.

2.2.3.2. Second batch

In the 2006 aman season, two crosses i.e., Binadhan-5 x Horkuch and Mut-1-1 x Pokkali were selected for evaluation of F_3 lines for morphological-physiological traits and salinity tolerance at the seedling and reproductive stages. F_3 lines were evaluated for Δ and salt tolerance in the subsequent year, since elite isomorphic lines contrasting in Δ can be used to evaluate selection strategies. The F_3 population of Mut-1-1 x Pokkali was damaged due to

unfavourable weather. Therefore, one cross population of Binadhan-5 (salt susceptible, high yielding mutant variety and low Δ) x Horkuch (salt tolerant, landrace and high Δ) was evaluated.

2.2.4. Year 4

For the Binadhan-5 x Horkuch cross, 38 F_3 plants were selected out of 900 in the F_2 population, based on similar phenology. These 38 F_3 lines along with their parents were tested in the BINA glasshouse for salinity tolerance at the seedling and reproductive stages following the IRRI standard protocol. Data for salt tolerance (SES score) at the seedling stage, yield and yield components (plant height, number of filled grains, 1000-seed weight, total DM and grain yield plant^{-1}), phenology (days to flowering, days to maturity) and heritability in the broad sense (Hb%) under salt stress and non-saline conditions at the reproductive stage, were recorded and analysed statistically.

3. RESULTS AND DISCUSSION

3.1. Year 1

There were no symptoms of salt injury in seedlings three days after initial salinization. The plants were uniform in height and leaves were green. Subsequently, however, the first and second leaves in the saline treatment became yellowish and scorched with whitish tips. Some plants were stunted and growth was non-uniform compared with plants in the non-saline treatment, which were tall and vigorous, with uniform green leaves.

The growth period in this experiment was 28 days after seeding. In the saline treatment, the 75 genotypes showed wide variation in tolerance with scoring from 3 to 9 (Table 1). The most salinity tolerant genotypes were Pokkali, Bawoi Jhak, Dhol Kochuri and one advanced mutant (RD-2586) from BINA. Six moderately salinity tolerant varieties/mutants were identified, viz., Charnock (DA 6), Chini Sagar, Kala Jira, Latisail 11-117, TNDB-100 and THDB.

Under saline conditions, the highest score was 9, where almost all plants were barren and stunted, with the majority of the leaves necrotic. Such plants did not survive. The basic principle in screening for salinity tolerance at the seedling stage was the ability of the seedlings to grow in saline culture solution [4]. The three salinity tolerant and six moderately tolerant varieties/mutants were used for the following year's breeding programme.

3.2. Year 2

3.2.1. Phenotypic performance at the seedling stage

Visual symptoms of salt stress appeared two or three days after the imposition of salinity. The 23 genotypes showed wide variation with respect to salt tolerance (Table 2). The variety Pokkali and the mutant THDB were salt tolerant. The moderately tolerant genotypes were Bara (Boro) Dhan, Dhol Kochuri, Latisail 11-117, RD-2586, TNDB-100, Y-1281, Mut-NS-16 and PNR-519.

Significant reductions in seedling height due to salinity ranged from 47% for Kaliboro 109-4 to 18% for Pokkali and THDB. Salinity-induced reductions in seedling total DM ranged from 75% for Kaliboro 109-4, Charnock (DA6) and Kaliboro 138-6 to 40% for Pokkali (Table 2). Reductions in seedling root length due to salinity ranged from 54% for Kaliboro 138-2 to 11% for PNR-519.

TABLE 1. SALINITY TOLERANCE SCORES OF SEEDLINGS OF 75 RICE GENOTYPES GROWN IN HYDROPONICS AT AN ELECTRICAL CONDUCTIVITY OF 12 DS M^{-1}

N°	SES score†	Variety/mutant
1-15	9.0	Dholasaitha (DA 32), HBJ Boro 2, Pani Sail, Pankaij, Ragu Sail, Boro 65-2, Boro 398, Dhaliboro 105-2, Jangliboro 581, Kaliboro 138-2, Kaliboro 139-2, Khayaliboro 595, Mala, Mut-1-1, RM-STL-3
16-24	8.3	Molla Diga, Bura Horin, Dholi Boro, Dhaliboro 70-1, Gasmal 110-2, Kaliboro 109-4, Bara (Boro) Dhan, Sonamukhi, Mut-NS-16
25-38	7.7	Aswina 322, Tupa Boro, Jinga Sail, Kartik Sail, Binni Dhan, Buta Sail, Dharga Sail, Binni Phul, Lal Dupa, Marich Ful, Surjamukhi, Bini, RM-250-124, RM-STL-2
39-55	7.0	HBJ Boro 5, HBJ Boro 4, Joyna, Kaika, Digha, Hida, Lal Hida, DA2 (Kataktara), Chini Atob, Dudraj, Katisail, Habiganj Boro 8, Mala/J 15, Mut-1-2, RM-250-83, Mut-NS-18, PNR-519
56-65	6.3	Habigonj Boro 6, Bhoro Diga, Kala Manik, Kala Manik, Banamuri, Jhora, Hashikalmi, IRRIBINI, MR-219, Y-1281, RD-21M
66-71	5.0	Charnock (DA 6), Chini Sagar, Kala Jira, Latisail 11-117, TNDB-100, THDB
72-75	3.0	Pokkali, Bawoi Jhak, Dhol Kochuri, RD-2586
CV (%)	20.5	

† On a scale of 1-9, where 1= highly tolerant and 9 = highly susceptible

TABLE 2. PHENOTYPIC CHARACTERISTICS OF SEEDLINGS OF 23 RICE VARIETIES

No.	Genotypes	Salinity-induced reduction (%) in			SES score†	ΔS (‰)	
		Plant height	Total DM	Root length		Non-saline	Saline
1	Pokkali	18	40	16	3	21.07	19.93
2	Kaliboro 139-2	45	72	52	7	21.36	19.58
3	Bara (Boro) Dhan	29	53	24	5	21.64	20.06
4	Chini Sagar	45	71	52	7	21.42	19.99
5	Bawoi Jhak	40	70	45	7	20.76	19.93
6	Jangliboro 581	43	75	46	7	21.61	19.55
7	Kaliboro 109-4	47	75	48	7	21.55	19.65
8	Charnock (DA6)	29	55	32	6	21.20	19.84
9	Kaliboro 138-2	41	75	54	7	21.80	20.15
10	Kalo Bhog	39	66	45	7	21.64	20.31
11	Dhol Kochuri	25	51	26	5	21.42	20.28
12	Kala Jira	39	66	21	6	21.26	19.90
13	Dhali boro 105-2	38	69	29	6	21.45	20.35
14	Latisail 11-117	27	56	27	5	21.70	20.66
15	RD-2586	23	43	26	5	21.63	20.63
16	TNDB-100	21	41	24	5	21.32	20.25
17	Y-1281	26	56	25	5	21.20	20.22
18	Mut-NS-16	26	57	24	5	21.10	20.34
19	PNR-519	24	46	11	5	21.99	20.79
20	THDB	18	41	17	3	21.64	20.38
21	Mut-1-1	30	57	32	6	22.05	20.60
22	BR-40	32	62	32	6	21.39	19.77
23	Binadhan-5	30	55	34	6	21.45	20.41
$LSD_{(0.05)}$		0.78	1.25	0.85	0.65	0.48	0.53
Mean		32.0	58.8	32.3	5.7	21.46	20.15
Range		18-47	40-75	11-52	3-7	20.76-22.05	19.55-20.79

†1-9 scale under salt stress, where 1= highly tolerant, 9 = highly susceptible.

Salinity stress significantly reduced $\Delta^{13}C$ of seedlings (ΔL) from an average of 21.46‰ under non-saline conditions to 20.15‰ (Table 2). Genetic variation in ΔL under salt stress ranged from 19.55 – 20.79‰, and from 20.76 – 22.05‰ under non-saline conditions. PNR-519 showed the higher ΔL (20.79‰) under saline conditions, while the lowest was Jangliboro 581 (19.55‰). The correlation between ΔL and the SES score was negative under salt stress (Fig. 1), signifying that a lower value of SES (salt tolerant) corresponded with a higher value of ΔL. On the other hand, correlations between ΔL and total DM or root length under salt stress were positive, while there were no significant correlations among these traits under non-saline conditions.

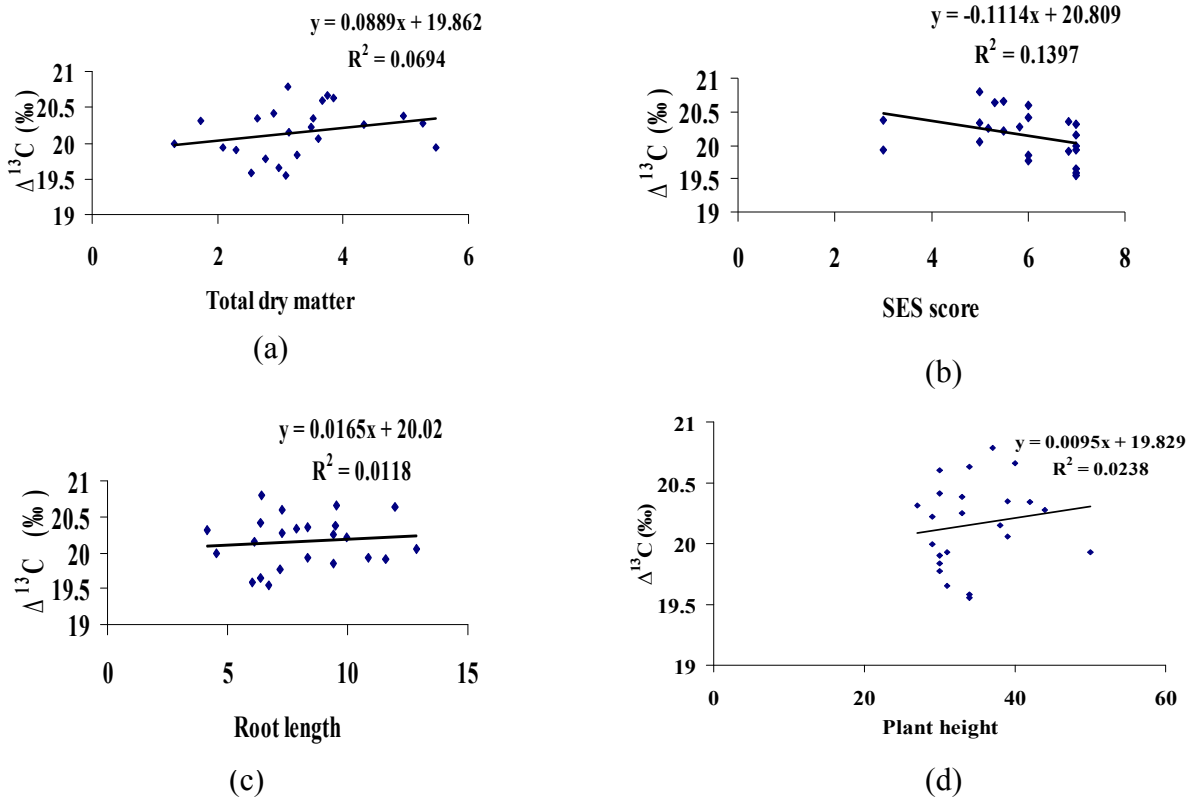


FIG. 1. Relationships between $\Delta^{13}C$ of rice seedlings and (a) total DM (b) SES score (c) root length and (d) plant height under saline conditions.

Highly significant and positive correlations were found between plant height and total DM under the saline regime (Table 3). Correlations of salt tolerance vs. plant height, total DM and root length were negative and significant, which implies that salt tolerant genotypes exhibited higher plant height, total DM and root length. Peng et al. [8] reported that increasing plant height would allow greater biomass production under salt stress in rice. Under salt stress, Pokkali and THDB showed higher plant height and total DM and were salt tolerant.

TABLE 3. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF RICE SEEDLINGS UNDER SALT STRESS

Parameter	Salt tolerance	Plant height	Total DM
Plant height	–0.406**		
Total DM	–0.740**	0.622**	
Root length	–0.278*	0.115	0.291*

*, ** = Significant at $P<0.05$ and $P<0.01$, respectively.

3.2.2. Phenotypic performance at the reproductive stage

The % reduction of plant height, total DM and number of filled grains due to salinity differed among 23 genotypes (Table 4). The lowest % reduction in plant height was found in RD-2586 (8.1%), whereas Kaliboro 139-2 showed the highest reduction (18.1%). The highest % reduction of total DM due to salinity was in Kaliboro 139-2 (64%), while the lowest was observed in RD-2586 (24.3%). THDB showed the lowest % reduction in number of filled grains (11.9%), while Jangliboro-581 showed the highest (95%). Jangliboro 581 had the highest reduction in grain yield plant^{-1} (99.1%), whereas Bara (Boro) Dhan showed the lowest reduction (32.7%).

TABLE 4. PHENOTYPIC CHARACTERISTICS OF 23 RICE GENOTYPES AT THE REPRODUCTIVE STAGE

N	Genotypes	Plant height (cm)		Total DM (g)		Filled grains		Grain yield (g plant^{-1})	
		Non-saline	Saline†	Non-saline	Saline†	Non-saline	Saline†	Non-saline	Saline†
1	Pokkali	110	99 (10.0)	14.56	5.38 (64.0)	305	169 (29)	4.80	2.60 (45.8)
2	Kaliboro 139-2	154	126 (18.1)	14.96	19.48 (35.6)	230	7.0 (83)	3.56	0.12 (96.6)
3	Bara (Boro) Dhan	131	116 (11.5)	30.23	3.70 (36.9	450	300 (21)	7.67	5.16 (32.7)
4	Chini Sagar	111	98 (11.7)	5.87	20.82 (34.9)	90	38 (20)	1.20	0.51 (57.5)
5	Bawoi Jhak	144	128 (11.1)	32.01	8.26 (60.6)	249	130 (34)	4.98	2.10 (57.8)
6	Jangliboro 581	175	146 (16.6)	20.95	7.09 (58.1)	176	3.3 (95)	5.62	0.05 (99.1)
7	Kaliboro 109-4	161	141 (16.1)	16.92	10.88 (32.0)	263	38 (79)	3.29	0.67 (79.6)
8	Charnock (DA6)	116	104 (10.3)	16.01	7.46 (62.5)	195	80 (57)	2.97	1.04 (65.0)
9	Kaliboro 138-2	184	151 (17.9)	19.87	3.54 (63.7)	355	40 (76)	6.29	0.55 (91.3)
10	Kalo Bhog	121	102 (15.7)	9.76	4.56 (37.5)	130	6.7 (52)	2.38	0.05 (97.9)
11	Kala Jira	138	119 (13.7)	7.30	20.34 (26.9)	32	21(17)	0.64	0.42 (34.4)
12	Dhol Kochuri	146	133 (8.9)	27.84	9.56 (59.3)	189	66 (23)	3.42	1.58 (53.8)
13	Dhali boro 105-2	161	136 (15.5)	23.51	16.26 (33.6)	375	13 (85)	6.11	0.24 (96.1)
14	Latisail 11-117	126	113 (10.3)	24.49	11.56 (24.3)	275	56 (29)	8.60	1.08 (87.4)
15	RD-2586	111	102 (8.1)	15.28	5.97 (27.3)	439	279 (23)	9.10	5.00 (45.1)
16	TNDB-100	90	82 (8.8)	8.21	3.98 (36.3)	273	174 (24)	5.78	1.39 (76.0)
17	Y-1281	98	86 (12.2)	6.24	6.91 (63.3)	140	58 (25)	2.26	0.43 (81.0)
18	Mut-NS-16	143	117 (18.2)	18.82	4.86 (28.4)	137	7.3 (75)	2.00	0.10 (95.0)
19	PNR-519	100	91 (9.0)	6.79	11.63 (31.4)	262	179 (27)	3.31	1.98 (40.2)
20	THDB	141	126 (10.6)	17.11	6.58 (43.5)	196	131 (12)	3.10	1.75 (43.5)
21	Mut-1-1	96	88 (8.3)	11.65	9.22 (32.4)	156	12.7(58)	2.10	0.20 (90.5)
22	BR-40	94	84 (10.6)	13.64	11.66 (38.0)	308	174 (25)	5.20	1.55 (70.2)
23	Binadhan-5	154	136 (11.7)	18.80	0.93 (0.97)	271	170 (25)	4.50	1.84 (59.1)
LSD $_{(0.05)}$		0.02	0.10 (0.87)	0.79	9.57 (40.11)	0.01	2.17 (2)	0.43	0.61 (0.75)
Mean		130.7	114 (12.4)	16.56	5.38 (64.0)	239	94 (43)	4.30	1.32 (69.4)
Range		90-184	82-151 (8.1-18.1)	5.87-32.01	3.54-20.82 (24.3-64.0)	32-450	3.3-300 (12-95)	0.64-9.10	0.05-5.16 (40.2-99.1)

†Data in parentheses are % reduction due to salinity.

With respect to phenology, Bawoi Jhak, Mut-NS-16 and BR-40 flowered earlier (84 days) whereas THDB showed late flowering (119 days) under salt stress (Table 5). Dhali Boro105-2 matured earlier (121 days) under salinity, while late maturity (131 days) was observed for genotypes Kaliboro 109-4 and Kala Jira.

THDB showed the highest $\Delta^{13}C$ of the flag leaf (ΔF) (21.58‰) under salt stress, whereas Kaliboro 139-2 had the lowest ΔF (19.09‰) (Table 5). Genotypic variation in ΔF under non-saline conditions ranged from 20.53 to 22.28‰. There were positive relationships between ΔF and total DM, number of filled grains and grain yield plant^{-1} under the saline regime (Fig. 2), but there were no significant relationships in the absence of salinity.

TABLE 5. PHENOTYPIC CHARACTERISTICS OF 23 RICE GENOTYPES AT THE REPRODUCTIVE STAGE

No	Genotypes	1000 seed weight (g)		Days to flowering		Days to maturity		ΔF (‰) †	
		Non-saline	Saline	Non-saline	Saline	Non-saline	Saline	Non-saline	Saline
1	Pokkali	22.58	20.37	104	92	134	122	20.88	20.12
2	Kaliboro 139-2	24.59	21.70	91	86	134	125	21.99	19.09
3	Bara (Boro) Dhan	26.50	21.00	96	91	136	124	21.36	19.83
4	Chini Sagar	19.20	17.10	92	90	137	126	20.63	19.24
5	Bawoi Jhak	20.90	19.83	86	84	137	126	20.98	20.43
6	Jangliboro 581	24.39	20.27	92	80	136	123	21.36	19.35
7	Kaliboro 109-4	22.35	19.43	92	85	153	131	20.85	19.34
8	Charnock (DA6)	21.20	17.30	95	90	156	125	20.79	19.98
9	Kaliboro 138-2	24.47	18.10	91	87	136	128	21.80	19.27
10	Kalo Bhog	19.98	13.03	96	86	136	126	21.77	19.18
11	Kala Jira	20.13	12.57	94	85	140	131	21.39	20.32
12	Dhol Kochuri	22.93	20.36	93	86	138	126	21.00	20.87
13	Dhali boro 105-2	24.50	23.87	96	85	137	121	20.95	19.39
14	Latisail 11-117	25.51	24.43	96	92	135	127	20.88	20.57
15	RD-2586	28.61	21.58	93	85	140	126	21.58	21.14
16	TNDB-100	20.99	20.28	99	90	140	127	21.96	21.48
17	Y-1281	22.95	20.93	99	90	140	130	21.07	20.22
18	Mut-NS-16	19.10	17.97	97	84	151	124	22.09	20.44
19	PNR-519	21.70	20.30	92	87	150	128	20.53	20.33
20	THDB	20.70	19.33	122	119	138	129	21.86	21.58
21	Mut-1-1	21.84	21.06	114	105	151	126	21.90	21.1
22	BR-40	23.35	16.24	121	84	134	122	22.28	20.79
23	Binadhan-5	24.10	20.69	95	92	138	127	20.95	19.94
LSD $_{(0.05)}$		0.01	0.01	23.8	1.2	4.3	5.6	0.62	1.02
Mean		22.72	19.47	98	89	140	126	21.34	20.17
Range		19.1-28.61	12.57-24.43	86-122	80-199	134-156	121-131	20.53-22.28	19.09-21.58

†ΔF = CID of the flag leaf.

A positive and highly significant correlation was observed between plant height and total DM at the reproductive stage under the saline regime (Table 6), suggesting that the higher the plant height the higher would be the total DM. Total DM and grain yield plant^{-1} were positively and significantly correlated with each other under salinity. Positive and highly significant correlations were also found among the number of filled grains, % fertility and grain yield plant^{-1}, which suggested that the increase of % fertility and grain yield plant^{-1} corresponded with an increase in the number of filled grains. Islam [9] found positive and

significant correlations among reduction in the number of filled grains, % fertility, grain yield plant^{-1} and total biomass at the reproductive stage in 80 recombinant inbred lines (RILs) of Pokkali/IR29 under salt stress.

Leaf damage was attributed to the accumulation of a high concentration of Na^+ that was transferred from the root to the shoot [10]. Ali et al. [11] observed that some rice lines showed a marked reduction in the root: shoot ratio at the seedling stage under salt stress. Reductions in root and leaf growth of rice seedlings as well as their delayed development were also observed by Bahaji et al. [12].

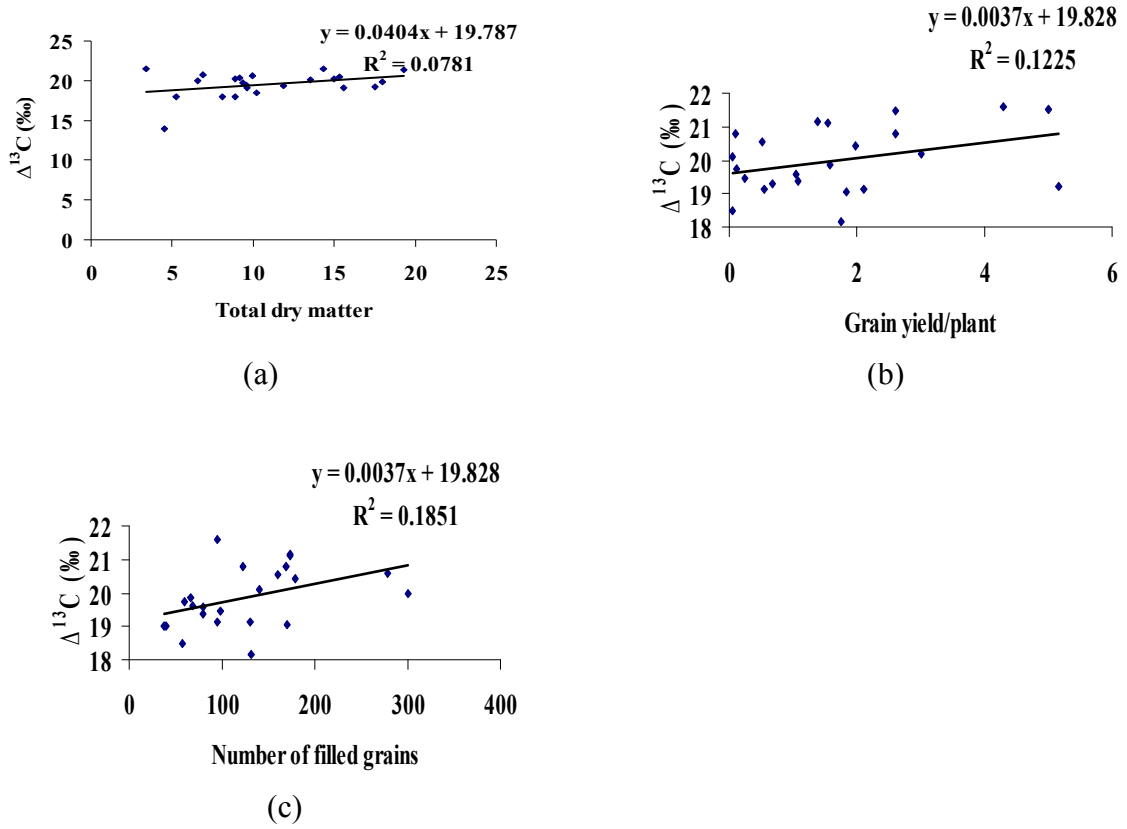


FIG. 2. Relationships between $\Delta^{13}C$ and (a) total DM (b) grain yield plant^{-1} and (c) number of filled grains in the saline treatment at the reproductive stage.

TABLE 6. CORRELATION COEFFICIENTS BETWEEN YIELD AND YIELD COMPONENTS OF RICE AT THE REPRODUCTIVE STAGE UNDER SALINE CONDITIONS

Parameter	Plant height	Total DM	No. of filled grains	% Fertility	Grain yield plant^{-1}
Total DM	0.536**				
No. of filled grains	–0.283	0.271			
% Fertility	–0.458*	0.073	0.743**		
Grain yield plant^{-1}	–0.146	0.433*	0.937**	0.651**	
1000 seed weight	0.086	0.271	0.209	0.091	0.258

*, ** = Significant at the 5% and 1% levels of probability, respectively.

The correlation between Δ and salinity tolerance (SES score) was negative and significant ($P<0.05$) at the seedling stage, suggesting that tolerant genotypes were able to maintain higher gas exchange and photosynthetic capacity under long term salt stress. Therefore selection for higher Δ of leaf tissue should result in higher salt tolerance. A large reduction in Δ as salinity increases indicates salinity tolerance, and reduced osmotic stress intolerance. It appears that salt-sensitive cultivars may exhibit high ^{13}C discrimination under non-saline conditions [13]. Dingkuhn et al. [14] reported that among 28 rice cultivars tested, Δ ranged from 19.8 – 21.5‰, with japonica types having the lowest mean Δ, aus types, the greatest, and indica types, intermediate, the opposite pattern to that observed for water use efficiency (WUE).

Salinity screening at the reproductive stage showed that genotypes differ in their salinity tolerance, with tolerant cultivars showing less growth reduction than sensitive genotypes [15, 16]. At the reproductive stage, salinity affects the yield and yield components more than at the vegetative stage [17]. A considerable effect due to salinity was observed for most of the traits evaluated during the reproductive stage. Growth reduction under salt stress could be attributed to excessive ion accumulation in the plant tissue and/or to water stress due to low external osmotic potential resulting from salinity. This result agrees with Khan et al. [18] who reported plant height of rice decreased under salinity at EC 8 dS m^{-1}. Tolerant genotypes showed lower reductions of yield components than that of susceptible genotypes. Asch et al. [19] found that 80 rice cultivars differed in their salt uptake, and that yield and yield components of tolerant cultivars were less affected by salinity than susceptible cultivars. The number of filled grain and total DM contributed the most variation in grain yield under salt stress, and these traits were used as selection criteria. Loss of biomass was less in tolerant genotypes which ultimately produced the higher number of grains. This result was consistent with the result observed by Islam [9] who worked with 80 RILs of Pokkali x IR29 and reported that total biomass of tolerant lines was reduced by 49.5% under salinity whereas susceptible lines were reduced by 64.0%. The reduction in total biomass is the major cause of stand loss in salt-affected rice fields. Yield sink capacity is always one of the primary objectives in plant breeding for increasing crop yield. The yield components are believed to have their own critical development periods that can affect final grain yield [20].

At the reproductive stage, Kaliboro 139-2, Jangliboro 581, Kalo Bhog, Kaliboro 138-2 and Dhali boro 105-2 had higher Δ than Pokkali under non-saline conditions, but a lower Δ than Pokkali under salt stress, suggesting that salinity induced a greater degree of stomatal resistance that provided less opportunity for discrimination against ^{13}C.

Positive correlations were noted between ΔF and grain yield which partially agrees with results reported for bread wheat and barley under both water-stressed and well watered conditions [21, 22]. Rebetzke et al. [23] observed that Δ was correlated negatively with transpiration efficiency in a backcross population of a commercial wheat variety, as well as other C_3 species. Merah et al. [24] reported that higher Δ in durum wheat was caused by a higher ratio of intercellular to atmospheric concentrations of CO_2 because of a larger stomatal conductance, which leads to higher rates of photosynthesis and higher yield. CID has been proposed as a criterion to select superior genotypes with improved WUE in C_3 crop species [25, 26].

3.3. Year 3

The genetic background, Δ and salt tolerance of the parents are shown in Table 7.

Thirty eight plants were selected out of 900 plants from the cross Horkoch x Binadhan-5. Only one plant showed the semi-dwarf characteristic (86 cm); 17 tillers were found in four plants; days to flowering and maturity were similar for all plants; the majority of the plants showed higher numbers of filled grains plant^{-1} than those of the parents (Table 8); and two plants exhibited higher grain yield plant^{-1} than those of the parents.

TABLE 7. $\Delta^{13}C$ AND SALT TOLERANCE OF PARENTS USED FOR CROSSING (1ST BATCH)

No.	Parents	Genetic background	ΔL †	ΔF †	Salt tolerance
1	Pokkali	Indian local cultivar	High	High	Tolerant
2	Bawoi Jhak	Bangladeshi landrace	High	High	Tolerant
3	Jangliboro 581	Bangladeshi landrace	Low	Low	Susceptible
4	Charnock (DA6)	Bangladeshi landrace	Low	Low	Susceptible
5	RD-2586	Advanced mutant	High	High	Moderately tolerant
6	TNDB-100	Advanced mutant	High	High	Moderately tolerant
7	Y-1281	Advanced mutant	High	High	Moderately tolerant
8	PNR-519	Advanced mutant	High	High	Moderately tolerant
9	Mut-1-1	Advanced mutant	High	High	Susceptible
10	Baradhan	Bangladeshi landrace	High	High	Susceptible
11	BRRI dhan40	Bangladeshi HYV	Low	High	Moderately tolerant
12	Binadhan-5	Bangladeshi HYV	Low	Low	Susceptible

†ΔL, ΔF = CID of seedling leaf, and flag leaf at the reproductive stage, respectively

TABLE 8. PHENOTYPIC CHARACTERISTICS OF PARENTS AND F_2 LINES OF BINADHAN-5 X HORKOCH CROSS GROWN IN THE BORO SEASON IN 2006

Parents/F_2 lines	Plant height (cm)	Effective tillers (plant^{-1})	Time to flowering (d)	Time to maturity (d)	Filled grains (plant^{-1})	Grains (plant^{-1})	Grain yield (g plant^{-1})
Horkoch (P)	135	17	123	140	1425	1510	68.2
Binadhan-5 (P)	100	12	126	150	980	1511	22.5
F_2 mean	124.4	14.6	121.1	139.4	2084.1	2700.7	46.2
SD	9.0	2.9	1.5	1.3	701.2	897.4	12.4
CV (%)	7.2	19.9	1.2	1.0	33.6	33.2	26.7
Range	86-138	10.0-19	117-123	138-142	1164-3494	1359-4614	26.8-80.4

3.4. Year 4

3.4.1. Phenotypic performance at the seedling stage

The 38 F_3 lines showed wide variation with respect to salt tolerance (Table 9). Five lines (P25S2, P25S5, P25S6, P25S8 and P18S4) were identified as salt tolerant and 17 lines were moderately tolerant. The parent Horkuch scored 1 (highly tolerant) while the parent Binadhan-5 scored 7 (susceptible).

3.4.2. Phenotypic performance at the reproductive stage

Phenology varied across the 38 F_3 lines (Table 10). Time to flowering ranged from 78 to 98 days in the non-saline treatment and from 73 to 91 days under salt stress. The tolerant parent Horkuch had delayed flowering (123 and 117 days under non-saline and saline regimes, respectively). Time to maturity ranged from 108 to 124 and from 106 to 121 days under non-saline and saline conditions, respectively. Horkuch matured late, i.e., 146 and 141 days under non-saline and saline conditions, respectively.

TABLE 9. SALINITY TOLERANCE OF F_3 RICE SEEDLINGS OF THE CROSS BINADHAN-5 × HORKUCH IN HYDROPONICS AT AN ELECTRICAL CONDUCTIVITY OF 12 DS M^{-1}

SES score †	Lines/parents
9	P15S3, P34S8
7	P36S5, P25S1, P15S1, P15S5, P25S6, P34S10, P16L4, P11S10, P14S, P14S2, P11S15, P29S10, P3S9, P11S2, Binadhan-5 (susceptible parent)
5	P27S1, P27S5, P1L3, P25S3, P25S4, P29S7, P25S7, P25S9, P25S10, P16L5, P24L2, P36S3, P14S3, P18S9, P18S2, P29S1, P29S12
3	P25S2, P25S5, P25S8, P18S4, P5L3
1	Horkuch (tolerant parent)
SD, 1.48; CV (%), 27.34	

†9 = highly susceptible, 7 = susceptible, 5 = moderately tolerant, 3 = tolerant, 1 = highly tolerant.

The lowest % reduction in plant height under salt stress was found for P25S10 (1.5%), whereas P25S3 showed the highest reduction (22.9%) (Table 10). The total DM $plant^{-1}$ ranged from 3.9 – 15.6 g and 2.5 – 8.8 g under non-saline and saline conditions, respectively. The lowest % reduction (5.2%) in total DM was observed in P25S8, while the highest (42.6%) was seen in P29S10.

The number of filled grains $plant^{-1}$ under salt stress ranged from 46 to 188, and under non-saline conditions from 107 to 206 (Table 11). With respect to the number of filled grains, P27S5 showed the lowest % reduction (9.7%), while line P29S1 showed the highest % reduction (70.9%). Lines P18S9 and P27S5 produced the highest grain yield $plant^{-1}$ (3.1 g) under salt stress. The lowest % reduction in grain yield $plant^{-1}$ was observed in P18S9 (6.1%). All the lines exhibited lower reductions in grain yield $plant^{-1}$ than the susceptible mutant variety Binadhan-5, which had the highest reduction (64.4%). Genotypic variation of yield $plant^{-1}$ under salt stress ranged from 1.0 – 3.1 g, and under non-saline conditions from 1.4 – 3.9 g (Table 11).

Heritability in the broad sense was high for the number of filled grains $plant^{-1}$ (58.1), 1000-seed weight (85.9) and grain yield $plant^{-1}$ (45.3) under salt stress (Table 11), while heritability was low for number of filled grains plant (14.6) and grain yield $plant^{-1}$ (29.9) in the absence of salt stress. Five lines, namely P27S5, P25S2, P25S3, P25S8 and P18S9 were salt tolerant at the reproductive stage based on the overall number of filled grains $plant^{-1}$, total DM and grain yield $plant^{-1}$. However, selection for a high survival rate under salt stressed conditions does not necessarily lead to an improvement in yield since the selected plants may be partially or completely sterile [27].

3.4.3. Relationships between Δ and phenotypic characteristics

Correlation between ΔL and SES score of 38 F_3 rice lines was negative under salt stress at the seedling stage (Fig. 3), illustrating that highly salt tolerant lines are associated with higher values of ΔL. There were positive correlations between ΔF and total DM, number of filled grains and grain yield plant^{-1} under saline conditions at the reproductive stage (Fig. 4).

TABLE 10. PHENOTYPIC CHARACTERISTICS OF PARENTS AND 38 F3 LINES OF RICE OF THE CROSS BINADHAN-5 X HORKUCH UNDER NON-SALINE AND SALINE CONDITIONS (EC 6 DS M^{-1}) AT THE REPRODUCTIVE STAGE

Lines/ parents	Plant height (cm)			Time to Flowering (d)		Time to Maturity (d)		Total DM (g plant^{-1})		
	Non-saline	Saline	% reduction	Non-saline	Saline	Non-saline	Saline	Non-saline	Saline	% reduction
P27S1	143	128	10.1	83	79	115	110	5.4	3.7	28.4
P27S5	152	123	18.8	85	78	116	113	6.0	5.2	14.3
P36S5	140	135	3.7	80	73	108	106	10.2	6.0	36.8
P25S1	120	108	10.5	98	88	124	118	8.8	6.6	23.4
P25S2	106	102	3.3	92	86	121	119	9.9	6.8	29.7
P25S3	102	78	22.9	92	89	120	120	7.5	7.8	–3.6
P25S4	100	91	8.6	94	91	119	119	6.9	4.1	39.1
P25S5	103	93	9.5	94	89	120	119	7.8	5.7	27.1
P1L3	103	95	7.9	85	83	115	114	6.1	3.1	40.6
P15S1	129	119	7.2	91	82	120	115	7.1	4.9	31.2
P15S3	116	111	4.6	92	86	122	117	7.3	5.4	23.5
P15S5	136	132	3.2	93	87	123	119	7.0	4.6	33.4
P29S7	118	99	14.6	82	77	121	113	5.1	3.6	30.3
P25S6	106	92	12.7	92	84	122	120	8.6	5.0	39.1
P25S7	108	90	15.9	85	82	124	121	7.2	5.9	17.3
P25S8	105	99	5.0	93	84	121	115	6.5	6.7	–5.2
P25S9	116	97	16.5	78	76	111	108	10.8	8.5	20.0
P25S10	107	105	1.5	90	86	120	120	6.5	5.4	18.3
P34S8	151	146	3.4	91	80	120	112	8.0	8.1	–1.7
P34S10	145	137	5.0	93	79	118	109	8.9	6.6	23.3
P16L4	144	129	10.6	92	89	121	118	9.1	7.0	21.7
P16L5	150	140	6.5	84	79	124	120	8.6	6.1	29.4
P24L2	111	102	7.9	92	81	121	111	3.9	2.5	32.8
P11S10	146	140	4.0	93	88	120	117	10.4	7.6	24.6
P14S1	168	154	8.4	91	80	118	111	10.4	7.4	28.1
P14S2	153	143	6.6	91	80	120	112	5.8	3.9	34.2
P14S3	147	131	10.2	88	81	122	118	10.7	6.7	37.3
P18S9	99	94	5.3	92	90	121	119	10.2	7.7	24.1
P18S2	108	103	4.9	93	89	121	120	8.8	6.5	23.5
P18S4	102	97	5.3	88	86	123	118	6.8	5.7	13.9
P11S15	144	139	3.9	85	75	114	108	11.6	7.8	29.0
P29S1	142	133	6.6	90	80	119	112	9.0	6.5	25.6
P29S10	142	137	3.7	84	79	122	119	15.6	8.8	42.6
P29S12	142	132	7.0	93	87	120	119	7.4	7.6	–2.8
P3S9	162	150	6.9	94	83	121	113	9.9	7.3	26.1
P5L3	119	103	13.7	96	86	123	116	7.5	5.2	29.7
P11S2	155	132	14.7	95	91	121	119	10.0	8.7	12.7
Horkuch	144	141	2.1	123	117	146	141	19.6	17.6	10.0
Binadhan-5	100	94	5.9	93	91	121	119	5.2	3.6	30.1
Mean	127	117	8.3	90	83	120	116	8.2	6.1	24.0
Range	99 - 168	78-154	1.5-22.9	78-98	73-91	108-124	106-121	3.9-15.6	2.5-8.8	–5.2-42.6
$LSD_{(0.05)}$	15	16	10.9	8	10	6	6	3.0	2.0	26.5

TABLE 11. PHENOTYPIC CHARACTERISTICS OF PARENTS AND 38 F_3 LINES OF RICE OF THE CROSS BINADHAN-5 X HORKUCH UNDER NON-SALINE AND SALINE CONDITIONS (EC 6DS M^{-1}) AT THE REPRODUCTIVE STAGE

Lines/ parents	Filled grains (plant^{-1})			1000 seed weight (g)			Grain yield (g plant^{-1})		
	Non-saline	Saline	% Reduction	Non-saline	Saline	% Reduction	Non-saline	Saline	% Reduction
P27S1	182	105	40.6	22.1	18.6	15.6	3.4	1.9	43.5
P27S5	180	188	–9.7	19.1	18.2	4.8	3.0	3.1	–1.4
P36S5	206	150	36.6	21.6	18.7	13.1	3.9	2.3	37.2
P25S1	168	87	37.7	18.8	14.5	22.8	2.0	1.3	28.9
P25S2	148	120	11.0	18.5	17.2	6.8	2.6	1.4	43.9
P25S3	151	128	15.0	19.0	18.0	5.0	2.6	2.7	–3.7
P25S4	174	70	56.1	19.5	16.4	15.9	2.8	1.1	56.5
P25S5	142	90	36.1	21.0	19.4	7.6	2.5	1.4	43.1
P1L3	164	133	24.5	18.6	17.6	5.6	2.5	2.2	13.2
P15S1	133	46	64.1	22.5	19.0	15.4	2.5	1.0	60.9
P15S3	107	90	18.0	18.7	18.3	1.9	1.6	1.3	19.2
P15S5	111	71	36.8	15.6	15.3	2.4	1.4	1.1	22.6
P29S7	181	105	41.0	22.2	19.0	14.6	3.5	2.0	41.9
P25S6	180	74	56.2	17.9	17.1	4.4	2.6	1.6	41.4
P25S7	174	56	66.5	18.7	18.0	3.6	2.6	1.1	57.5
P25S8	160	141	11.1	22.3	17.5	21.5	2.9	2.6	11.5
P25S9	159	133	15.9	17.5	16.6	5.1	2.3	1.9	19.7
P25S10	129	106	18.6	19.5	15.2	22.3	2.0	1.7	16.5
P34S8	127	65	46.6	16.4	14.5	12.0	1.7	1.0	34.7
P34S10	200	93	51.5	19.7	17.6	10.8	3.3	1.5	53.8
P16L4	156	90	43.6	19.7	17.3	12.2	2.6	1.4	47.0
P16L5	116	63	47.2	15.7	13.5	14.0	1.4	1.0	31.8
P24L2	190	156	15.9	18.4	16.2	12.0	2.8	2.5	10.9
P11S10	187	100	44.6	21.5	17.4	18.9	3.4	1.6	52.0
P14S1	156	87	39.2	21.7	18.3	15.3	2.9	1.4	48.0
P14S2	175	108	36.4	21.7	17.7	18.5	3.2	1.7	45.2
P36S3	149	131	12.2	21.3	18.8	11.5	2.7	2.1	24.8
P14S3	132	141	–6.9	21.9	18.3	16.3	3.2	1.7	45.4
P18S9	166	141	15.7	22.4	18.7	16.8	3.0	3.1	–6.1
P18S2	174	148	14.3	17.8	15.5	13.3	2.5	1.9	24.3
P18S4	173	150	13.8	22.3	18.4	17.4	3.3	2.9	12.4
P11S15	176	78	54.1	18.7	16.6	11.2	2.8	1.2	53.7
P29S1	164	48	70.9	22.1	18.6	15.8	3.0	1.3	56.8
P29S10	199	97	48.7	21.5	19.6	8.8	3.7	1.6	55.1
P29S12	189	89	52.3	18.7	16.1	13.5	3.0	1.4	51.9
P3S9	175	119	30.4	20.8	18.1	13.2	3.1	1.9	37.8
P5L3	176	151	14.8	17.6	16.3	7.2	2.5	2.1	17.8
P11S2	165	94	42.4	20.1	18.2	9.4	2.8	1.5	46.2
Horkuch	140	128	8.6	18.1	18.0	0.5	1.6	1.4	12.5
Binadhan-5	204	82	63.2	21.3	19.3	9.4	3.4	1.2	64.4
Mean	163	105	36.5	19.8	17.3	12.3	2.7	1.5	33.3
Range	107-206	46-188	–9.7-70.9	15.6-22.5	13.5-19.5	1.7-22.8	1.4-3.9	1.0-3.1	–6.1-60.9
Hb (%)	14.5	58.1	-	72.7	85.9	-	29.9	45.3	-
$LSD_{(0.05)}$	73	47	22	1.4	1.5	7.0	1.1	0.87	22.6

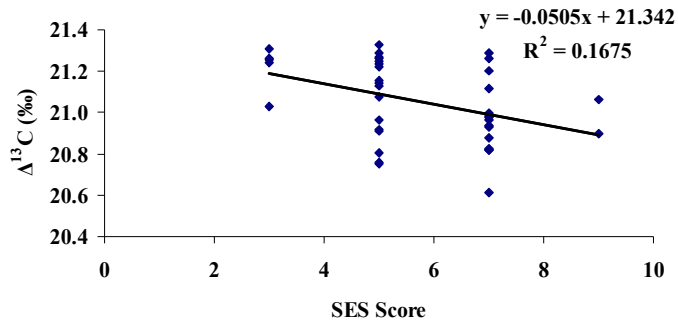
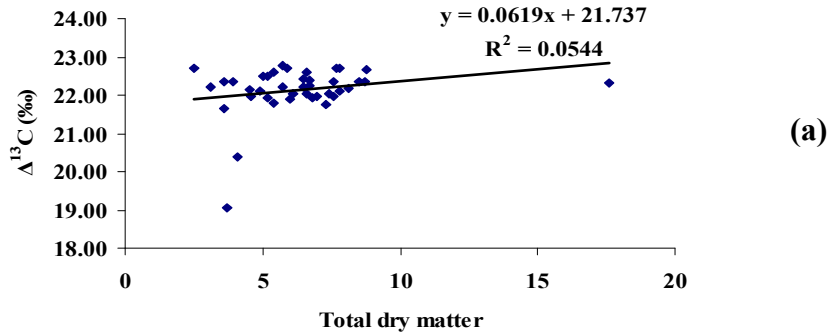
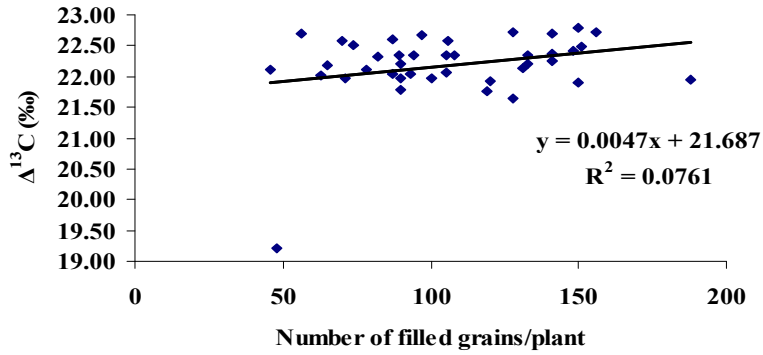


FIG. 3. Relationship between $\Delta^{13}C$ and SES score of $38F_3$ lines under salt stress at the seedling stage.



(a)



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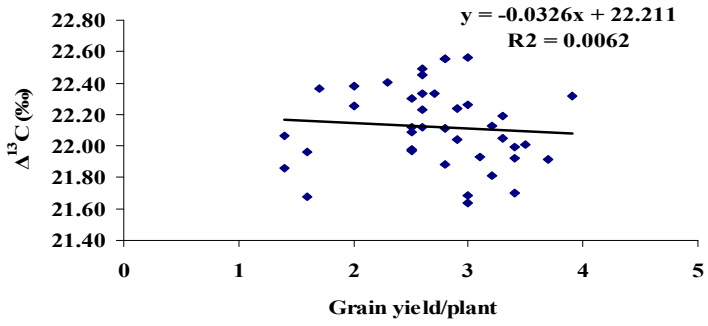

(c)

FIG. 4. Relationships between $\Delta^{13}C$ and (a) total DM (b) grain yield plant^{-1} and (c) number of filled grains plant^{-1} of $38F_3$ lines under salt stress at the reproductive stage.

4. GENERAL DISCUSSION

Seedlings grown under salt stress showed several symptoms of salt injury including yellowing and drying of leaves, reductions in root and shoot growth and stem thickness, and in many cases seedling mortality. Overall, seedling growth was suppressed under salt stress. These symptoms were also observed by Islam [9], Bhuiyan [28] and Bonilla et al. [29]. Under salinity stress, the stomatal limitation of photosynthetic capacity is reflected by a lower Δ. Stomatal closure, at least in part, contributed to the decline in growth and increases in leaf rolling and scorching. A great reduction in Δ as salinity increases indicates salinity intolerance and, in particular, salinity-induced osmotic stress intolerance.

The reduction in total biomass is the major cause of stand loss in salt-affected rice fields. Yield sink capacity is always one of the primary objectives in plant breeding for increasing crop yield. Heritability in the broad sense was higher under salt stress than under non-saline conditions at the reproductive stage, which suggests that improvement of such a character through simple selection would be effective in early generations.

5. CONCLUSIONS

Selection for higher CID may provide a useful method for indirect selection of salt tolerance, and perhaps biomass and grain yield, in a breeding programme to develop rice varieties for saline environments.

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DEVELOPMENT AND APPLICATION OF TECHNIQUES IN BREEDING FOR GREATER GRAIN YIELD OF RICE UNDER FAVOURABLE CONDITONS IN SOUTHERN CHINA

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Abstract

Experiments were carried out to investigate the correlation of carbon isotope discrimination (CID) with yield and yield components, to identify the quantitative trait loci (QTLs) controlling CID of irrigated rice, during 2005 to 2007 under field conditions. The 127 recombinant inbred lines (RILs) derived from a cross between Zhenshan97 and Minghui63 were employed for identification of QTLs. Six hybrid varieties (Chuanxiagyou727, IIyou838, Liangyoupei9, 99you029, Shanyou63 and Liangyou125) were used to investigate relationships between CID and grain yield and/or its yield components under six fertilizer N topdressing rates of 0, 90, 135, 180, 225 and 270 kg N ha^{-1}. A significant transgressive segregation for CID was found in the RILs, and the frequency of CID was almost normally distributed. The CID at the heading stage was negatively correlated with heading date. Negative correlations of CID at 30 days after transplanting were observed with biomass and spikelets per panicle, while positive correlations were found with harvest index and number of panicles. Grain yield, grain filling percentage and 1000 grain weight were not correlated with CID at 30 days after transplanting. Similarly, CID at the heading stage showed poor negative correlations with grain yield, harvest index and 1000 grain weight, while significant negative correlations with biomass, spikelets and productive panicle number were found. A total of five and three QTLs for CID at the heading stage were identified in 2006 and 2007, respectively. They were located at chromosomes 1, 2, 3, 7, 8, 10 and 12. The QTL located at R1440-C1023 on chromosome 7 and the QTL located at C909b-R496 were consistently detected in two years. The QTL for CID located at RG236-C86 on chromosome 1 was associated with the QTLs to control grain yield. CID varied among rice genotype and fertilizer N had a negative effect on CID in three field experiments. Although there was poor correlation of CID with grain yield for inbred germplasm and RILs, a significant negative correlation occurred between CID and grain yield for hybrid varieties. The CID values of high yielding hybrid rice are lower than those of lower yielding varieties, opening up the possibility of using CID for screening high yielding hybrid rice under favourable irrigated field conditions.

1. INTRODUCTION

Rice is the most important food crop in the world. Rice accounts for more than 40% of caloric intake in tropical Asia, reaching more than 60% in many countries, and is the staple for many poor people. It is estimated that world rice production has to increase by 1% annually in the next 20 years to meet the growing demand for food [1]. It is a big challenge to continuously increase world rice production because rice productivity is showing signs of decline, investments in irrigation have virtually ceased, high fertilizer use is causing concern, and good rice lands are being lost to other purposes [2, 3]. The only option available is to

raise the rice yield potential in favourable environments and to enhance adaptability of rice cultivars in less favourable environments through genetic improvement. Improving yield potential of crop varieties through plant breeding has been a critical component of the strategy for global food security, especially for rice and the other major cereals [1, 4, 5].

Irrigated rice contributes more than 75% of total rice production, although it accounts for only about 50% of the total rice area. Irrigated rice covers 80 million hectares worldwide. The majority of these areas are considered favourable for rice production; water and nutrients are not major limiting factors for rice growth. In tropical Asia, the yield potential of current high yielding cultivars grown under favourable environments is about 7 t ha^{-1} during the wet season and about 10 t ha^{-1} during the dry season. The dry season has about 50% higher solar radiation than the wet season. Maximum yield potential has been estimated at 9.5 and 15.9 t ha^{-1} in this region during the wet and dry seasons, respectively, based on the level of solar radiation. The challenge is to narrow the gap between the present and maximum yield potential through genetic improvement [6]. Great progress in increasing rice yield potential has been achieved recently in China by developing new rice varieties using the combination of ideotype approach and utilization of inter-subspecific heterosis. These hybrid varieties produced grain yield of 12 t ha^{-1} in on-farm demonstration fields, 8 – 15% higher than the hybrid check varieties. In a very high yielding environment in Yunnan province, 15 – 18 t ha^{-1} of rice yield has been reported by growing these super hybrid rice varieties [7, 8].

Yield potential is defined as the yield of a variety when grown in environments to which it is adapted, with nutrients and water non-limiting, and with pests, diseases, weeds, lodging and other stresses effectively controlled. Yield potential is determined by biomass production and harvest index. Biomass production is a function of the rates and duration of photosynthesis and respiration [9]. Optimum canopy architecture for maximum crop photosynthesis, increased photosynthetic capacity of individual leaves, and delayed leaf senescence for longer photosynthetic duration are effective approaches for increasing biomass production. Harvest index is affected by sink size, canopy photosynthetic rate during the ripening phase and grain filling. Increased number of grains per panicle and reduced partitioning of dry matter to unproductive tillers will result in an improved harvest index. Understanding physiological processes governing yield potential and identifying plant traits associated with high yield are essential for crop improvement through plant breeding and molecular technology.

Carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) has been shown to have substantial potential application as a screening tool in breeding programs to increase the rate of genetic increase in yield [10, 11]. The isotopic ratio of ^{13}C to ^{12}C in plant tissue is less than the isotopic ratio of ^{13}C to ^{12}C in atmospheric CO_2, indicating that plants discriminate against ^{13}C during photosynthesis, particularly C_3 plants such as wheat, peanuts and cotton, in which the primary CO_2 acceptor is ribulose bi-phosphate (RuBP) [12, 13]. Variation in discrimination against ^{13}C during photosynthesis is due to both stomatal limitations and enzymatic processes. Positive correlations between CID and productivity (biomass production and economic yield) have been reported in wheat, beans, coffee, and cowpea [14, 15, 16]. The positive relationship between CID and productivity indicates that a potential source of variation in CID is variation in stomatal conductance. Recent studies provide strong evidence that grain yield may be improved by increasing stomatal conductance. It was reported that phosphoeno/pyruvate carboxylase (PEPC) and pyruvate orthophosphate dikinase (PPDK) transgenic rice plants enhanced net photosynthetic rate by increasing stomatal conductance. The yield potential of wheat varieties released by CIMMYT has increased by 0.83% per year over the last 30 years. This increase was mainly attributed to increased stomatal conductance and canopy

temperature depression. A strong correlation between grain yield and canopy conductance was observed across rice sub-species. The $\Delta^{13}C$ method has conceptual and practical advantages over measuring stomatal conductance by instantaneous measurements of gas exchange or measuring yield by destructive sampling in whole plant harvests. Since the plant samples (e.g. leaves) can be easily collected for analyses, the method enables analysis of a large number of samples for yield potential in a breeding program [17 - 20].

The genetic gain in yield potential of semi-dwarf rice varieties developed in the late 1950s and early 60s was attributed to the improvement in harvest index. However, high yield of recently developed varieties was achieved by increasing biomass production. For example, hybrid rice has about 15% higher yield than inbreds mainly due to an increase in biomass production rather than in harvest index. Further improvement in rice yield potential will continue to be dependent on increased biomass production [7, 8]. It is unclear whether the increase in biomass production of newly developed varieties was due to the improvement in single leaf photosynthetic rate (greater stomatal conductance) or in canopy photosynthetic rate (better canopy architecture). There has been controversy about the contribution of single leaf photosynthetic rate to the genetic improvement of rice yield potential. One argument is that the poor relationship between single leaf photosynthetic rate and grain yield was due to the limitation of instantaneous measurements of gas exchange. CID was proposed as an efficient method to estimate the long term integrated single leaf photosynthetic rate and stomatal conductance. Measurements of CID in leaf tissue or grain could therefore provide an indirect measure of biomass production and could clarify whether selection for greater CID could result in genetic gain in yield potential [21].

Understanding the genetic relationship between morpho-physiological traits and yield traits could contribute to breeding for improving crop yield potential. Genetic studies for quantitative traits have been greatly facilitated by the development of various molecular markers. The use of quantitative trait locus (QTL) mapping has contributed to a better understanding of the genetic basis of many agronomically important traits such as grain yield. In rice, many researchers have identified QTLs for grain yield and its components. However, attention was only paid to basic yield traits in those studies. The lack of a physiological understanding limits the interpretation of those QTLs, and further limits genetic manipulation in crop improvement [22 - 28].

Since permanent segregating populations, including the recombinant inbred lines (RIL) populations, provide researchers with the characteristics of genetic homozygosity and further advantages for QTL mapping, those populations have been widely employed to identify genes for quantitative traits of importance. In this study, we identified QTLs controlling source, sink, transport tissue, yield traits and various ratios among them, using a RIL population derived from a cross between the parents of an elite hybrid. Emphasis was given to the relations between the various ratios and yield traits. The objective of the study was to reveal the underlying genetic relationship between source-sink-transport characters and yield traits [29, 30, 31].

The experiment included a population consisting of 241 RILs (F_{12}) constructed by a single-seed descendant from a cross between two indica cultivars Zhenshan97 and Minghui63 (the parents of Shanyou63, the most-widely grown hybrid in China) available at Huazhong Agricultural University. We used those RIL seeds for the research in 2006 and later years.

2. MATERIALS AND METHODS

2.1. Experiments

2.1.1. Experiment 1

To evaluate different strategies for using CID as a selection tool for identifying high yielding genotypes of rice, 500 stable lines (screened from 50 typical F_4 lines within each of 20 populations) were planted in an irrigated rice field. All of the materials were field tested for dry matter (DM) accumulation and grain yield at heading and/or at maturity. After harvest, 10 of the typical lines varying in grain yield were selected from the given population to measure the flag leaf and panicle CID. The objective was to investigate the relationship between CID and rice biomass production and/or grain yield, and to establish the criteria of $\Delta^{13}C$ for selecting high yielding rice under irrigation.

The field experiment was conducted in Xiaonan county, Hubei province, China. All the test lines were planted in a farmer's field with a sub-plot size of 2 × 2 m. Thirty-day old seedlings were transplanted on 8th June 2005. Transplanting spacing was 16.7 × 16.7 cm with two seedlings per hill. 150 kg N ha^{-1} in the form of urea was applied in three equal splits, one day before transplanting, 20 days after transplanting (DAT) and at 45 DAT, to ensure N sufficiency. A total of 80 kg K ha^{-1} as potassium chloride (KCl) was applied in two equal splits, one day before transplanting and at 20 DAT. Phosphorus (40 kg ha^{-1} as single superphosphate) and zinc (5 kg ha^{-1} as zinc sulfate heptahydrate) were applied and incorporated in all plots one day before transplanting. Pests and diseases were intensively controlled using chemicals.

Plants were sampled from a 0.20 m^2 area (5 hills) at heading and at maturity. Leaf area was expressed as leaf area index (LAI). The dry weight of each component was determined after oven-drying at 70 °C to constant weight to obtain aboveground total DM. Specific leaf weight (SLW) was calculated as the ratio of leaf DM to leaf area at heading. At maturity, panicles were hand-threshed and the filled spikelets were separated from unfilled spikelets by submergence in tap water. Three sub-samples of 30 g of filled spikelets and 5 g of unfilled spikelets were taken to count the number of spikelets. The filled spikelets were then oven dried at 70 °C to constant weight for determining grain weight. Grain filling percentage (100 × filled spikelet number/total spikelet number) and harvest index were calculated. Grain yield was determined from a 1-m^2 area in each plot and adjusted to the standard moisture content of 0.135 g H_2O g^{-1}. The flag leaf samples collected at flowering from two replicates were ground to a fine powder for $\delta^{13}C$ analysis.

2.1.2. Experiment 2

A population consisting of 241 recombinant inbred lines (RILs at F_{12} generation) was developed from a cross between two Indica varieties, Zhenshan97 (ZS97) and Minghui63 (MH63). The RILs were developed by a single-seed descendant (SSD) method. A total of 140 lines [including two parents, the F_1 and randomly selected 127 RILs from the whole established 241 RILs, two line hybrid rice (Liangyoupei9, F_1) and its parents, and another 4 inbred cultivars] were arranged in a randomized complete block design with three replications. Plot size for each line was 2.2 × 2.6 m.

Thirty-day old seedlings were transplanted on 4-6 June in 2006. Transplanting spacing was 16.7 × 20 cm with three seedlings per hill. A total of 130 kg N ha^{-1} of N fertilizer in the form of urea was applied in three splits (50 kg N ha^{-1} at one day before transplanting, 40 kg N

ha^{-1} at 20 DAT and 40 kg N ha^{-1} at 45 DAT) to ensure N sufficiency. A total of 50 kg K ha^{-1} as KCl was applied in two equal splits (one day before transplanting and at 20 DAT). Phosphorus (40 kg ha^{-1} as single superphosphate) and zinc (5 kg ha^{-1} as zinc sulfate heptahydrate) were also incorporated in all plots one day before transplanting. Pests and diseases were intensively controlled using chemicals. The above experiment was repeated in 2007 at a location in Wuxue County, Hubei province, with the same design as year 2006.

For initial soil and site characterization, samples were collected from each block before basal fertilizer application. Sequential plant sampling was carried out to determine above ground biomass and grain yield at heading and at maturity. At maturity (separately based on different lines), twelve hills in the middle of each plot were harvested and oven dried to determine grain yield, the biomass DM, the grain filling percentage, the 1000 grain weight and harvest index. On the same date panicles from an area of 1 m^2 were harvested for grain yield determination. All the tested lines were harvested on 7th October. Samples were then processed and analyzed.

The flag leaf of the main-stem was sampled at heading to determine the isotopic ratio of ^{13}C to ^{12}C. About 450 samples were collected, dried and then ground. 221 samples were analyzed by the International Atomic energy Agency (IAEA) in Austria, and another 140 samples were analyzed by the Analysis Service Laboratory (ASL) at the International Rice Research Institute (IRRI), Philippines.

For leaf area determination a 5-hill sample was collected at heading. Leaf area was determined by a leaf area meter (LICOR 3100C). The specific leaf weight was calculated based on the leaf area and the oven-dry weight. SPAD (Soil-Plant Analysis Development) readings were taken sequentially during the mid-tillering stage, panicle initiation stage and heading stage. Other agronomic traits investigated were growth duration, plant height, panicle length, and grain yield components.

The molecular-marker linkage map and the markers [25] consisted of 221 marker loci and covered a total of 1,796 centimorgan (cM). The average values of each line from the three replicates of the trial were used in detecting QTLs. The chromosomal locations and the number of putative QTLs were determined using QTLMapper (version 1.0) which was based on a mixed-model approach [32]. QTLs were declared with a LOD (Logarithm of Odd, a statistical estimate of whether two loci are likely to be linked) threshold of 2.5 corresponding to a probability of 0.001 for the detection.

2.1.3. Experiment 3

An experiment was carried out in a farmer's field in Wuxue county, Hubei province in 2007 to develop optimal N management for Liangyoupei9 (LYP9, the two-line super hybrid rice variety widely adapted in China), and to determine the effect of N fertilizer rate on the relationships among CID, grain yield and yield components.

Treatments were arranged in randomized block design using the two-line super hybrid variety-LYP9, with eight fertilizer-N rates ranging from 0 to 365 kg N ha^{-1}. The experiment was replicated four times with a plot size of 25 m^2. LYP9 is an inter-subspecific hybrid rice developed with Pei'ai64S as the female parent and 9311 as the male parent using the two-line method [33]. Pei'ai64S is a thermo-sensitive genetic male sterile line and belongs to the intermediate type between Indica and Japonica. The restorer line 9311 is a typical Indica type.

Eight N treatments included four real-time N management (RTNM) treatments with SPAD thresholds of 36 to 45 at an interval [34]. A chlorophyll meter (SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan) was used for weekly measurements on 15 topmost fully expanded leaves per plot from 15 to 75 DAT. In these RTNM treatments, N in the form of urea was applied by broadcasting when the mean SPAD value was below the threshold from 15 DAT to heading. N rates of each topdressing increased by 5 kg N ha^{-1} with each increment of SPAD threshold in order to reduce the number of N application in RTNM treatments with high SPAD thresholds. The N rates of 30, 35, 40, and 45 kg N ha^{-1} were used for the SPAD thresholds of 36, 39, 42, and 45, respectively. Basal N of 50 kg N ha^{-1} was applied one day before transplanting in RTNM treatments in the above N treatments. Additional RTNM treatment was assigned with SPAD thresholds of 39 and without basal N application.

Fixed-time adjustable-dose N management (FTNM) was tested [35, 36, 37]. Fifty kg N ha^{-1} was applied as a basal dressing in the FTNM treatment. For N top-dressing at mid-tillering and panicle initiation, if the SPAD reading was greater than 39, a base N rate minus 10 kg N ha^{-1} was applied; if less than 37, a base N rate plus 10 kg N ha^{-1} was applied; if between 37 and 39, a base N rate only was applied. A zero-N control and a farmers' fertilizer practice (FFP) treatment were included. In FFP, the basal application rate was 126 kg N ha^{-1}, and a top-dressing at mid-tillering, one week after transplanting, was 54 kg N ha^{-1}.

Pre-germinated seeds were sown in the seedbed. 27-day old seedlings were transplanted on 12th June 2007 at a hill spacing of 0.2 × 0.2 m with two seedlings per hill. Phosphorus (40 kg P ha^{-1}) and zinc (5 kg Zn ha^{-1}) were applied and incorporated in all subplots one day before transplanting. Potassium (100 kg K ha^{-1}) was equally split as a basal dressing and at panicle initiation. Crop management followed standard cultural practices. The experimental field was kept flooded from transplanting till 10 days before maturity. Insects were intensively controlled by chemicals to avoid biomass and yield loss. Herbicide was used to control weeds. The final harvest was on 2nd October, 2007.

Plants were sampled from a 0.48 m^2 area (12 hills) at mid-tillering, panicle initiation, heading and maturity. Leaf area was expressed as leaf area index (LAI). The dry weight of each component was determined after oven-drying at 70°C to constant weight to obtain above ground total DM. Specific leaf weight (SLW) was calculated as the ratio of the leaf dry weight to the leaf area at heading. At maturity, panicles were hand-threshed and the filled spikelets were separated from unfilled spikelets by submergence in tap water. Three sub-samples of 30 g filled spikelets and 5 g unfilled spikelets were taken to count the number of spikelets. The filled spikelets were then oven dried at 70°C to constant weight for determining grain weight. Grain filling percentage (100 × filled spikelet number/total spikelet number) and harvest index were calculated. Grain yield was determined from a 5-m^2 area in each plot, and adjusted to the standard moisture content of 0.135 g H_2O g^{-1}. The samples of mixed leaves and stems collected at panicle initiation from two replicates were ground to a fine powder for $\delta^{13}C$ isotope ratio analysis.

2.1.4. Experiment 4

Five hybrid varieties and an inbred variety were tested in a farmer's field in Wuxue county, Hubei province in 2007 to compare the growth and yield responses under various fertilizer N regimes and to understand the CID of different high yielding hybrid varieties.

Treatments were arranged in a split plot design with N rates as the main plots and varieties as subplots. The experiment was replicated three times and the subplot size was 20

m^2. Six fertilizer-N rates ranged from 0 to 270 kg N ha^{-1}. The tested varieties included three-line hybrid varieties Chuanxiangyou727 (CXY727), IIyou838 (IIY838), and 99you029 (99Y029), two-line hybrid varieties Liangyoupei9 (LYP9) and Liangyou125 (LY125), and the inbred variety Huanghuazhan (HHZ) as the control. Fertilizer N rates were 0, 90, 135, 180, 225 and 270 kg N ha^{-1}, with basal, mid-tillering and panicle initiation splits of 40, 20 and 40%, respectively. Other fertilizers such as phosphorus (40 kg P ha^{-1}) and zinc (5 kg Zn ha^{-1}) were applied and incorporated in all subplots one day before transplanting. Potassium (100 kg K ha^{-1}) was equally split as a basal dressing and at panicle initiation.

Pre-germinated seeds were sown in the seedbed. 27-day old seedlings were transplanted on 12th June 2007 at a hill spacing of 0.2 × 0.2 m with two seedlings per hill. Crop management followed standard cultural practices. The experimental field was kept flooded from transplanting till 10 days before maturity. Insects were intensively controlled by chemicals to avoid biomass and yield loss. Herbicide was used to control weeds. The final harvest was between 25th September and 2nd October, based on the proper harvest dates for each variety.

Plants were sampled from a 0.32 m^2 area (8 hills) at mid-tillering, panicle initiation, heading and maturity. Leaf area was expressed as leaf area index (LAI). The dry weight of each component was determined after oven-drying at 70°C to constant weight to obtain above ground total dry weight. Specific leaf weight (SLW) was calculated as the ratio of the leaf dry weight to leaf area at heading. At maturity, panicles were hand-threshed and the filled spikelets were separated from unfilled spikelets by submergence in tap water. Three sub-samples of 30 g filled spikelets and 5 g unfilled spikelets were taken to count the number of spikelets. The filled spikelets were then oven dried at 70 ^{o}C to constant weight for determining grain weight. Grain filling percentage (100 × filled spikelet number/total spikelet number) and harvest index were calculated. Grain yield was determined from a 5-m^2 area in each plot, and adjusted to the standard moisture content of 0.135 g H_2O g^{-1}. Samples of mixed leaves and stems collected at panicle initiation from two replicates were ground to a fine powder for $\delta^{13}C$ isotope ratio analysis.

2.2. Analyses

2.2.1. Isotope ratios

Carbon isotope composition ($\delta^{13}C$) was determined with an isotope ratio mass spectrometer and calculated as:

$$\delta^{13}C\,(‰) = \left[\left(R_{sample}/R_{reference}\right) - 1\right] \times 1000 \quad (1)$$

with R being the $^{13}C/^{12}C$ ratio.

CID (Δ) was calculated using the formula [13]:

$$\Delta\,(‰) = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000 \quad (2)$$

where δ_p is the $\delta^{13}C$ of the plant sample and δ_a is the $\delta^{13}C$ of atmospheric CO_2 (–8‰).

2.2.2. Quantitative trait locus (QTL)

The molecular linkage map was constructed using Mapmaker 3.0 [38], employing the Kosambi function to calculate genetic distance. The chromosomal locations and the number of putative QTLs with additive and epistatic effects were determined by using a mixed linear-model and the corresponding computer software QTLMapper 1.6 [32]. A LOD threshold of 2.0 and a significance level of 0.005 were used. Standard nomenclature for QTLs was used [39].

3. RESULTS AND DISCUSSION

3.1. Relationships between Δ and grain yield or related parameters with cross populations

CID of flag leaves at heading, grain yield and dry weight of above ground biomass of the test lines during 2005 are presented in Fig. 1. It is clear that there are no visible differences among all the test lines for flag leaf Δ at heading, while the grain yield and dry weight of above ground biomass varied for the different test lines.

We therefore selected four populations within all 520 lines from 10 populations of F_4 developed by crossing of Fengaizhan1 with IR5802513, C70, Doddabyranellu, Govind, Pusa, CDR22, C71, IR64a, Aizhidao, and Khazar to establish the relationship between flag leaf Δ at heading and harvest grain yield. To ensure various grain yields were covered, we selected 10 lines from lowest to highest for each population with two replications. These populations were crosses between Fengaizhan1 and IR5802513, C70, Doddabyranellu and Govind. All tested lines showed a high grain yield level ranging from 5.27 to 9.35 t ha^{-1} with a mean of 6.94 t ha^{-1}, while the variation in biomass ranged from 8.02 to 16.55 t ha^{-1} with a mean of 11.74 t ha^{-1} (Fig. 1). These values were then used to establish a regression of flag leaf Δ with grain yield and biomass at maturity.

Further analysis was carried out on the data set from the above populations. The relationships between grain yield and biomass production with $\delta^{13}C$ are shown in Fig. 2.

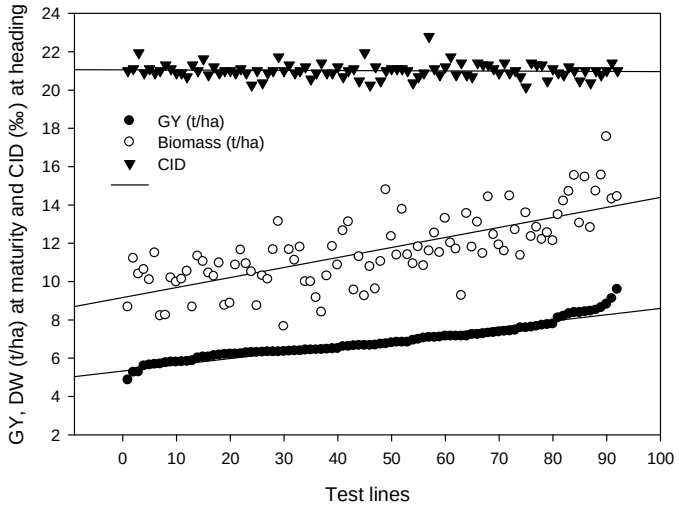

FIG. 1. The grain yield (GY), dry weight of above ground plant material at maturity (DW) and $\Delta^{3}C$ of selected lines (2005).

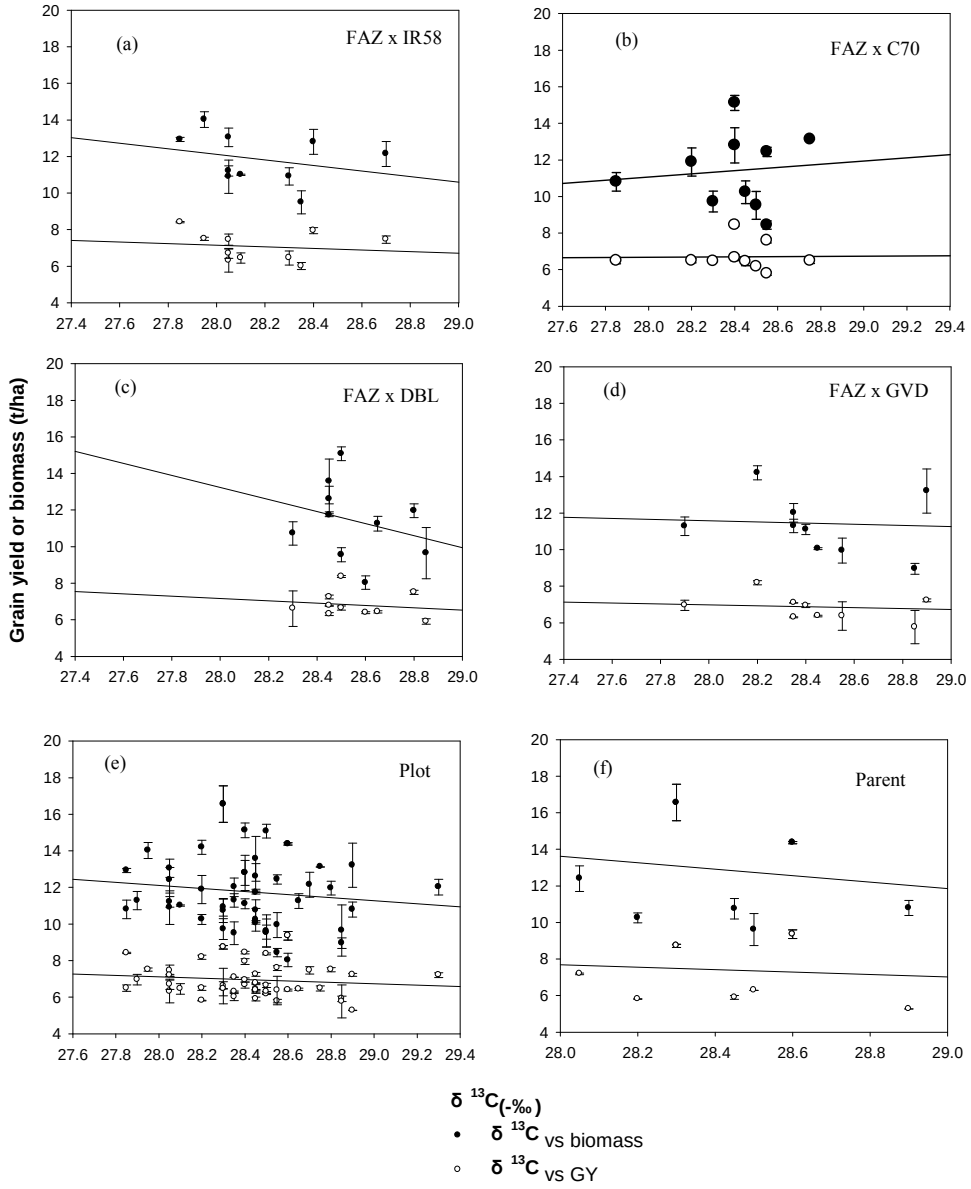

FIG. 2. Relationships between $\delta^{13}C$ of flag leaves at heading with biomass and grain yield (2005) for populations developed from crosses between (a) Fengaizhan1 and IR5802513 (b) Fengaizhan1 and C70 (c) Fengaizhan1 and Doddabyranellu (d) Fengaizhan1 and Govind (e) All tested lines from 4 populations (f) Different parents.

When all the tested lines were plotted, the scatter showed a very poor relationship of $\delta^{13}C$ of the flag leaf with grain yield and biomass (Fig. 2d). In order to eliminate the effect of different genetic backgrounds, relationships for each population were analysed, but poor regressions were found. However, these traits in relation to $\delta^{13}C$ distribution differed for different populations. The $\delta^{13}C$ of lines of the population developed from FAZ and IR5802513 was distributed dispersedly from –28.7 to –27.9‰ (Fig. 2a). The same trend occurred within the population developed from FAZ and Govind, which ranged from –28.9 to –27.9‰ (Fig. 2d). The other two populations had centralized distributions of $\delta^{13}C$, i.e. the population developed from FAZ and C70 mostly ranged from –28.7 to –28.0‰ (Fig. 2b), and the population developed from FAZ and Doddabyranellu ranged from –28.9 to –28.3‰ (Fig. 2c). These findings can be associated with the differences of the $\delta^{13}C$ of experimental parent cultivars (Fig. 2f). Neither LSW nor LAI in the above lines were correlated with $\Delta^{13}C$ of flag leaves under favourable conditions (Fig. 3).

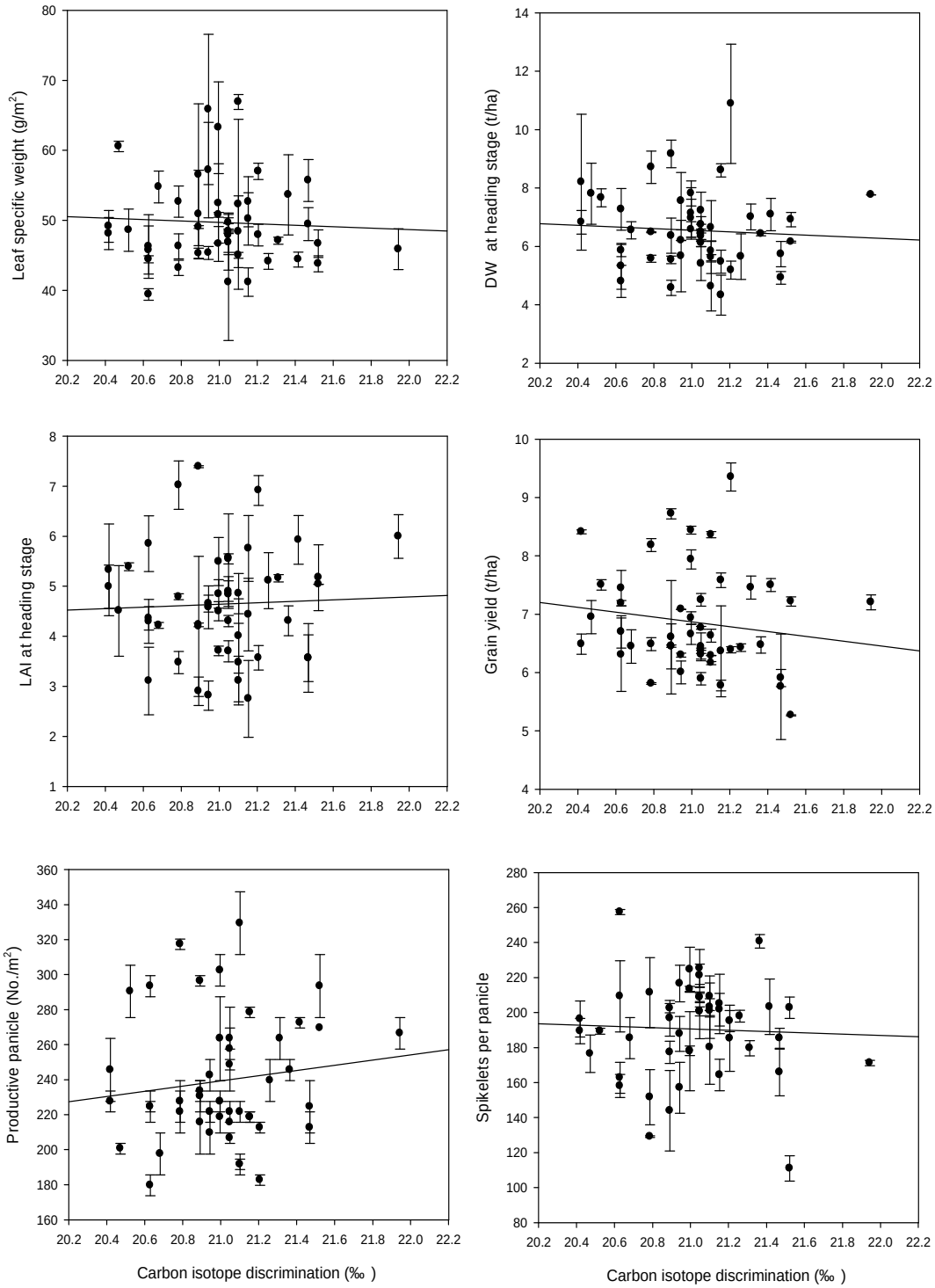

FIG. 3. Relationships between CID and leaf specific weight, dry weight of above ground plant material (DW) at heading, leaf area index at heading, harvest grain yield, productive panicle number and spikelets per panicle (2005).

Similarly, no significant relationships were found between flag leaf Δ at heading and dry weight at heading, spikelets number per panicle, thousand grain weight and harvest index. A small negative relationship of grain yield and dry weight at maturity was found with flag leaf Δ at heading (Fig. 4). A smaller positive relationship was found for productive tiller numbers m^{-2} and flag leaf Δ. A relatively prominent (negative) relationship of flag leaf Δ at heading was found with the grain filling percentage.

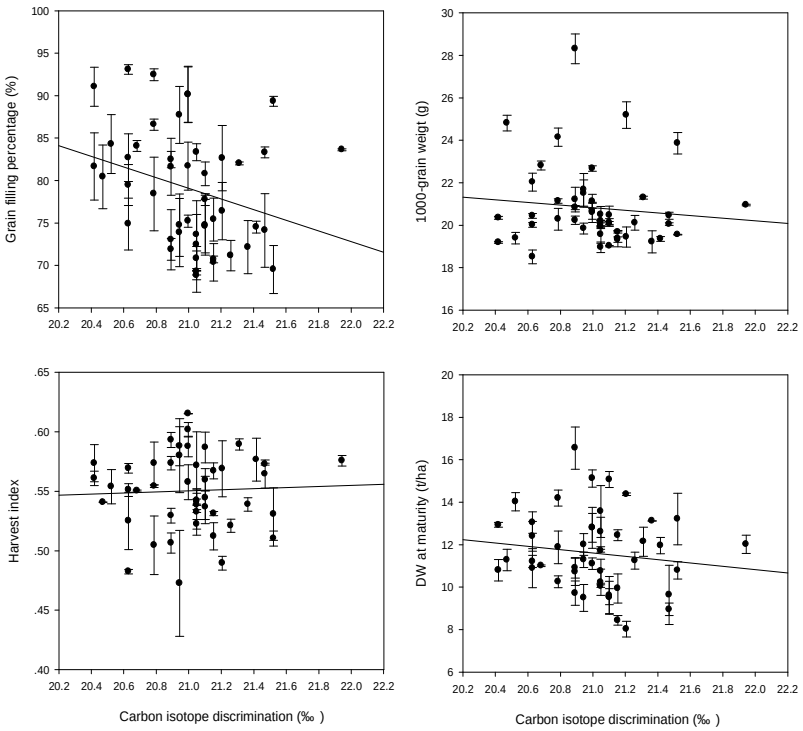

FIG. 4. Relationships between CID and grain filling percentage, 1000 grain weight, harvest index, and dry weight of above ground plant material at maturity (2005).

One explanation for these poor relationships of flag leaf Δ with various traits could be that the differences in $\Delta^{13}C$ between different rice lines under favourable irrigated conditions are too small. Another reason may be that $\Delta^{13}C$ in the flag leaf is not sensitive enough to reflect the differences among the tested lines. So it can be concluded that we cannot use $\Delta^{13}C$ values as an indicator for predicting or screening high grain yielding rice varieties or high yield potential varieties under favourable irrigated field systems. Thus results differed from the finding that biological yield and grain yield were both positively correlated with Δ using some wheat varieties under field conditions of non-limited water [14].

To further illustrate the relationships between important traits like flag leaf Δ at heading, grain yield, biomass production and harvest index, the lines were divided into six groups. The ratios of relative percent values of these traits were compared with the maximum percent values shown by any group of the crossing lines (Fig. 5).

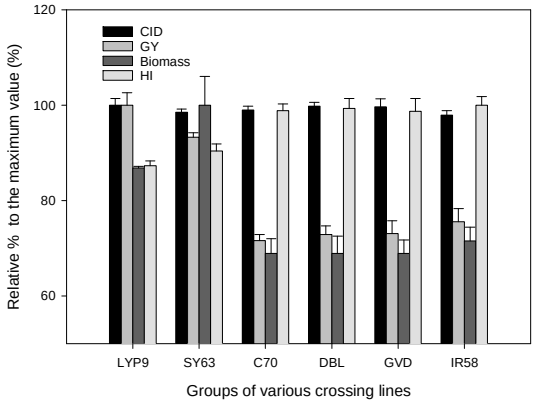

FIG. 5. Comparison of the relative percentage (%) of CID of flag leaves at heading, harvest grain yield, dry weight of above ground plant material at maturity and harvest index, based on the maximum value of various groups of crossing lines (2005).

Δ values for all the groups of the crossing lines were almost the same (Fig. 5). The ratios of the relative percent values for grain yield and biomass production to maximum percent values of LYP9 and SY63 crossing groups were markedly higher than other groups. The ratios of the relative percent harvest index of LYP9 and SY63 crossing groups were a little lower than the other groups, but the ratios were almost the same for the other groups.

3.2. QTLs for leaf CID and other related traits in irrigated rice

The objectives of this study were to identify chromosomal regions involved in leaf CID variation and other yield related traits of rice varieties Zhenshan97 and Minghui63 along with their RIL (recombinant inbred line) population derived from a cross between these parents under field conditions.

3.2.1. Phenotypic analysis of the RILs and parents in 2006 and 2007

The average SPAD readings at panicle initiation (PI) and heading (HD) of Minghui63 (MH63) were 35.1 and 37.6 and those of Zhengshan97 (ZS97) were 36.9 and 42.0, respectively. The difference between MH63 and ZS97 was significant (P<0.001) at both stages. The average SPAD-PI and SPAD-HD of RILs were 35.5 and 37.1, respectively, skewed towards that of MH63 (Table 1).

TABLE 1. PHENOTYPIC PERFORMANCE † OF RI LINES AND OTHER VARIETIES IN 2006

Vars./	LSW ($g\ m^{-2}$)		DW ($t\ ha^{-1}$)		PN	GNP	KGW	GY	HI	GFP	DTH	$\Delta^{13}C$	SPAD	
RILs	at PI	at HD	at HD	at MA	(m^{-2})		(g)	($t\ ha^{-1}$)		(%)	(d)	(‰)	at PI	at HD
PA64	45.17	48.71	8.88	-	457	-	-	6.56	-	-	90.0	21.01	38.9	41.9
YD6	37.57	44.17	13.57	21.26	296	138	26.68	9.97	0.45	87.0	87.0	21.42	38.1	39.9
SY63	36.62	39.06	10.42	-	348	125	25.39	7.07	0.45	83.2	64.3	21.27	34.7	36.4
LYP9	37.84	47.18	12.43	18.64	323	156	23.11	9.58	0.49	79.1	81.4	21.32	37.7	38.8
MH63	33.43	42.73	12.54	19.32	390	94	26.18	6.80	0.40	81.1	90.7	21.14	35.1	37.6
ZS97	36.02	44.85	6.39	5.52	398	-	21.63	6.62	-	-	87.7	21.64	36.9	42.0
RILs														
Min.	31.07	35.38	6.38	6.00	253	68	18.18	3.90	0.25	52.5	57.0	20.21	32.2	31.9
Max.	44.71	54.55	12.46	19.44	479	138	29.88	8.37	0.66	91.1	97.7	21.96	40.3	31.9
Ave.	37.09	41.69	9.37	13.44	355	99	24.02	6.38	0.42	80.4	82.0	21.22	35.6	37.1
Skew	0.64	0.86	0.26	–0.48	0.5	0.2	0.13	–0.28	0.87	–1.3	–0.45	–0.35	0.28	0.72
Kurt	0.32	0.60	–0.83	–1.27	0.5	–0.2	–0.26	–0.05	2.86	1.8	0.54	0.22	–0.23	1.09

† LSW, leaf specific weight; DW, dry weight of above ground biomass; PN, panicle number; GNP, spikelet number per panicle; KGW, 1000 grain weight; GY, grain yield; HI, harvest index; GFP, grain filled percent; DTH, days from sowing to heading; PI, panicle initiation; HD, heading; MA, maturity.

The average of SLW at PI, SLW at HD, PN, CID of flag leaf, SPAD at PI, and SPAD at HD of Minghui63 (the male-parent of the population) had lower values than those of the female-parent of Zhenshan97 (ZS97), while the means of the population were greater than those of MH63, but lower than those of ZS97, except for SLW at PI, which was greater than both of their parents. The average of LAI at PI and HD, DW at HD and MA, KGW, GY and DTH of ZS97 had the higher value than MH63, while the averages of the population were lower than MH63, except for DTH, which was lower than the two parents.

3.2.2. Mapping of QTLs for CID and other related traits

QTLs for plant height at different growth stages were detected on chromosomes 1, 3 and 7 in 2007. The QTLs for plant height at panicle initiation had one QTL on chromosome 3 (C63-C316) contributing 13.9% of the variation. Plant height at heading had two QTLs on chromosomes 1 and 7. Plant height at maturity had two QTLs on chromosomes 1 and 7; both the QTLs have large additive effects and individually explained 23.4% and 22.5% of the total variation (see Fig. 6 and Table 2).

In 2006, QTLs for SPAD were detected on chromosomes 1 and 8 at panicle initiation and heading. One QTL was detected in 2006 on chromosome 1, which explained 17.1% of the total variation. Two QTLs were detected on chromosomes 1 and 8; explaining 30.5% of the total variation (see Fig. 6 and Table 2).

QTLs for CID were detected on chromosomes 1, 2, 3, 7, 8, 10 and 12 in two years. Five QTLs were detected in 2006, explaining 40.7% of the phenotypic variation. Alleles for increasing phenotypic score were mostly from Zhenshan 97. Three QTLs were detected in 2007, which individually explained 9.2 to 11.6% and together explained 31.4% of the total phenotypic variation. No common genomic region was found in two years (see Fig. 6 and Tables 2 and 3).

A QTL for days to heading was detected on chromosome 7 in two years. The QTL detected in 2007 had a strong effect explaining 32.9% of the total variation, and overlapped in a similar region with that of a QTL for plant height at maturity and a QTL for effective panicle number (see Fig. 6 and Tables 2 and 3). Two QTLs for grain yield were mapped on chromosomes 1, 6 and 7 in two years (see Fig. 6 and Tables 2 and 3). Across years and growth stages, QTLs for dry weight were detected on chromosomes 6 and 7. No QTL was detected for dry weight in 2006 at maturity. Except for the QTL on chromosome 6, the alleles from Minghui63 increased the dry weight. The QTL for dry weight at heading in 2007 had the largest effects contributing 41.3% of the variation. Two QTLs for dry weight at different growth stages in two years were in the same region.

Three QTLs for panicle number were indentified in 2006, which explained 59.9% of the total variation. Among these three QTLs, the alleles from Zhenshan 97 increased the value. Three QTLs for panicle number were mapped in 2007, which explained 42.3% of the total variation, to which two parents contributed alleles. Two QTLs detected in two years were found in the same region (R1440-C1023) on chromosome 7. A total of 8 QTLs were confirmed to be associated with grain number per panicle in two years. Seven QTLs were detected in 2007, which together explained 77.9% of the total phenotypic variation.

Three QTLs for grain weight were located on chromosomes 1, 3 and 5, accounting for 64.9% of the phenotypic variation. The QTL located on the interval RZ403-C1087 had large effects and individually explained 31.2% of the total variation. Seven QTLs were mapped on chromosomes 1, 3, 5, 6 and 7, contributing 65.8% of the total phenotypic variation. Two chromosome intervals, RZ403-C1087 on chromosome 3 and RG360-R3166 on chromosome 5, were shared by QTLs detected in two years. For harvest index, two QTLs on chromosomes 5 and 7 and one on chromosome 4 were indentified in 2006 and 2007, respectively. The variation of these QTLs ranged from 10.0 to 19.9%.

A total of four QTLs were associated with filled grain number per panicle in two years. Two QTLs were identified in 2006, explaining 34.21% of the phenotypic variation, and two parents contributed alleles that increased filled grain number per panicle. The QTL located at C86-C2340 on chromosome 1 had the largest effect and accounted for about 29.0% of the total phenotypic variation. There were no QTLs for filled grain number per panicle detected in the two years.

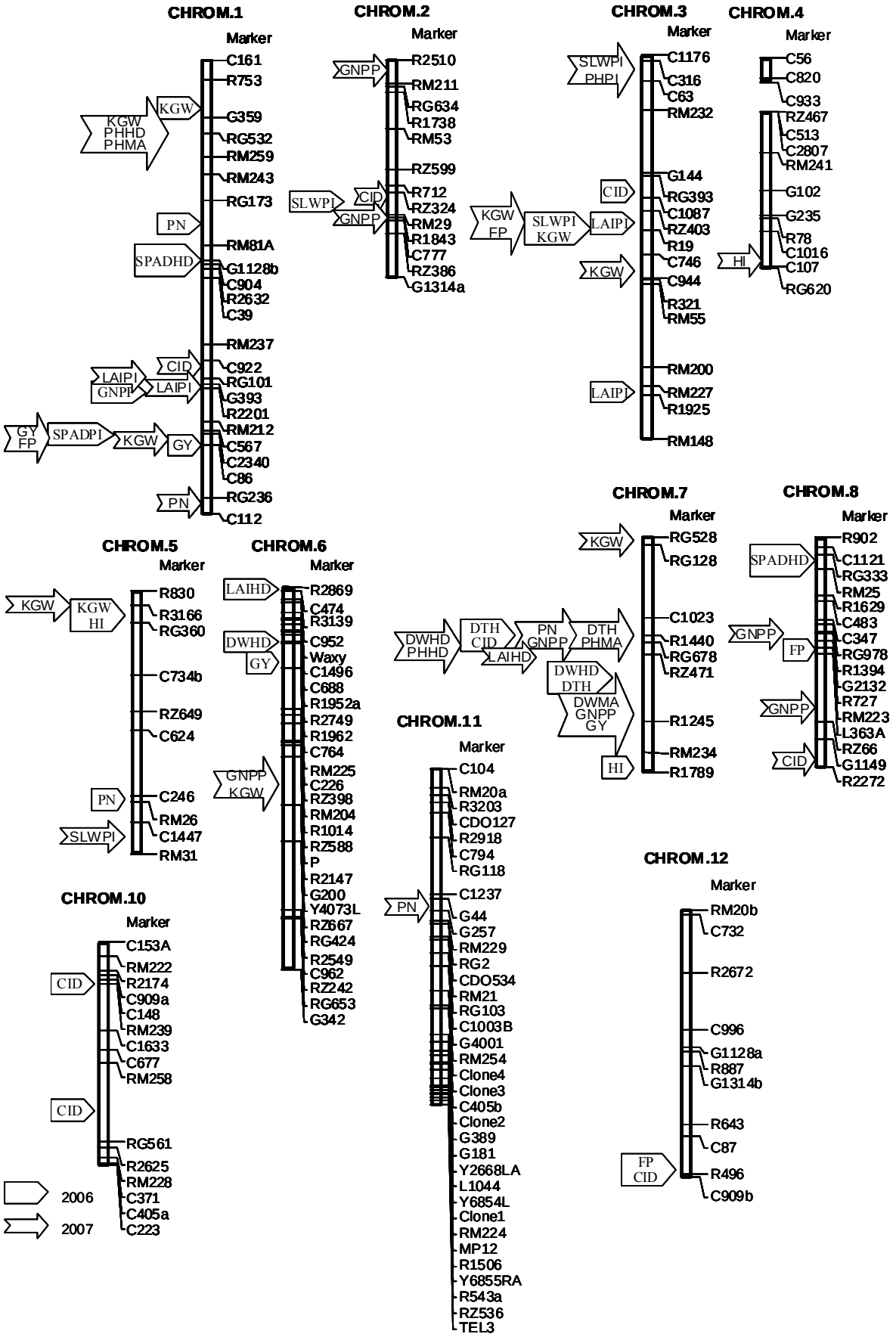

FIG. 6. Genetic linkage map of RILs derived from Zhenshan97 × Minghui63 and distribution of putative QTLs.

TABLE 2. ADDITIVE EFFECT OF QTLS FOR INVESTIGATED TRAITS OF RI LINES IN 2006

Trait†	QTL	Chr.	Interval	Site‡ (cM)	LOD	Additive effect #	Variation (%)
LSW-PI	Qslwpi2-1	2	RM29-RZ324	0	7.95	–1.201	20.8
	Qslwpi3-1	3	RZ403-C1087	6	3.74	–0.813	9.5
LAI-PI	Qlaipi3-1	3	R1925-RM227	4	2.81	0.126	6.8
	Qlaipi3-2	3	R19-RZ403	0	10.18	0.252	27.1
LAI-HD	Qlaihd6-1	6	C474-R2869	0	6.83	–0.400	19.7
DW-HD	Qdwhd6-1	6	RZ398-C764	0	3.37	–0.417	9.0
	Qdwhd7-1	7	R1245-RZ471	32	5.71	0.559	16.2
PN	Qpn1-1	1	RM81A-RG173	22	4.00	–9.351	10.7
	Qpn5-1	5	RM26-C246	0	4.35	–9.192	10.3
GNP	Qgnpp1-1	1	R2201-G393	2	6.58	–6.320	29.5
KGW	Qkgw1-1	1	G359-R753	0	16.31	–0.958	25.4
	Qkgw3-1	3	RZ403-C1087	0	19.19	1.062	31.2
	Qkgw5-1	5	RG360-R3166	0	7.01	–0.549	8.3
GY	Qgy1-1	1	C2340-C567	0	7.04	0.322	19.8
	Qgy6-1	6	RZ588-R1014	2	3.16	0.204	7.9
HI	Qhi5-1	5	RG360-R3166	6	5.31	–0.024	19.9
	Qhi7-1	7	R1789-RM234	2	4.59	–0.024	18.8
GFP	Qfp8-1	8	L363A-RM223	0	3.54	–2.235	14.8
	Qfp12-1	12	C909b-R496	2	4.83	2.558	19.4
DTH	Qdth7-1	7	R1245-RZ471	32	4.75	2.682	12.2
$\Delta^{13}C$	Qcid3-1	3	C1087-RG393	10	4.91	0.103	10.4
	Qcid7-1	7	R1440-C1023	0	8.36	–0.133	17.5
	Qcid10-1	10	RG561-RM258	16	2.60	–0.082	6.7
	Qcid10-2	10	RM239-C148	2	3.06	–0.078	6.1
	Qcid12-1	12	C909b-R496	2	4.74	0.102	10.2
SPAD-PI	Qspadpi1-1	1	C567-RM212	2	5.10	–0.550	17.1
SPAD-HD	Qspadhd1-1	1	C904-G1128b	0	5.76	0.711	16.8
	Qspadhd8-1	8	RM25-RG333	4	4.09	0.640	13.8

†LSW, leaf specific weight; LAI, leaf area index; DW, dry weight of above ground biomass; PN, panicle number; GNP, spikelet number per panicle; KGW, 1000 grain weight; GY, grain yield; HI, harvest index; GFP, grain filled percent; DTH, days from sowing to heading; PI, panicle initiation; HD, heading.

‡Genetic distance between the most likely position of putative QTL and the left flanking marker in marker interval.

#Positive value indicates the Minghui63 genotype having positive effect on the trait; negative value represents Zhenshan97B positive effect, respectively.

TABLE 3. ADDITIVE EFFECT OF QTLS FOR INVESTIGATED TRAITS OF RI LINES IN 2007

Trait†	QTL	Chr.	Interval	Site‡ (cM)	LOD	Additive effect #	Variation (%)
SLW-PI	Qslwpi3-1	3	C63-C316	8	4.41	0.94	13.7
	Qslwpi5-1	5	RM31-C1447	2	2.86	–0.75	8.7
LAI-PI	Qlaipi1-1	1	G393-RG101	2	7.64	0.17	24.6
LAI-HD	Qlaihd7-1	7	RZ471-RG678	4	4.14	0.19	8.9
DW-HD	Qdwhd7-1	7	RG678-R1440	0	14.79	0.55	41.3
DW-MA	Qdwma7-1	7	R1245-RZ471	32	6.23	0.67	20.1
PN	Qpn1-1	1	C112-RG236	2	7.21	11.05	18.1
	Qpn7-1	7	R1440-C1023	0	4.90	–8.14	9.8
	Qpn11-1	11	G257-G44	0	5.81	–9.83	14.3
GNP	Qgnpp2-1	2	R1843-RM29	0	6.75	–5.04	7.8
	Qgnpp2-2	2	RM211-R2510	12	10.82	–6.97	15.0
	Qgnpp6-1	6	R2549-RG424	6	5.84	–5.26	8.5
	Qgnpp7-1	7	R1245-RZ471	0	6.92	–5.41	9.0
	Qgnpp7-2	7	R1440-C1023	0	16.70	9.11	25.5
	Qgnpp8-1	8	RZ66-L363A	0	6.29	–5.21	8.4
	Qgnpp8-2	8	R727-G2132	0	3.25	3.39	3.5
KGW	Qkgw1-1	1	C567-RM212	2	2.86	0.51	4.1
	Qkgw1-2	1	RG532-G359	2	12.31	–0.97	15.3
	Qkgw3-1	3	C944-C746	8	6.20	0.79	10.2
	Qkgw3-2	3	RZ403-C1087	2	5.71	0.78	9.9
	Qkgw5-1	5	RG360-R3166	0	12.70	–1.05	17.7
	Qkgw6-1	6	R2549-RG424	10	3.73	0.56	5.1
	Qkgw7-1	7	RG128-RG528	2	3.29	0.48	3.7
GY	Qgy1-1	1	C86-C2340	4	6.35	0.27	20.6
	Qgy7-1	7	R1245-RZ471	0	4.16	–0.20	11.7
HI	Qhi4-1	4	RG620-C107	0	3.18	0.01	10.0
GFP	Qfp1-1	1	C86-C2340	0	10.25	0.04	29.0
	Qfp3-1	3	RZ403-C1087	0	2.65	–0.02	6.4
DTH	Qdth7-1	7	R1440-C1023	0	11.58	3.68	32.9
$\Delta^{13}C$	Qcid1-1	1	RG101-C922	8	4.75	–0.11	11.6
	Qcid2-1	2	RM29-RZ324	10	3.84	0.09	9.2
	Qcid8-1	8	R2272-G1149	6	3.34	0.10	10.6
PH-PI	Qphhi3-1	3	C63-C316	10	4.60	1.95	13.9
PH-HD	Qphhd1-1	1	RG532-G359	0	5.31	–3.31	12.7
	Qphhd7-1	7	RG678-R1440	2	9.28	4.73	25.9
PH-MA	Qphma1-1	1	RG532-G359	0	8.48	–4.77	23.4
	Qphma7-1	7	R1440-C1023	0	9.43	4.68	22.5

†LSW, leaf specific weight; LAI, leaf area index; DW, dry weight of above ground biomass; PN, panicle number; GNP, spikelet number per panicle; KGW, 1000 grain weight; GY, grain yield; HI, harvest index; GFP, grain filled percent; DTH, days from sowing to heading; PH, plant height; PI, panicle initiation; HD, heading; MA, maturity

‡Genetic distance between the most likely position of putative QTL and the left flanking marker in marker interval

#Positive value indicates the Minghui63 genotype having positive effect on the trait, negative value represents Zhenshan97B positive effect, respectively

Four QTLs for specific leaf weight at panicle initiation in the two years were detected, two on chromosome 2 and 3 in 2006, explaining 30.3% of the phenotypic variation. Two QTLs were detected in 2007, which together explained 22.4% of the total phenotypic variation. No QTLs were detected at heading in the two years (see Fig. 6 and Tables 2 and 3). Across years and growth stages, QTLs for leaf area index were detected on chromosomes 1, 3, 6 and 7. Except the QTL on chromosome 6, the alleles from Minghui63 increased the leaf area index (see Fig. 6 and Tables 2 and 3).

3.2.3. Detection of epistatic interaction loci

Epistatic interaction analysis of $\delta^{13}C$ of flag leaves at heading showed six pairs of epistatic QTLs which involved 9 loci. These epistatic QTLs were on chromosomes 1, 2, 5, 6, 7 and 12, with the LOD value ranging from 3.0 to 5.6. Variation explained by single interaction pairs ranged from 3.4 to 8.0%. Three pairs of E-QTLs were detected in 2005, with the LOD ranging from 5.9 to 7.4 and they explained 3.6 to7.9% of the phenotypic variation (Table 4).

TABLE 4. EPISTATIC EFFECT OF QTLS FOR $\Delta^{13}C$ OF RI LINES

Chr.	Marker int.	Site † (cM)	Chr.	Marker interval	Site † (cM)	LOD	AA ‡	H2 (AA)# (%)
1	C86-C2340	4	7	RG128-RG528	2	3.53	–0.072**	4.71
1	RM237-C39	2	6	G342-RG653	0	3.40	0.074**	4.97
2	R1843-RM29	0	5	RM31-C1447	16	4.48	–0.088**	7.07
2	R1843-RM29	0	7	RM234-R1245	0	5.59	0.093**	7.88
6	C764-R1962	0	12	R643-G1314b	0	3.01	–0.063*	3.63

†Genetic distance between the most likely position of putative QTL and the left flanking marker in marker interval
‡Direction of epistatic effect: positive value shows parent type > recombinant type, negative value shows parent type < recombinant type; *, ** represent the significance level at $P< 0.001$, $P<0.0005$, respectively
#H2 (AA) indicates the variation explained by additive QTL

Grain yield was negatively correlated with CID at heading but not with CID at 30 days after transplanting. Our results agree with those reported for other cereals [40]. The results suggest that CID at heading can be used as a substitute index for grain yield. The location of QTLs for CID was affected by growth stages. Most of the QTLs for CID at heading were found on chromosomes 7 and 12, whereas the QTLs for CID at 30 days after transplanting were detected on chromosomes 1, 2, 3 and 8. These findings suggest that the expression of QTLs for CID differs during crop development. The effect of growing conditions on QTLs for CID needs to be clarified [24].

Some QTLs for CID and other traits were located in the same region or a similar position. The occurrence of QTLs associated with different traits in the same locus may be explained by the fact that the QTLs are closely linked genetically, or a single locus controls multiple traits and a gene may have pleiotropic effects. Among the QTLs for CID, many were co-located with the QTLs for harvest index, panicles m^{-2}, grain filling, 1000 grain weight and biomass. These results indicated the possibility of a genetic relationship of CID with grain yield and its components. However, further study is obviously needed to examine this possibility. In this study, the mechanism underlying the genetic relationship between CID and grain yield could not be identified.

3.3. Relationships between CID and other traits of the RILs

Among the detected QTLs controlling CID, one allele was associated with QTLs controlling SPAD readings at heading, and a second one was associated with QTLs controlling productive panicle number, and a third one was associated with QTLs controlling dry weight at maturity and spikelet number per panicle (Fig. 6). These results showed that there was a relationship between CID and other agronomic traits. The regressions between CID and other traits such as SPAD-HD, productive panicle number, dry weight at maturity, spikelet number per panicle, harvest grain yield, and 1000 grain weight are shown in Fig. 7. There were no significant relationships between CID and harvest grain yield or 1000 grain weight. However, there were significant positive relationships between CID and SPAD readings at heading, and productive panicle number m^{-2}, while there were negative relationships between CID and spikelet number per panicle, and dry weight at maturity. The significant regression equations were based on the pair data between CID and the above parameters, respectively. The equations were:

$Y = -22.0435 + 2.6855 \times$ (CID vs SPAD-HD, $r^2 = 0.1288^{**}$, n = 125)
$Y = -511.9190 + 40.8433 \times$ (CID vs productive panicle no., $r^2 = 0.1134^{**}$, n = 125)
$Y = 76.7046 - 2.9823 \times$ (CID vs dry weight at maturity, $r^2 = 0.0530^{*}$, n = 122)
$Y = 436.6686 - 15.9232 \times$ (CID vs spikelet no. per panicle, $r^2 = 0.1072^{**}$, n = 77)

These significant linear relationships supported the results obtained from the identification of QTLs (Fig. 7).

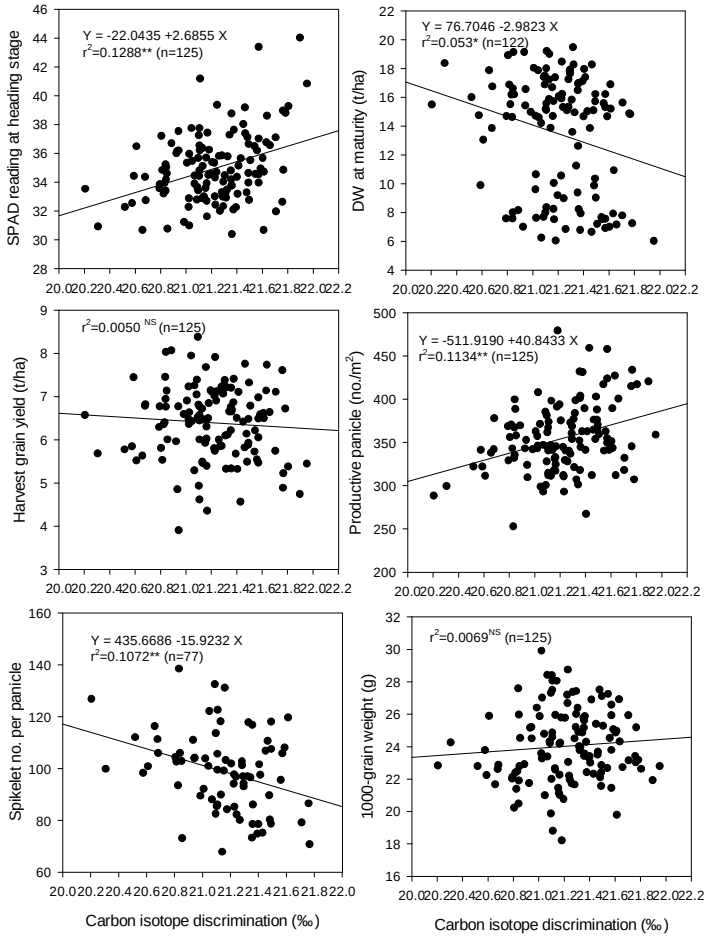

FIG. 7. Relationships between CID and SPAD-PI, SPAD-HD, panicle number, maximum tiller number, plant height at heading and average panicle length, based on the RILs during 2006.

3.4. Effect of fertilizer N on CID

The effect of N-fertilizer application on the CID characteristics of different hybrids is shown in Fig. 8. The experiment was conducted during 2006 and 2007. The data show the same trend for both years i.e. that the Δ of all the hybrids decreased with N fertilizer application compared to the control (no N fertilizer).

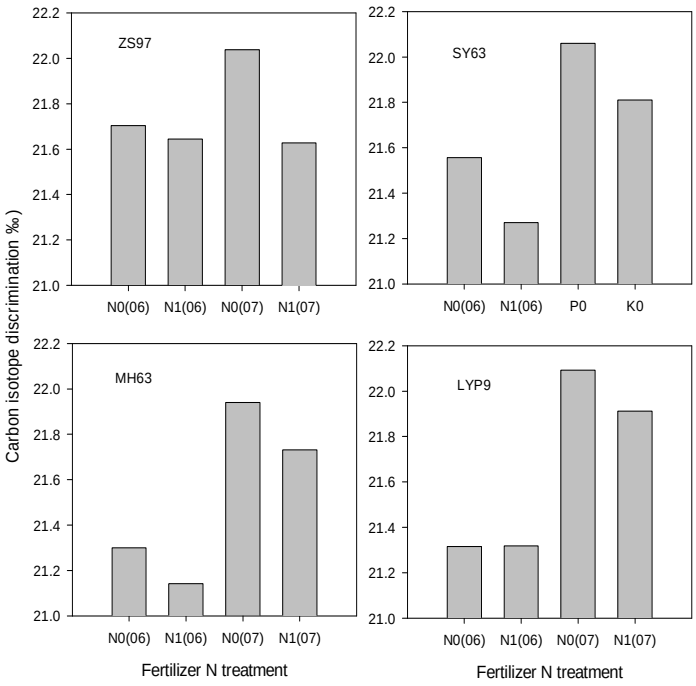

FIG. 8. The effect of fertilizer N application on CID using several varieties in 2006 and 2007.(Note that ZS97 is the female parent of the three-line hybrid variety of SY63; MH63 is the male parent of the three-line hybrid variety of SY63; LYP9 is two-line hybrid variety. N_0 and N_1 represent 0 and 135 kg N ha^{-1}, respectively).

The effect of fertilizer N on Δ was further confirmed in another field experiment (Experiment 3). Using a given two-line hybrid of LYP9 under various fertilizer N application rates, a significant linear relationships between N rate and Δ was found, with Δ decreasing as the fertilizer N rate increased (Fig. 9).

3.5. Relationship between CID and grain yield of hybrid rice

The relationships between CID and harvest grain yield under various N fertilizer treatments were evaluated using the two line hybrid rice LYP9. A strong negative relationship was observed between CID and grain yield for the various N fertilizer levels (Fig. 10). This result supports the findings of our other experiments in which we found that CID decreased with N fertilizer application, with a concomitant increase in the grain yield. Thus N fertilizer application simultaneously affected the two parameters oppositely i.e. grain yield increased and CID decreased. These results were also confirmed by the field data obtained for evaluating the relationship of CID with grain yield for other high yielding adapted hybrids in relation to N fertilizer application during 2007.

Relationships between CID and grain yield, spikelet number m^{-2}, productive panicle number m^{-2}, spikelet number per panicle, seed setting percentage and 1000 grain weight are shown in Fig. 11.

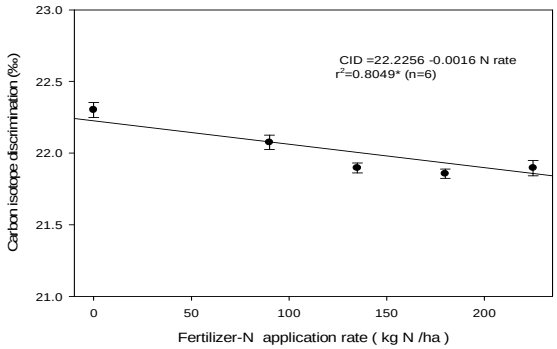

FIG. 9. Effect of fertilizer N application rate on CID (2007).

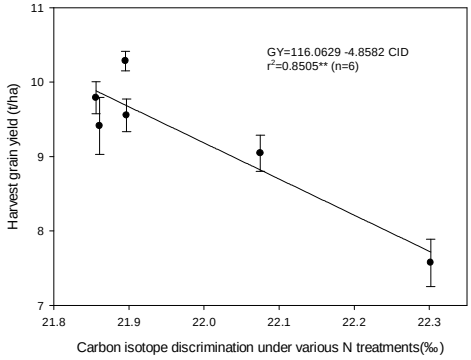

FIG. 10. The relationship between CID and harvest grain yield under various fertilizer N treatments using the two-line hybrid rice LYP9 (2007).

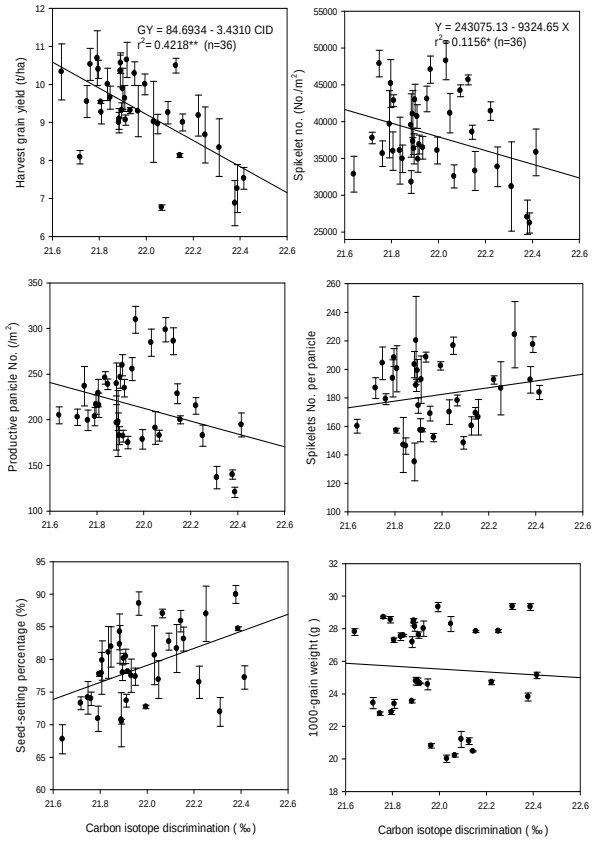

FIG. 11. Relationships between CID and harvest grain yield, spikelet number m^{-2}, productive panicle number, spikelet number per panicle, seed-setting percentage, 1000 grain weight of six varieties under 0 – 270 kg N ha^{-1} treatments.

Grain yield showed a significant negative relationship with CID of different hybrids under varying N fertilizer levels. Similarly, spikelet number m^{-2} and productive panicle number m^{-2} of different varieties also showed negative relationships with CID under different N fertilizer levels. In contrast, spikelet number per panicle and seed setting percentage showed positive relationships with CID under different N fertilizer levels, while 1000 grain weight of different hybrids showed a poor negative (almost no) relationship with CID. So it can be argued that the strong negative relationship of grain yield with CID under different N fertilizer levels is mainly affected by spikelet number m^{-2} and productive panicle number m^{-2} rather than other yield components.

4. CONCLUSIONS

Based on the findings of our experiments we can conclude that there were no significant relationships between flag leaf Δ at heading with grain yield and other agronomic traits. So CID of flag leaves cannot be used as a useful indicator for screening high yielding inbred rice germplasm under favourable irrigated field conditions.

The variation in CID was transgressively observed among RILs. The most plausible cause is accumulation of complementary alleles in the progeny. Two of the three QTLs detected for CID were increased by the Minghui 63 allele, whereas the other one was increased by the Zhenshan 97 allele. The region (RG236-C86) on chromosome 1 controlled CID and grain yield. Some QTLs for CID and other traits are located in the same region or a similar position.

We also detected several QTLs on different chromosomes for different traits controlling different levels of variation. Similarly when the RILs population was used for C isotope studies we found significant relationships between CID and some agronomic traits like productive panicles number, SPAD reading at heading, spikelet number per panicle and dry weight at maturity.

Besides the CID variation of rice genotype, the effect of fertilizer N on CID was illustrated in three field experiments. There was a consistent trend of Δ decreasing with fertilizer N increasing, illustrating the importance of applying the correct amount of fertilizer N when using CID as a tool to screen high yielding rice germplasm.

Alhough CID was poorly related to grain yield of inbred germplasm, we found a significant linear relationship between Δ and harvest grain yield for hybrid varieties. The CID values of high yielding hybrid rice are lower than those of lower yielding varieties. These findings will be useful for future research activities aimed at using CID for screening high yield hybrid rice under favourable irrigated field conditions.

ACKNOWLEDGEMENTS

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CARBON ISOTOPE DISCRIMINATION AND SALINITY TOLERANCE IN RICE

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Abstract

The relationship between carbon isotope discrimination (CID, $\Delta^{13}C$, Δ) and salinity tolerance in rice was investigated in six experiments during 2004–09. In Experiment 1, quantitative trait loci (QTLs) for Δ were analyzed in an IR29 / Pokkali mapping population of 79 recombinant inbred lines (RILs) grown under salt stress imposed at the seedling stage. Three QTLs for flag leaf-Δ were detected on chromosomes 1, 3, and 11 with R^2 values ranging from 18 to 33%, and all three co-located with mapped QTLs for salinity tolerance and Na^+: K^+ ratio. In Experiment 2, a set of 80 breeding lines and varieties were evaluated at the seedling stage for Δ and salinity tolerance using visual standard evaluation system (SES) scores, growth, salt uptake, and $\delta^{13}C$ composition of leaves. While the first experiment using a mixture of sensitive and tolerant RILs showed strong correlations between Δ and salt-stress traits, the data from the breeding lines showed relatively weaker correlation, possibly due to the low genetic variability within this set and the high tolerance level of most of the lines. The correlation between Δ and salinity tolerance at the reproductive stage was tested in Experiment 3 using flag leaf and grain samples across a set of 80 tolerant and sensitive lines, including 46 breeding lines and 34 varieties and landraces. The flag leaf-Δ values correlated well with grain-Δ, and both correlated positively with grain yield of single plants, suggesting that flag leaf-Δ can potentially be used to select for salinity tolerance during the reproductive stage, which agreed with results obtained at the early vegetative stage. While the varieties and landraces showed larger variation across the different traits, the breeding lines were much more uniform. This data was found useful to select contrasting parental lines used for the development of new populations for subsequent studies. In Experiment 4, the same set of 80 tolerant and sensitive lines were evaluated at the seedling stage for comparison with results from the reproductive stage. A strong negative correlation between Δ and seedling shoot biomass was observed but not with SES scores, possibly due to this set being less variable than the one used in the first experiment. In Experiment 5, a set of 38 lines which included both near isogenic lines (NILs) and a few RILs with equal tolerant and sensitive lines was evaluated. This experiment aimed to test whether the Pokkali introgressions that affected salinity tolerance from the *Saltol* QTL also influenced the extent of CID. No significant relationships of Δ with SES scores or with Na^+ uptake and Na^+: K^+ ratios were observed under both saline and non-saline conditions, but Δ correlated negatively with shoot length ($R^2 = -0.34$). Finally in Experiment 6, a set of 80 F_2 families from a second mapping population (FL478 / Azucena) was tested at the seedling stage to compare with the original IR29 / Pokkali RIL population results. Here Δ had a weak correlation with seedling vigor and less so with SES scores, and no QTLs above LOD 3.0 were detected for the Δ values using these 80 F_2 families, which is probably because these families were still segregating. This also suggests that future studies should rely more on stable lines to allow reliable phenotyping and better correspondence between the genotype and phenotype data. In summary, Δ correlated positively with salinity tolerance in rice, both at the seedling and reproductive stages. However, variation in Δ was greater in more diverse genotypes and the correlations of Δ with salt tolerance became stronger with increasing diversity. Δ could therefore be successfully used for selection of parental lines for breeding.

1. INTRODUCTION

Salt stress is a major constraint for cereal production worldwide. In Asia, the Pacific and Australia, more than 400 million ha are affected by salinity and / or sodicity. Additionally, about 10 million ha of agricultural lands are lost annually due to salinization and waterlogging, and the problem is further exacerbated by global warming where sea levels

are rising and coastal storms are becoming more frequent, resulting in higher salt intrusion into coastal lowlands. Deposition of salt in irrigated areas is also increasing because of higher evaporative demand coupled with irrational use of irrigation water and poor drainage.

Rice is considered the most important cereal crop globally with about half of the world's population depending on it as a staple food. In Asia alone it supplies 50 – 80% of the daily intake of calories. Rice is a C_3 grass that evolved in semi-aquatic, low-radiation habitats, and is grown in a wide range of environments from the humid tropics to arid and semi-arid areas and even temperate zones. As such, it carries a peculiar range of adaptations compared with other crops. Rice is relatively salt-sensitive, yet it is the only cereal that has been recommended for reclamation of salt-affected soils because it can grow well under flooded conditions, and because the standing water in rice fields can help leach salts from the topsoil to levels low enough for subsequent crops. It is also the only crop that can be grown during the monsoon season in most coastal ecosystems because of the persistence of waterlogged conditions and often stagnant water. Despite its high sensitivity, considerable variation in responses to salinity stress has been observed in rice; however, the extent of tolerance varies with the stage of development. It is comparatively tolerant during germination, active tillering, and towards maturity, but sensitive during early seedling and reproductive stages. Salinity tolerance at the seedling and reproductive stages are only weakly correlated [1], and hence pyramiding of contributing traits at both stages is needed for developing resilient salt-tolerant cultivars.

The physiological basis of salt tolerance in rice during the early seedling stage is reasonably well understood, and few key traits associated with tolerance have been identified. Most of these traits are apparently independent and are neither all present nor expressed to a higher level in a single phenotype. It is hypothesized that pyramiding of these traits would result in a higher level of tolerance than is presently observed among tolerant landraces. A logical approach is to identify physiological / genetic determinants underlying the different mechanisms, and then tag them through physiological or DNA markers to help combine them through breeding [2]. Selection based on physiological mechanisms is often difficult to implement in large breeding programs, and is mostly limited to selection of parental lines; however, selection based on their surrogates that are easier to measure and can provide an integrated assessment of performance over a period of time will be more attractive and effective in breeding.

Tolerant genotypes are able to regulate stomatal conductance upon exposure to salt stress, whereby the stomata close quickly for a few days of acclimation, and then partially reopen afterwards, whereas the stomata of sensitive cultivars close gradually in a manner consistent with reduction in leaf water potential and salt damage [3, 4]. This mechanism is essential for seedling survival as the uptake of salt is proportional to the amount of water that moves through the plant. However, since this mechanism involves both short-term as well as long-term responses, it seems logical to determine whether selection for greater or lower stomatal conductance (g_s) will be more adaptive under salt stress. It is also possible that genotypes that maintain greater g_s after acclimation may be more tolerant to salinity due to their active growth and salt dilution, which will consequently result in higher Δ values. Maintenance of g_s could be associated with effective exclusion, recirculation or compartmentalization of salts coupled with osmotic adjustment. The ability to maintain the integrity of the photosynthetic system under salt stress is also crucial for survival and growth and could ensure dissipation of excess energy load from direct sunlight. Mechanisms associated with effective compartmentalization of salt into the apoplast as well as detoxification of reactive oxygen species (ROS) generated during stress will help maintain the

integrity of cellular membranes and preserve the photosynthetic apparatus. Rice shows substantial variation in its ability to detoxify ROS under salt stress [4].

A physiological measure that integrates these responses under salt stress could be useful as a selection criterion for breeding for salinity tolerance. Besides, current phenotyping approaches entail the use of solution culture in controlled environments to avoid the inconsistency encountered because of soil and stress heterogeneity when screening under field conditions. However, this approach may not be practical for large-scale breeding programs in different countries because of the lack of such facilities and expense. Hence, Δ was proposed as an alternative criterion to measure long-term integrated responses of g_s and photosynthetic carbon fixation. Measurements of Δ in leaf tissue could therefore provide an indirect measure of these parameters and could clarify whether selection for higher or lower Δ would be more adaptive under salt stress. This will also provide insights into the usefulness of this technique in breeding, if adequate correlation with adaptation to such conditions can be established.

The objectives of the present investigation were (i) to determine the extent of genetic variability in Δ in rice, and the relationships with morpho-physiological traits that are known to be associated with salt tolerance (ii) to identify extreme phenotypes to be used for crossing to generate genetic stocks for further analyses (iii) to evaluate different strategies of using Δ as a selection tool for identifying higher yielding genotypes of rice in salt affected areas (iv) to develop a set of elite isomorphic lines of rice that contrast in yield potential, Δ and tolerance to salt stress (v) to evaluate the elite lines in contrasting environments to determine the most effective breeding strategies for application of CID in target environments, and (vi) to establish which of the underlying physiological mechanisms of salt tolerance are strongly associated with Δ.

2. MATERIALS AND METHODS

2.1. Experimental

Six experiments were conducted to test the relationships between Δ and stress traits at the seedling and reproductive stage in rice. The first two experiments studied salinity tolerance at the seedling stage; one used an F_8 mapping population of recombinant inbred lines (RILs) and the other used a set of breeding lines from the salinity tolerance breeding program at IRRI. The third experiment used a diverse set of 80 genotypes with contrasting tolerance to salt stress to evaluate the relationship between Δ and salinity tolerance during the reproductive stage. In Experiment 4, the same 80 genotypes used in Experiment 3, comprising tolerant and sensitive lines, were used to compare seedling performance with that observed during the reproductive stage. A set of 38 lines including NILs and a few RILs with equal numbers of tolerant and sensitive lines was chosen for Experiment 5, based on the location of the Pokkali introgression in the graphical genotypes and the SES scores, and Na^+ concentration data, to test whether the Pokkali introgressions that affected salinity tolerance from the *Saltol* quantitative trait locus (QTL) also influenced the CID measurements. Finally in Experiment 6, a set of 80 F_2 families from a second mapping population (FL478 / Azucena) was evaluated to compare with the results from IR29 / Pokkali obtained in the first experiment. Furthermore, crosses were made between breeding lines with contrasting Δ under salt stress at the reproductive stage. Four populations of 400 lines each were advanced to the F_3 generation and are available for follow-up studies.

2.1.1. Experiment 1

A set of 79 RILs together with their parental lines, Pokkali (tolerant) and IR29 (sensitive), were used in this study. This population was previously used for mapping of salinity tolerance where a major QTL was identified on chromosome 1. The lines were grown in saline nutrient solution [5] in a phytotron in September 2004. Initial scoring for visual salt injury using the IRRI Standard Evaluation System of rice [6] was made 10 days after salinization (DAS) at 12 dS m^{-1} and plants were harvested at 17 DAS for measurements of plant height, root length, green leaf area, shoot Na^+ and K^+ concentrations, and shoot and root dry weights. The following day (18 DAS) samples were harvested for analysis of chlorophyll concentration [7] and $\delta^{13}C$ composition, and the final SES scores were recorded.

2.1.2. Experiment 2

This experiment included 80 breeding lines that were known to be mostly tolerant of salt stress during the vegetative stage. The experimental conditions were similar to those in the first experiment. Seeds were pre-germinated and grown in a normal solution for 14 d prior to the imposition of salt stress. During September 2005, salt stress of 5 dS m^{-1} was first applied for one week and subsequently increased to 10 dS m^{-1} until the tissue was harvested. Final SES scores were taken at 17 DAS, and at the same time samples were collected for measurements of growth attributes, salt uptake and $\delta^{13}C$ composition.

2.1.3. Experiment 3

This experiment was conducted at the reproductive stage with 46 breeding lines and 34 varieties and landraces. The latter set was chosen to provide a balance between tolerant (14 lines), intermediate (8 lines), and salt-sensitive genotypes (12 lines). Plants were grown on soil in perforated pots in the greenhouse for both control and salt-stress treatments. Seeds were pre-germinated on September 6, 2006, with five seeds per pot, which were later thinned to one plant per pot across three replications for both the saline and control (non-stress) treatments. Plants were grown for one month prior to the imposition of stress. Salt stress of 3 dS m^{-1} was applied on October 6 and gradually increased to 6 dS m^{-1} over 10 days, and then maintained until harvest. Visual SES scores were recorded when plants were about 90 days old. Simultaneously, flag leaf samples were collected for salt uptake and $\delta^{13}C$ analyses. Flag leaf and harvested grain samples were analyzed for $\delta^{13}C$ composition. A number of morphological traits and yield components were also measured to compare reproductive stage responses of these diverse genotypes to salt-stress, and to test the relationship between Δ and reproductive stage salinity tolerance.

2.1.4. Experiment 4

The same 80 diverse lines as used in Experiment 3, were grown in the phytotron during June – July 2007 to estimate seedling stage salinity tolerance. The experimental protocol and measurements were the same as in Experiment 1.

2.1.5. Experiment 5

This experiment was conducted in May 2008 with 38 lines that included both near isogenic lines (NILs) and recombinant inbred lines (RILs) from the tolerant donors Pokkali and FL478. This set was chosen on the basis of location of the Pokkali introgression in the graphical genotypes and previous SES and Na^+ concentration data. Both extremes were chosen to provide a balanced set of tolerant and sensitive genotypes. The lines were grown in

saline nutrient solution in a phytotron with 9 lines per tray across three replications for both saline and control treatments. Nine day-old plants were subjected to salt stress beginning with 6 dS m^{-1} for 3 days, then increased to 12 dS m^{-1}. Initial scoring for visual salt injury was done at 10 DAS at 12 dS m^{-1} at which time shoot fresh weight, dry weight, and average shoot length were recorded. A final SES was performed 21 DAS, and total shoot and root dry weights were obtained. Simultaneously, leaf samples were collected for measurements of shoot Na^+ and K^+ concentrations, chlorophyll concentrations and $\delta^{13}C$ analysis.

2.1.6. Experiment 6

A final experiment was initiated to test a set of 80 F_2 families from a new mapping population which was previously phenotyped to select the extremes of salinity tolerance. The 40 most susceptible and 40 most tolerant lines of the F_2 families from the FL478 / Azucena population were selected for this experiment. The lines were grown in saline nutrient solution in a phytotron in September 2008. The experimental conditions were similar to those in the previous experiment. Seeds were pre-germinated and grown in a normal solution for 14 d prior to the imposition of stress. Salt stress of 6 dS m^{-1} was first applied for 5 days and subsequently increased to 12 dS m^{-1} until harvest. Initial scoring was done 14 days after initial salinization, with the final scoring and tissue harvest completed 21 days post-salinization. Tissue samples were analyzed for $\delta^{13}C$ composition as well as for other physiological traits.

2.2. Analytical

Leaf samples from seedlings (developed during stress) or flag leaves from mature plants were collected, dried at 70°C and then ground to a fine powder. The $\delta^{13}C$ composition of plant samples was analyzed at the IRRI Analytical Services Laboratory (ASL) using an isotope-ratio mass spectrometer. Δ values were calculated from $\delta^{13}C$ data [8]. QTL analysis on Δ and other traits was conducted using QGENE software (www.qgene.org). Shoot Na^+ and K^+ concentrations were determined by atomic absorption spectrophotometry (AAS 3100, Perkin Elmer, USA). Green leaf area was measured using a LiCor leaf area meter.

3. RESULTS AND DISCUSSION

3.1. Experiment 1

Salinity stress significantly reduced Δ from an average of 23.2 ‰ under control conditions to 21.7 ‰ under salt stress. A reasonable genetic variation in Δ under salt stress was also observed in this population, ranging between 21.0 ‰ to 22.8 ‰, and with a smaller range under normal conditions (22.7 ‰ to 23.8 ‰). Similar genotypic variation and drought effects were previously reported in cowpea [9] and rice [10]. The correlation between Δ and SES scores under salt stress was negative and highly significant ($R = –0.75$; $P<0.01$; Table 1; Fig. 1A), suggesting that tolerant genotypes were able to maintain higher g_s and / or photosynthetic capacity in the long-term under salt stress, and selection for higher Δ of leaf tissue will probably result in higher levels of tolerance. A similar correlation between Δ and WUE was previously reported for upland rice [10], and also between Δ and salinity tolerance [11]. Δ was positively correlated with green leaf area, and root and shoot dry weights as well as with K uptake, but was negatively correlated with the uptake of Na^+ and the Na^+: K^+ ratio in leaf tissue (Table 1, Fig. 1).

TABLE 1. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF RICE SEEDLINGS OF 79 RILS GROWN UNDER 12 DS M^{-1} SALT STRESS IN EXPERIMENT 1

Characteristic	SES score	Green leaf (cm^2)	Shoot DM †	Root DM †	Shoot Na^+ (%)	Shoot K^+ (%)	Shoot Na^+: K^+
Green leaf (cm^2)	–0.756**						
Shoot DM †	–0.742**	0.646**					
Root DM †	–0.719**	0.671**	0.780**				
Na^+ (%)	0.556**	–0.576**	–0.461**	–0.450**			
K^+ (%)	–0.448**	0.576**	0.402**	0.447**	–0.720**		
Shoot Na^+: K^+	0.502**	–0.558**	–0.421**	–0.421**	0.920**	–0.901**	
$\Delta^{13}C$ (‰)	–0.749**	0.657**	0.628**	0.579**	–0.460**	0.423**	–0.462**

†DM, dry matter normalized; **, significant at $P<0.01$.

Since the 79 RILs in this experiment were previously genotyped with AFLP (amplified fragment length polymorphism) and SSR (simple sequence repeat) markers, a QTL analysis was performed to compare the location of CID QTLs with previously detected salt tolerance QTLs. Using the leaf Δ measurements and single point analysis with QGene software, three QTLs with a LOD > 3.0 were identified (Table 2). One of these, linked with the SSR marker RM7075, is located in the same region as the *Saltol* QTL on the short arm of chromosome 1, a major QTL for salt tolerance derived from the Pokkali allele [12]. Analysis using leaf Δ showed a LOD of 3.8 and an R^2 of 20%. An even larger QTL for Δ under salt stress was detected on chromosome 3, linked with the AFLP marker P1 / M5-3, with an LOD of 6.4 and an R^2 of 33%. The third QTL was located on chromosome 11 with an LOD of 3.0 and an R^2 of 18%. The fact that all of the significant QTLs for Δ under salt stress also mapped to the same locations as QTLs for salt tolerance and Na^+: K^+ ratio suggests that these traits are closely integrated in their physiological mechanisms and genetic control.

TABLE 2. QTLS DETECTED FOR SALT TOLERANCE AND Δ USING 79 RILS FROM AN IR29 X POKKALI CROSS IN EXPERIMENT 1

Chromosome†	cM	Peak	SES score		Na : K		$\Delta^{13}C$	
			LOD	R^2 (%)	LOD	R^2 (%)	LOD	R^2 (%)
1	64.9–73.7	RM7075	10.5	45	9.0	43	3.8	20
3		P1 / M5-3	7.4	37	4.0	21	6.4	33
11	79.1	RM21	4.4	24	3.1	19	3.0	18

† Source, Pokkali; ‡values are significant at $P<0.05$.

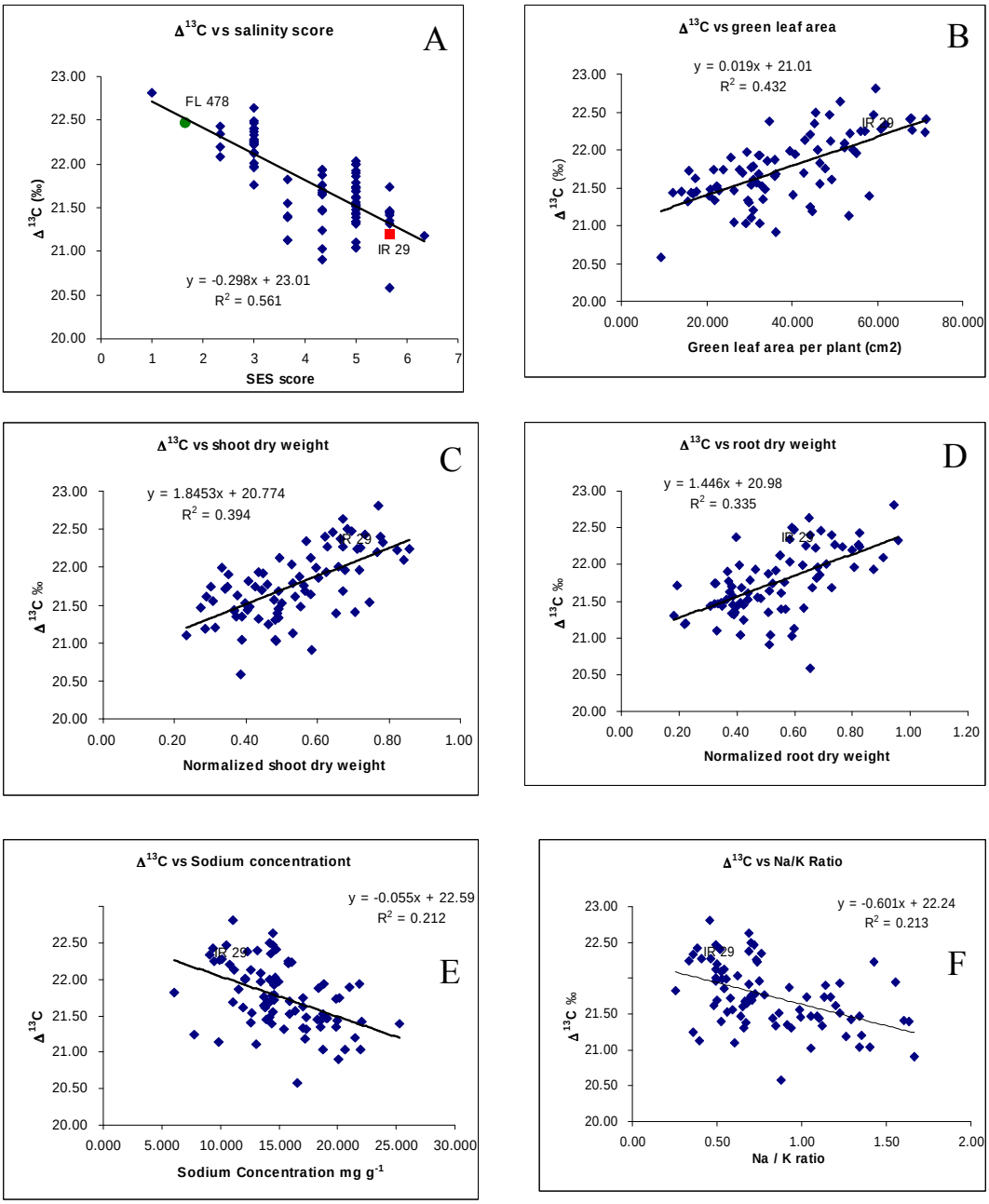

FIG. 1. Relationships between Δ and (A) visual SES scores (B) green leaf area (C) shoot dry weight (D) root dry weight (E) Na^+ concentration in seedling tissue and (F) Na^+: K^+ ratio using a set of 79 RILs and two parents in Experiment 1. Shoot and root dry weights were normalized to that obtained under control conditions. The green and red symbols in (A) represent the tolerant (FL478) and sensitive (IR29) parental values, respectively.

3.2. Experiment 2

Weak correlations existed between Δ and salt stress traits, in contrast to the earlier experiment using the RILs (Table 3; Fig. 2). However, the correlation between Δ and Na^+ uptake was negative and reasonably high (R = –0.39). For example, in the first experiment the correlation between Δ and the SES score under salt stress was negative and highly significant (R = –0.75, Table 1), while in the second experiment it was negative and relatively low (R = –0.13, Table 3). This is likely due to the fact that the RILs constituted a more variable set of sensitive and tolerant lines, while most of the breeding lines used in this trial were tolerant.

The higher correlation of Δ with Na^+ concentration also suggested that Na^+ uptake is probably more reliable than the more subjective SES scores in assessing salinity tolerance.

TABLE 3. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF RICE SEEDLINGS OF 80 TOLERANT BREEDING LINES GROWN UNDER 12 DS M^{-1} SALT STRESS IN EXPERIMENT 2

Characteristic	SES	Vigor	$\Delta^{13}C$	Total C	Na (ppm)	K (ppm)	Na (%)	K (%)
Vigor	0.181ns							
$\Delta^{13}C$	–0.128ns	0.451**						
Total C	–0.620**	–0.300**	0.058 ns					
Na (ppm)	0.473**	0.096 ns	–0.389**	–0.617**				
K (ppm)	–0.467**	–0.507**	0.024 ns	0.581**	–0.636**			
Na (%)	0.473**	0.075 ns	–0.386**	–0.601**	0.967**	–0.616**		
K (%)	–0.443**	–0.554**	–0.004 ns	0.552**	–0.564**	0.960**	–0.521**	
Na : K	0.593**	0.331**	–0.269**	–0.699**	0.883**	–0.864**	0.855**	–0.824**

ns, not significant; **, significant at $P<0.01$.

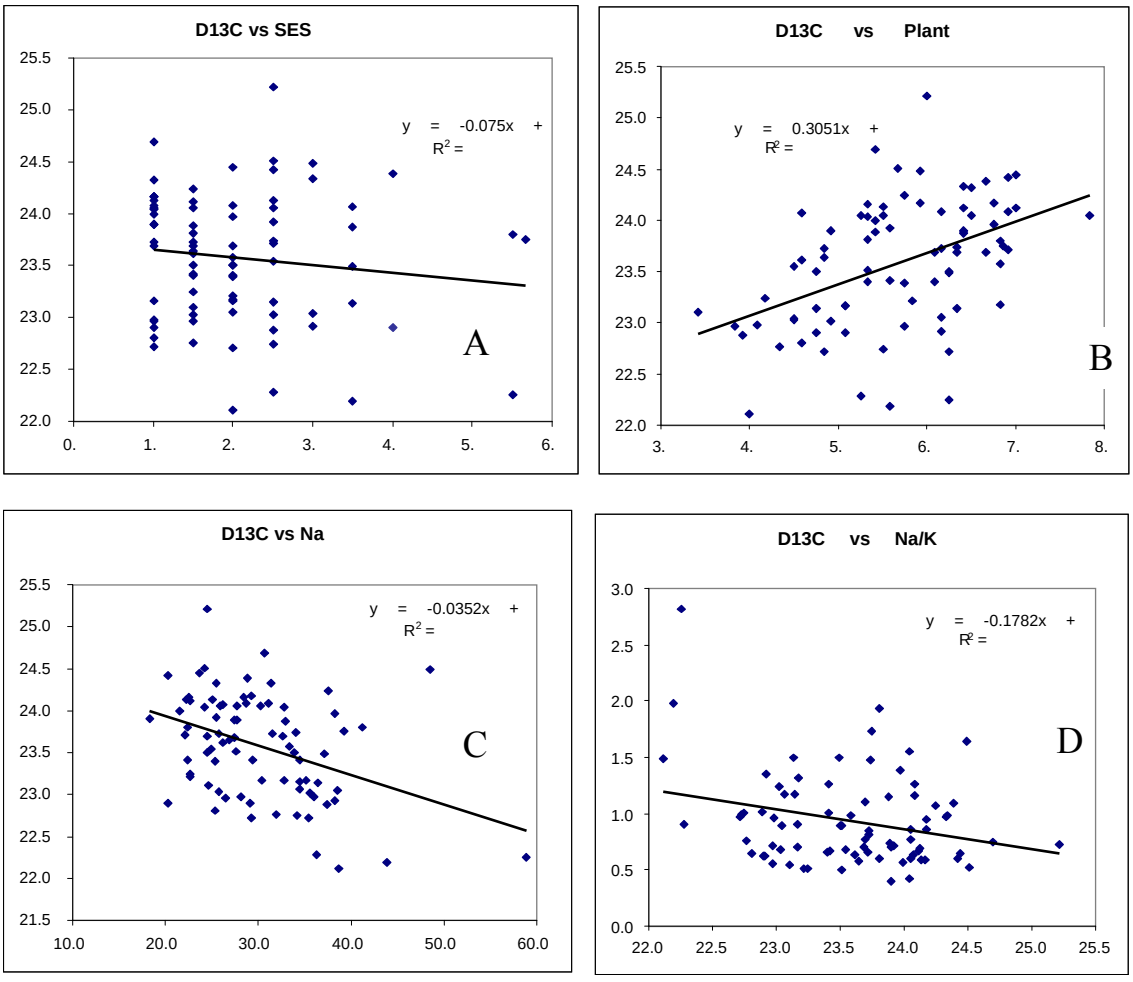

FIG. 2. Relationships between Δ and (A) visual SES scores (B) plant vigor (C) Na^+ concentration and (D) Na^+: K^+ ratio in seedling tissue using 80 mostly tolerant breeding lines in Experiment 2.

3.3. Experiment 3

Strong correlations were found between grain yield (total grain weight per plant) and grain number, and between flag leaf-Δ and grain-Δ both under normal and salt stress

conditions. Flag leaf-Δ also correlated negatively with visual SES scores under salt stress. Grain yield per plant correlated strongly and positively with grain number but not with fertility under salt stress, suggesting that salinity damage in this trial was probably more serious during spikelet formation than during pollination and fertilization (Tables 4 and 5). The strong correlation of flag leaf-Δ with grain-Δ and grain yield suggest that selection for salinity tolerance during the reproductive stage could be based on flag leaf-Δ, which is easier and could be done relatively early in case selected lines need to be further backcrossed during breeding.

The two sub-sets of varieties showed clear differences, with the 34 varieties and landraces having a larger range of plant height at maturity compared with the shorter and more uniform set of 46 mostly semi-dwarf breeding lines, seen under both saline and normal conditions (Fig. 3A and Fig. 3B).

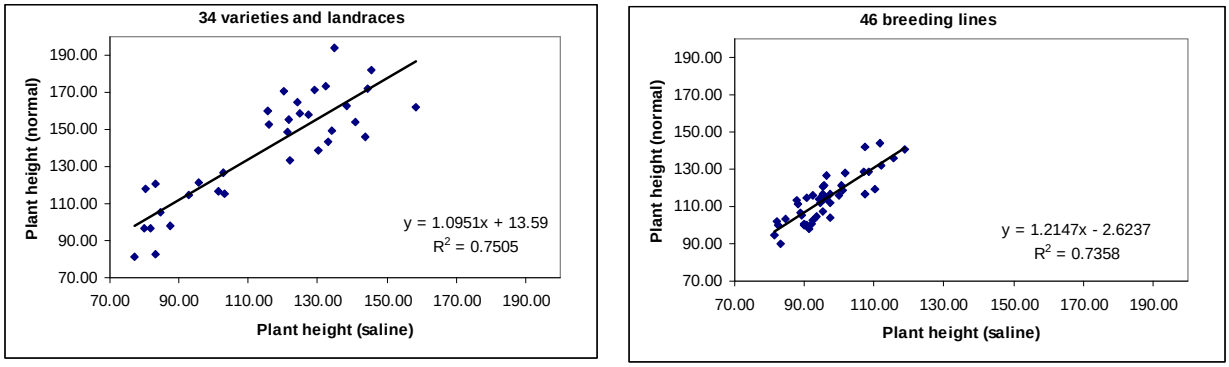

FIG. 3. Relationships between plant height under normal conditions and under salt stress at maturity for (A) the subset of 34 varieties and landraces and (B) the set of 46 IRRI breeding lines in Experiment 3.

TABLE 4. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF MATURE RICE PLANTS OF 80 DIVERSE GENOTYPES GROWN UNDER 6 DS M^{-1} SALT STRESS IN EXPERIMENT 3

Characteristic	Height	Fertility	Yield	Grain N^{o}	$Na^+ : K^+$	Flag leaf-Δ	Grain-Δ
Fertility	0.18 ns						
Yield	–0.14 ns	0.14 ns					
Grain N^{o}	–0.07 ns	0.19 ns	0.72**				
$Na^+ : K^+$	0.01 ns	0.07 ns	0.21 ns	0.11 ns			
		ns	0.54**	0.46**	0.06 ns		
Grain-Δ	–0.59**	–0.10 ns	0.50**	0.43**	0.13 ns	0.87**	
SES	0.09 ns	0.10 ns	–0.53**	–0.51**	–0.25*	–0.26*	–0.27*

ns, not significant; *, **, significant at $P<0.05$ and 0.01, respectively.

TABLE 5. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF MATURE RICE PLANTS OF 80 DIVERSE GENOTYPES GROWN UNDER NON-SALINE CONDITIONS IN EXPERIMENT 3

Characteristic	Height	Fertility	Yield	Grain N^o	$Na^+ : K^+$	Flag leaf-Δ
Fertility	–0.39**					
Yield	–0.44**	0.71**				
Grain N^o	–0.17 ns	0.60**	0.60**			
$Na^+ : K^+$	0.04 ns	–0.03 ns	0.06 ns	–0.15 ns		
Flag leaf-Δ	–0.52**	0.46**	0.51**	0.34**	0.14 ns	
Grain-Δ	–0.57**	0.32**	0.41**	0.31**	0.09 ns	0.83**

ns, not significant; **, significant at $P<0.01$

However, salinity seems to affect plant height of both groups to similar degrees. To compare the usefulness of the flag leaf-Δ compared with grain-Δ, the values were plotted from the 80 lines under both saline and normal conditions (Fig. 4A and Fig. 4B). Strong correlations (R = 0.87 and 0.83 for saline and normal conditions, respectively) were observed between both measurements, suggesting that either flag leaves or grain samples can be used interchangeably for Δ measurements to assess reproductive stage salinity tolerance in rice. For the remainder of the comparisons, only the flag leaf-Δ is shown, although grain-Δ gave similar results. A comparison of the two sub-sets of varieties showed clear differences in the range of Δ values under saline conditions, where the landraces seem to have a broader range than the breeding lines, and also the values tended to be lower for the landraces. This illustrates the sensitivity of the technique to differentiate between rice genotypes based on their yield potential, and that the more diverse set of varieties and landraces have greater variability in their ability to maintain gas exchange than the more uniform set of breeding lines (Fig. 4C and Fig. 4D). Under a Mediterranean climate, better correlations of grain yield over years were observed with grain-Δ than with flag leaf-Δ in durum wheat [13], suggesting that different approaches may work better for different crop species.

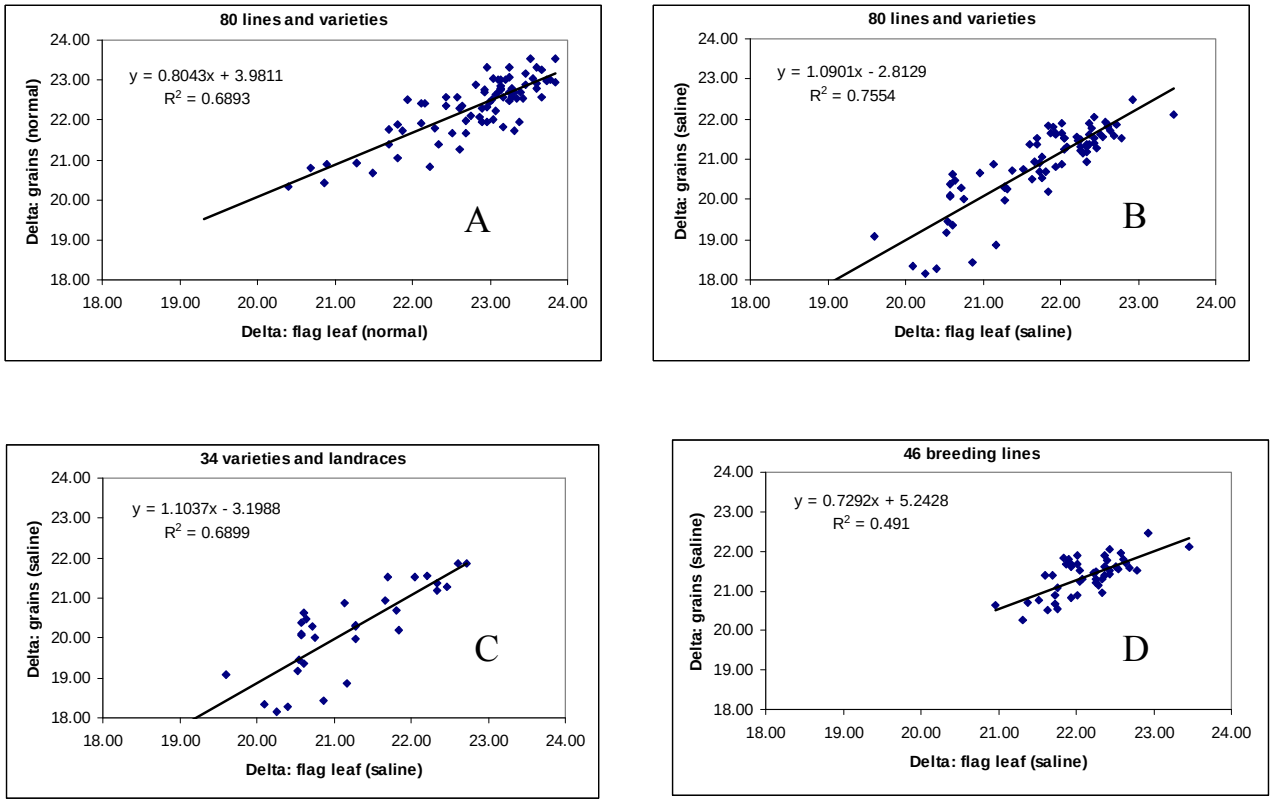

FIG. 4. Relationships between flag leaf-Δ and grain-Δ for (A) the 80 genotypes under salt stress (B) the 80 genotypes under normal conditions (C) the subset of 34 varieties and landraces under salt stress and (D) the set of 46 breeding lines under salt stress in Experiment 3.

To determine the relationship of Δ with the extent of salinity tolerance during the reproductive stage, the Δ values were compared with grain yield per plant across the 80 lines and varieties for both saline and normal conditions (Fig. 5A and Fig. 5B). The strong positive correlation (R = 0.54) under saline conditions suggests that the more tolerant varieties (which can sustain high yields under salt stress) also maintain greater g_s and gas exchange rates, while the sensitive varieties have much lower stomatal conductance when under salt stress (Fig. 5A). In addition, a positive correlation (R = 0.51) was obtained under normal conditions, but overall the Δ values were higher for all varieties, suggesting better g_s under non-stress conditions as expected (Fig. 5B). Interestingly, when the two sub-sets of varieties were analyzed separately, the correlations between yield and Δ were lower (R ranged from 0.21 to 0.33). This is likely to be due to the uniformity within each of the two sets; all 34 diverse varieties have lower yields and lower Δ values, while all 46 breeding lines had higher yields and higher Δ values under both saline and normal conditions (Figs. 5C, 5D, 5E, 5F). Other factors in the greenhouse that might have affected grain setting (light intensity and temperature) may have also confounded this analysis.

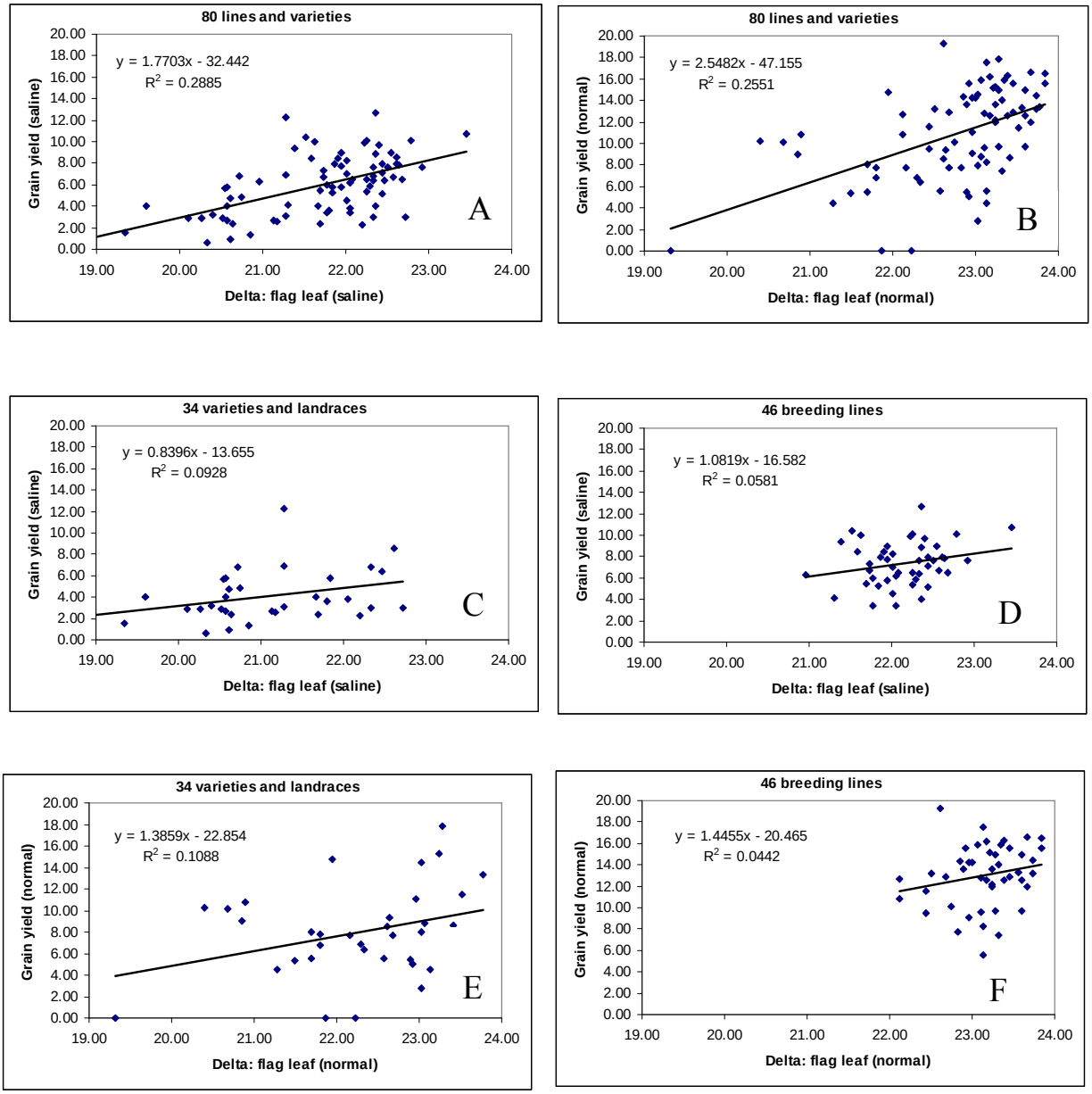

FIG. 5. Relationships between grain yield (g plant^{-1}) and Δ values (‰) from the flag leaves for (A) the set of 80 genotypes under salt stress (B) the set of 80 genotypes under normal conditions (C) the subset of 34 varieties and landraces under salt stress (D) the set of 46 breeding lines under salt stress (E) the subset of 34 varieties under normal conditions and (F) the set of 46 breeding lines under normal conditions.

Moreover, an interesting trend was seen in the comparison of plant height and Δ values; a negative correlation (R = –0.52) was observed under normal conditions, while an even stronger negative correlation (R = –0.68) was seen under saline conditions (Fig. 6A and Fig. 6B). Analysis of the two groups separately suggested that the taller landraces tend to have much lower Δ values under salt stress than the semi-dwarf breeding lines (Fig. 6C and Fig. 6D). This may be due more to an indirect selection for higher g_s and gas exchange rates during the breeding process of modern, high-yielding rice lines, rather than a physiological link between Δ and plant height.

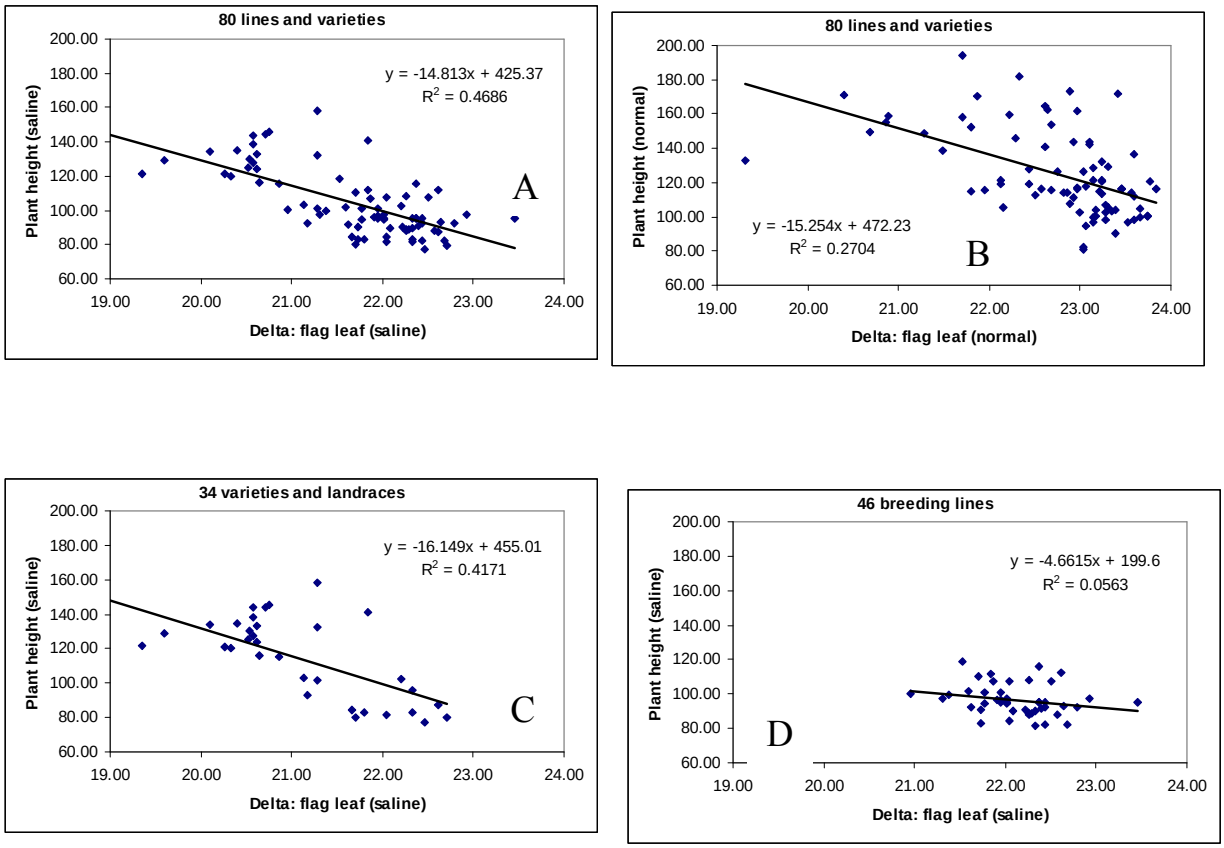

FIG. 6. Relationships betweens plant height and Δ values from flag leaves for (A) the 80 lines under salt stress (B) the 80 lines under normal conditions (C) the subset of 34 diverse lines under salt stress and (D) the set of 46 breeding lines under salt stress.

The correlation between grain yield and visual SES scores was negative as expected (Fig. 7A), and the relationship was also evident when comparing percent reduction in grain yield due to imposed salinity (Fig. 7B). However, this trend is not as strong when comparing Δ values with SES scores. Although the mapping population previously evaluated at the seedling stage showed a stronger correlation (R = –0.75), the experiments using this diverse germplasm had lower correlations during the reproductive stage (Fig. 8A and Fig. 8B). This is also apparent when comparing the differences in Δ values between the normal and salt stress environments with the SES score; the mapping population showed a stronger positive correlation, but the 80 lines and varieties did not (Fig. 9A and Fig. 9B). This may be due to differences in the reliability of the visual scoring at the seedling stage compared with the reproductive stage, where scoring is mainly based on the overall plant conditions, rather than just grain yield during reproduction. This might also suggest that salinity causes additional effects on grain setting when imposed during reproduction than can be predicted from the overall plant appearance. Another possibility for the poor correlation could also be the complications when studying diverse germplasm compared to a mapping population that is segregating for salinity tolerance. This last point is further confirmed when the Δ values between the normal and saline treatments are directly compared for each experiment. For the mapping population, which was derived from the two parents IR29 and Pokkali, the Δ values are tightly clustered (Fig. 10A). The set of 81 mostly breeding lines (from Experiment 2) and the 46 breeding lines (from Experiment 3) also clustered together, although not as tightly as the mapping population (Fig. 10B and Fig. 10D), while the 34 varieties and landraces showed a much wider range of Δ values, reflecting a greater physiological variation likely resulting from the greater genetic diversity of these varieties.

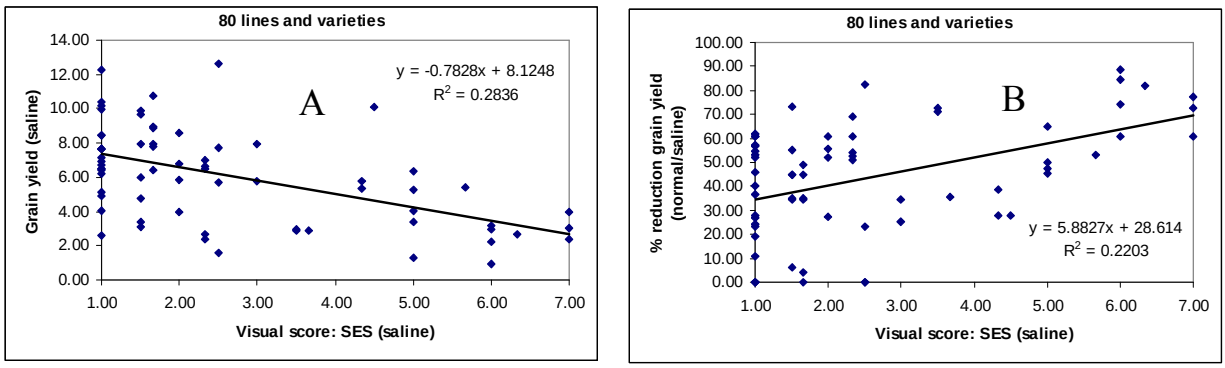

FIG. 7. Relationships between SES scores for the set of 80 lines and varieties and (A) grain yield plant^{-1} under salt stress and (B) the percent reduction of grain yield between the normal and salt stress conditions.

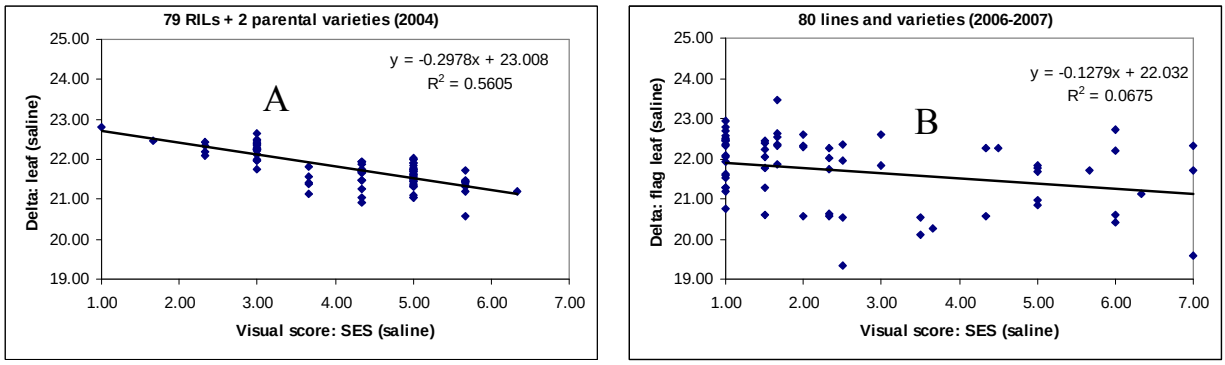

FIG. 8. Relationships between SES scores and Δ values under salt stress for (A) the leaves of seedlings of 79 RILs in solution culture in Experiment 1 and (B) the flag leaves of 80 lines and varieties at the reproductive stage in soil culture in Experiment 3.

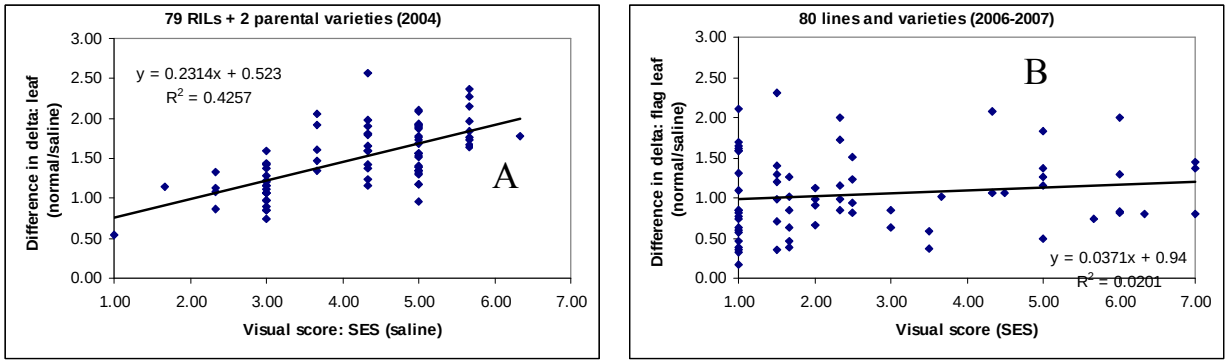

FIG. 9. Relationships between SES scores and the difference in Δ values between normal and salt stress treatments for (A) the leaves of seedlings of 79RILs in solution culture and (B) the flag leaves of 80 lines and varieties in soil culture.

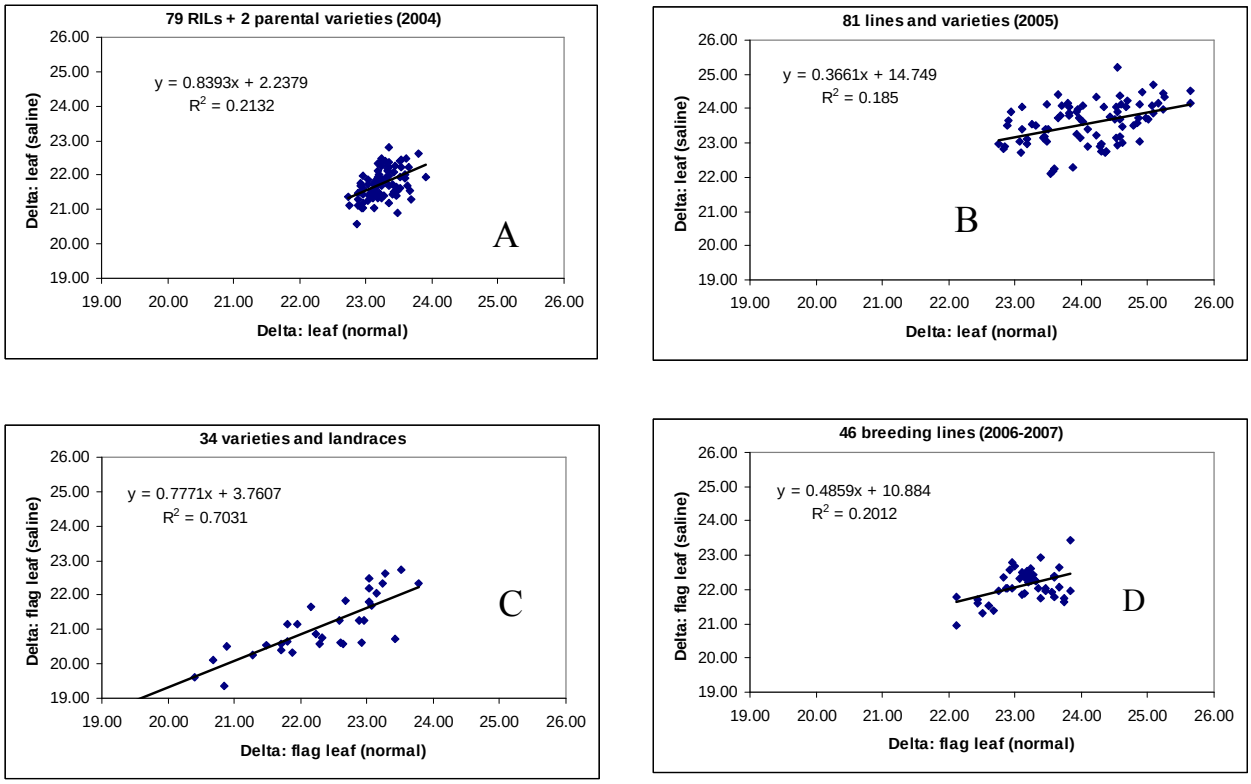

FIG. 10. Relationships between leaf tissue Δ values under salt stress and normal conditions for (A) 79 RILs in Experiment 1 (B) 81 lines and varieties in Experiment 2 (C) 34 varieties and landraces and (D) 46 breeding lines. The Δ values were measured using the youngest fully expanded leaves at the early vegetative stage in A and B (solution culture), and the flag leaves in C and D (soil culture, Experiment 3).

Flag leaf-Δ values correlated well with grain-Δ values, and both correlated positively with the grain yield of single plants, suggesting that Δ can potentially be used to select for salinity tolerance during the reproductive stage based on values obtained from flag leaves. The widely different results seen between the two sets of germplasm (34 diverse genotypes compared with the 46 more uniform breeding lines) suggests that future experiments to study the correlation between Δ and salinity tolerance should always include both germplasm groups during the initial studies. Subsequently, after the thresholds for specific Δ values in different environments are defined, then Δ measurements can be used within these uniform sets of germplasm.

3.4. Experiment 4

There were no significant relationships between Δ and SES scores, Na concentration or Na: K ratio, while there was a strong negative correlation between Δ and seedling shoot biomass (Fig. 11). In contrast to the strong correlations seen with the 79 RILs in Experiment 1, the experiments using 80 breeding lines (Experiment 2) and this set of 80 more diverse lines and varieties did not show correlation between Δ and seedling SES scores. It is possible that the relationship is only significant when using sets of extremely tolerant and sensitive lines (such as the RILs) to provide enough contrast to detect the subtle correlations between these traits.

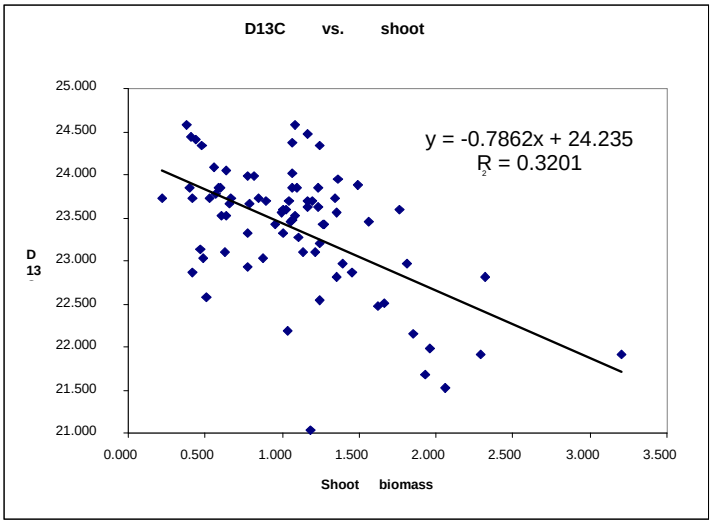

FIG. 11. Relationships between Δ and shoot biomass in seedlings of 80 diverse lines and varieties in Experiment 4.

3.5. Experiment 5

There were no significant relationships between Δ values and both initial and final SES scores under saline conditions, or Na uptake and Na: K ratios under both saline and non-saline conditions. Salinity affected plant height significantly, and Δ was negatively correlated with shoot length (R^2 = –0.34), but not with total chlorophyll concentration (R^2 = 0.13).

3.6. Experiment 6

An expected correlation was seen between the visual SES score with % Na from the salinity stress experiment (Table 6, Fig. 12). However, Δ had a weak correlation with seedling vigor (R^2 = 0.19) and no significant correlation with SES scores (R^2 = 0.01). This is in contrast with the RILs from Experiment 1, which showed a higher correlation between Δ values and SES scores. In addition, no QTLs above LOD 3.0 were detected for the Δ values using these 80 F_2 families. One complicating factor is that the F_2 families are still segregating, so the variation in the F_3 progeny will affect the results. This suggests that future studies should rely more on RIL populations for genetic mapping, as the fixed RILs allow for more precise screening and better correspondence between the genotype and phenotype data.

TABLE 6. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF RICE SEEDLINGS OF 80 F_2 (FL478 / AZUCENA) FAMILIES GROWN UNDER SALT STRESS IN EXPERIMENT 6

Characteristic	Shoot length	Fresh mass	Vigour	SES initial	SES final	Na^+ (%)	K^+ (%)	Na^+ : K^+	Chlor.†
Fresh mass	0.83**								
Vigour	–0.95**	–0.95**							
SES initial	–0.51**	–0.60**	0.54**						
SES final	–0.44**	–0.56**	0.49**	0.77**					
Na^+ (%)	–0.43**	–0.52**	0.47**	0.60**	0.73**				
K^+ (%)	–0.32**	–0.17ns	0.27*	0.16ns	0.14ns	0.26*			
Na^+ : K^+	–0.25*	–0.41**	0.31**	0.52**	0.65**	0.86**	–0.21*		
Chlor.†	0.17ns	0.32**	–0.22*	–0.46**	–0.50**	–0.51**	–0.11ns	–0.44**	
$\Delta^{13}C$	–0.32**	–0.25*	0.33**	0.11ns	0.07ns	0.14ns	0.31**	–0.02ns	0.09ns

†Chlorophyll a + b; ns, not significant; *, **, significant at P<0.05 and P<0.01, respectively

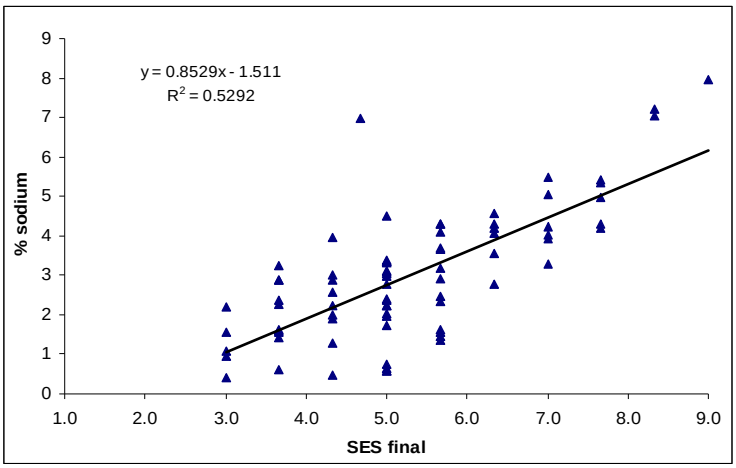

FIG. 12. Relationship between % Na and SES scores for 80 FL478 / Azucena F_2 families at the seedling stage in Experiment 6.

3.7. Population development for lines with contrasting Δ values

To study the inheritance of tolerance to salinity, several crosses were made for lines that gave contrasting Δ values at the reproductive stage. The contrasting parents were selected from Experiment 3, including two high-Δ x high-Δ crosses: IR63731-3-5-14-1-2 x IR65209-3B-1-2-1 and IR65209-3B-1-2-1 x IR70868-B-5-3, and two high-Δ x low-Δ crosses: IR63731-3-5-14-1-2 x Moroberekan and IR70868-B-5-3 x IR71995-3R-1-2-2 (SAL 547). The F_1 seeds were advanced to F_2, approximately 400 F_2 plants were grown for each of the 4 populations, and at least 200 F_3 seeds were harvested from each of these 1,600 F_2 individuals. Leaf tissue was harvested from F_2 plants from one population (IR63731-3-5-14-1-2 x Moroberekan) to allow for rapid QTL mapping after phenotyping the F_3 progeny. These crosses can be used to develop elite isomorphic lines, and the F_3 progenies can also be evaluated under field conditions for grain yield and grain- and / or flag leaf-Δ to confirm the relationships with the level of salt tolerance under field conditions using contrasting populations. The F_4 seeds will be made available for future experiments in the field or greenhouse where correlations between tolerance to salinity and Δ values will be further studied.

4. CONCLUSION

These studies provided a rigorous evaluation of the relationships between $\Delta^{13}C$ and salinity tolerance in rice in diverse sets of germplasm, ranging from breeding lines, landraces and genetic stocks developed for mapping of QTLs associated with salinity tolerance. In all cases, Δ correlated positively with salinity tolerance both at the seedling and reproductive stages. Grain-Δ correlated positively with flag leaf-Δ, suggesting that flag leaves could successfully be used for selection of salinity tolerance during reproduction. Variation in Δ was greater in landraces than in advanced breeding lines and varieties, and the correlations of Δ with salt tolerance became stronger with increasing diversity. Apparently, Δ could successfully be used for selection of parental lines from diverse populations, but the variability could be relatively small to detect differences between breeding lines that are more homogenous or closely related. Three large QTLs were detected on chromosomes 1, 3, and 11 that are associated with Δ under salt stress, which co-localize with QTLs that were previously mapped for salinity tolerance [12].

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UTILIZATION OF CARBON ISOTOPE DISCRIMINATION AND ASH CONTENT TO SELECT WHEAT FOR TOLERANCE TO WATER LIMITATION IN THE PENINSULAR ZONE OF INDIA

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India

Abstract

Relationships between grain yield, ash content (m_a) and carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) were studied in 30 wheat genotypes (14 durum and 16 aestivum) during 2003–2004, and 20 durum genotypes during two successive seasons (2004–2005 and 2005–2006) under three water regimes, i.e., residual soil moisture stress (RSMS), post-anthesis water stress (PAWS) and well watered (WW) conditions. Correlations between grain yield, ash content and Δ depended greatly on the environmental conditions and the drought scenario. Grain yield, biomass, Δ and m_a decreased significantly with the reduction in water availability. Yield was significantly and positively correlated with ΔL_a and ΔG_m for each water regime only in 2005–2006, but the relationships were significant under WW across seasons. Strong negative correlations were observed between yield and m_aG_m for each water regime only in 2004–2005, but the relationships were significant across seasons under WW conditions. Several significant and negative correlations were observed between yield and canopy temperature depression, but the relationships were significant only under PAWS across seasons. Results obtained during 2004–2005 and 2005–2006 show that Δ and m_aG_m could consistently predict yield only under WW conditions. ΔG_m and ΔL_a were consistently and significantly correlated with m_aL_a only under RSMS. In RIL population studies a major QTL was detected on chromosome 4B, responsible for around 15% of the variation in Δ. Major QTLs for canopy temperature depression (CTD) were identified on chromosomes 2A and 6A, and major QTLs for ash content were detected on chromosomes 5A and 6A, under PAWS and full irrigation, respectively. Near Infra-red transmittance (NIT) was used to speed up the determination of grain ash content. The overall range for wheat samples analyzed by the reference and NIT methods were 1.2 – 2.3 % and 1.22 – 1.99% (dry weight basis), respectively. The slope of the regression was 1.000 with an r^2 value of 0.961. Samples covering a wider range of concentrations are needed to calibrate the NIT methodology precisely.

1. INTRODUCTION

With an average annual production of 76 million tons, India is the second largest producer of wheat in the world [1]. Based on the diversity of agro- climatic conditions, wheat cultivation in India has been divided into five mega-zones, namely Northern Hills, North Western Plains, North Eastern Plains, Central and Peninsular [2]. With 8.6 million ha of wheat, the Central and Peninsular zones account for one third of the total wheat area of India. These zones have a great potential to boost national wheat productivity and production [3]. Yield is limited, however, by inadequate availability of irrigation water and high temperatures. Wheat is cultivated from November to April (Rabi season), mainly under residual soil moisture and limited irrigation, and is exposed to extremely high temperatures during the grain filling period [4].

Several morpho-physiological traits have been proposed as screening criteria for drought tolerance [5] such as relative water content (RWC) and transpiration efficiency (TE), the ratio of dry matter produced to water transpired. The use of Δ as a related criterion affords an easy way of screening for TE. The negative relationship established between Δ and TE [6] led to the suggestion that Δ could be used as an indirect selection criterion for TE [7, 8, 9].

During photosynthesis, plants discriminate against the heavy isotope of carbon (^{13}C) which leads to ^{13}C depletion of the plant dry matter. Δ is a measure of the plant $\delta^{13}C$ compared with $\delta^{13}C$ in atmospheric CO_2 [6]. In C_3 species, including bread wheat and barley, Δ was found to be positively correlated with C_i/C_a, the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration, and negatively correlated with TE [6, 9, 10, 11, 12]. Δ appears to provide a useful integration of TE for C_3 species, and therefore has been proposed as a potential index for TE [13].

However, natural abundance carbon isotope analysis ($\delta^{13}C$) using mass spectrometry is expensive, especially for the screening of large collections of genetic resources. Attempts have been made to develop alternative screening methods. Ash content and dry mass per unit of leaf area have been proposed as surrogates for Δ [14, 15, 16] and as an alternative selection criterion for TE and yield.

Other traits associated with yield, such as grain number and harvest index, can be used in visual selection of breeding lines. Canopy temperature depression (CTD) shows high correlation with yield in both warm and temperate environments, indicating that the trait is heritable and therefore amenable to early generation selection [17]. Since CTD can be easily measured on scores of plants in a small breeding plot (thus reducing error), work has been conducted to evaluate its potential as an indirect selection criterion for genetic gains in yield.

The objectives of the present study were: (i) to investigate the relationships among grain yield, $\Delta^{13}C$ and other morpho-physiological traits (ash content, canopy temperature depression), in order to define the conditions under which Δ or ash content may be used as selection indices under different water regimes (residual soil moisture, moderate irrigation and optimal irrigation) (ii) to develop segregating populations involving high yielding and high Δ parents for genetic studies, and for screening under limited irrigation using Δ and ash content as criteria for selection (iii) to identify quantitative trait loci (QTLs) for Δ, CTD and ash content in a recombinant in-bred line (RIL) population developed from durum wheat cross PDW 233 × Bhalegaon 4 (iv) to investigate the use of Near Infra-red Transmittance to analyze plant tissue ash content.

2. MATERIALS AND METHODS

2.1. Experimental

All experiments were conducted at the Experimental Farm of Agharkar Research Institute (ARI), located in the Hol District, Pune, India (18.04º N, 74.21º E and 548.6 m above sea level) on a clay loam soil having a water holding capacity of 65% and a pH of 8.1. Temperature is a major factor limiting yield in the Peninsular Zone, as the crop suffers heat stress after February (Table 1). Early maturity is consequently a requirement for this zone.

TABLE 1. MINIMUM (MIN) AND MAXIMUM (MAX) TEMPERATURES (°C) DURING CROP SEASONS 2003–2004 TO 2006–2007

Season	October		November		December		January		February		March	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
2003–04	19.9	34.5	16.8	32.5	15.3	31.0	11.7	30.6	14.1	34.5	16.5	37.2
2004–05	18.5	31.4	16.6	30.5	11.3	29.6	12.2	29.2	13.4	33.5	17.8	38.9
2005–06	18.2	30.8	12.3	30.1	10.8	30.0	9.9	30.1	18.6	33.9	15.9	35.2
2006–07	19.3	31.4	18.5	29.9	12.1	29.8	10.6	29.3	12.4	32.0	16.0	36.5

The varietal trials conducted from 2003–04 to 2005–2006 were in a randomized block design with 3 treatments, i.e., residual soil moisture stress (RSMS), post-anthesis water stress (PAWS, i.e. limited irrigation) and well watered (WW) conditions, with 3 replicates per genotype. Water was applied as flood irrigation. The first irrigation was given immediately after sowing to ensure good germination. Three irrigations were given before anthesis to PAWS and six irrigations to the WW treatment (Table 2).

Fertilizer was applied as per recommended doses i.e., (N, P, K; kg ha^{-1}, respectively) of 60, 30 and 40 at sowing (RSMS), 80, 40 and 40 (PAWS) and 100, 50 and 40 (WW). N application in PAWS and WW were given in two splits, half at sowing and half 25 days later with irrigation. Seeds were sown in six 4 m rows spaced at 23 cm (260 seeds m^{-2}). Wheat in this zone generally takes from 120 to 125 and from 90 to 100 days to mature under WW and RSMS conditions, respectively.

TABLE 2. RAINFALL AND IRRIGATION (MM) UNDER THREE WATER REGIMES DURING SEASONS 2003–2004 TO 2006–2007

Season	May to October	Nov	Dec	Jan	Feb	March	Total rainfall	Irrigation †		
								RSMS	PAWS	WW
2003–04	77.2	17.0	0.0	0.0	0.0	0.0	94.2	60	210	330
2004–05	548.5	12.5	0.0	3.5	0.6	3.5	568.6	30	210	330
2005–06	652.9	0.0	0.0	0.0	0.0	0.0	652.9	30	210	330
2006–07	668.3	25.6	0.0	0.0	0.0	0.0	693.9	–	150	270

†RSMS, residual soil moisture stress; PAWS, post-anthesis water stress; WW, well watered.

2.2. Varietal trials

2.2.1. 2003–2004 season

Fourteen *durum* (B. Yellow, MACS 1967, HI 8498, MACS 2846, MACS 3125, UAS 401, MACS 3490, MACS 3463, MACS 3444, MACS 3501, PDW 233, MACS 3560, MACS 3547, MACS 3557) and 16 *aestivum* (MACS 2496, NIAW 34, HI 977, HD 2189, GW 322, MACS 6145, LOK 45, HD 2781, HM 994, MACS 6158, CAS 439, GW 373, PBW 512, MACS 6198 and GW 353) wheat genotypes were selected from varieties released in India and ARI's germplasm bank.

2.2.2. 2004–2005 and 2005–2006 seasons

Twenty semi-dwarf durum wheats that yielded well under limited irrigation trials at ARI during previous years and from the All India Co-ordinated Programme were selected i.e., HD 4672, HI 8498, HI 8627, HI 8641, HI 8666, UAS 401, UAS 405, MACS 2694, MACS 2846, MACS 3125, MACS 3518, MACS 3571, MACS 3572, MACS 3618, MACS 3640, DD 07, RKD 111, NIDW 350 and NIDW 295.

2.3. Breeding and RIL populations

On the basis of results obtained during two seasons, two sets of F_2 population that were developed from crosses between high yielding varieties and high Δ lines (MACS 3125/MACS 3571 and HI 8498/MACS 3571) were raised during the off-season for generation advancement. About 150 individual plants were harvested to raise seed for undertaking a F_3 progeny trial. About 130 F_3 families were retained for planting in a replicated trial during the regular 2006–2007 crop season under limited irrigation and well watered conditions with

2 replicates. Each plot consisted of two rows of 3 m length with 30 cm between rows and 10 cm between plants. Data on phenology (days to flowering and maturity), agronomic traits (plant height, yield, biomass, harvest index) and physiological parameters (Δ, canopy temperature depression, ash content) were recorded. Flag leaves were harvested at anthesis for determination of ash content and Δ.

Another F_4 population having 25 progenies involving low Δ (Bhalegaon 4) and high Δ (PDW233) parents was empirically selected from breeding material during the 2004–2005 season. Another trial was conducted during the 2006–2007 season under limited irrigation and well watered conditions involving 140 RILs derived from PDW 233 and Bhalegaon 4 parents. Grain Δ for this population was used for composite interval mapping to locate QTLs.

2.4. Measurements

2.4.1. Carbon isotope discrimination

CID was determined on the flag leaf at anthesis (30 leaves $plot^{-1}$) and on grain at maturity (one 10 g sample $plot^{-1}$). Samples were dried at 60 ^oC for 48 h and ground to a fine powder. Samples of the three replicates were carefully mixed in equal weights, to constitute a bulk sample. $\delta^{13}C$ was measured on a 1 mg sample with an isotope ratio mass spectrometer at the University of Agricultural Sciences, Bangalore.

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{reference}) - 1] \times 1000 \quad (1)$$

where R, the isotope ratio = $^{13}C/^{12}C$. A secondary standard (potato starch), calibrated against the primary standard, Pee Dee Belemnite (PDB) fossil carbonate, was used as the reference.

$\Delta^{13}C$ in the flag leaf at anthesis (ΔL_a) and in grain at maturity (ΔG_m) were calculated [13] as:

$$\Delta^{13}C\ (‰) = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000 \quad (2)$$

where $\delta_p = \delta^{13}C$ of the plant sample and $\delta_a = \delta^{13}C$ of atmospheric $CO_2 = –8‰$.

2.4.2. Ash content (%)

Ash content was determined on plantlets, the flag leaf at the booting stage, at anthesis and at maturity, and grain at maturity for all replicates. Samples collected for carbon isotope analysis were used for mineral ash analysis. Ash content of plant material was determined by the official AACC method [18]. Approximately 1.0 g of dry material was incinerated at 575^oC for 16 h (until light gray ash was obtained). Ash content (%) was expressed on a dry mass basis. The ash contents of plantlets, the flag leaf at booting, anthesis and maturity, and grain at maturity are referred as m_aL_p, m_aL_b, m_aL_a, m_aL_m and m_aG_m, respectively.

Grain ash content was estimated by NIT (Near Infra-red Transmittance) [19], and was calibrated against the standard ashing procedure, in order to provide a rapid screening tool for breeding programmes.

2.4.3. Agronomic and phenological data

For the varietal trials, biomass and yield were determined by harvesting four 3.5 m rows at maturity, and g $plot^{-1}$ data were extrapolated to t ha^{-1}. Flag leaf samples (10 $plot^{-1}$) taken from breeding (F_3) and RIL populations at anthesis, and grain at maturity, were

analyzed for $\delta^{13}C$ and ash content. Phenology (days to flowering and days to maturity) and physiological traits (tillers m^{-1}, 1000-kernel weight and plant height at maturity) were recorded.

2.4.4. Canopy temperature depression (CTD)

CTD for genotypes, breeding populations and RILs was measured using a Telatemp infrared thermometer [17] at boot (CTD_b), anthesis (CTD_a) and at grain filling (CTD_g) for PAWS and WW treatments, with a minimum gap of 10 d after irrigation.

2.5. Statistical analysis

Data were analysed using Agrobase 99 software for replicated observations. A combined ANOVA analysis determined differences across genotypes (G) and water treatments (T) for different plant traits, and G × T interactions. Relationship between grain yield and traits were determined by phenotypic correlations (r).

3. RESULTS

3.1. The effect of water regime on agronomic and physiological traits

3.1.1. 2003–2004 season

Results have been published [20], and will therefore be summarized here. Grain Δ was lower under PAWS and RSMS than under WW, while seedling- and leaf Δ did not differ significantly among water regimes. Grain yield was positively correlated with grain Δ under PAWS and negatively correlated with grain ash content under RSMS. A significant positive correlation between yield and ash content in the flag leaf at maturity was noted under RSMS and WW treatments. When durum and bread wheats were considered together, the relationship between ΔG_m and grain yield was significant under PAWS. No correlation was found between yield and either ΔL_p or ΔL_a. A significant negative correlation between yield and m_aG_m was observed under RSMS. Under PAWS, m_aL_a was negatively correlated with ΔG_m.

3.1.2. 2004–2005 season

Genotypes differed significantly in grain yield, biomass, harvest index, leaf ash content at anthesis and maturity, grain ash content, and flag leaf and grain Δ (Table 3). Water regimes had similar significant effects on the agronomic and physiological parameters. G × T interactions were highly significant for all traits except ΔL_a (Table 3).

TABLE 3. MEAN SQUARES OF COMBINED ANOVA FOR AGRONOMIC AND PHYSIOLOGICAL TRAITS OF WHEAT IN THE 2004–2005 SEASON

Source of variation	df	Grain yield	Biomass	HI	Ash content †					CID ‡	
					m_aL_p	m_aL_b	m_aL_a	m_aL_m	m_aG_m	ΔL_a	ΔG_m
Genotype (G)	19	1.0*	5.7**	57**	1.9ns	7.4ns	4.8*	8.9***	0.13***	0.83***	0.75***
Treatments (T)	2	75***	642***	214***	211***	19ns	144***	81***	1.5***	70***	27***
G × T	38	0.65***	3.2***	24***	1.7***	5.4***	2.3***	2.5***	0.05***	0.21ns	0.23***

†Ash content in plantlet (m_aL_p), flag leaf at boot stage (m_aL_b), at anthesis (m_aL_a) and at maturity ($m_aL_{m,}$) and grain at maturity (m_aG_m).
‡CID, carbon isotope discrimination ($\Delta^{13}C$) in flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m).
*, **, ***, significant at P<0.05, 0.01 and 0.001, respectively; ns = not significant.

The highest yield was recorded under WW followed by PAWS and RSMS (Table 4). The highest biomass was obtained under WW and PAWS followed by RSMS. The highest m_aG_m and m_aL_a values were found in WW and PAWS with RSMS significantly lower. The ash contents of the flag leaf at anthesis and maturity and grain were significantly lower in RSMS compared with PAWS and WW (Table 4). Similarly, flag leaf Δ at anthesis and grain Δ were lower under RSMS than under PAWS and WW (Table 4). Days to flowering ranged between 50–65, 60–75 and 65–80 under RSMS, PAWS and WW, respectively (data not shown).

TABLE 4. AGRONOMIC AND PHYSIOLOGICAL TRAITS OF WHEAT UNDER THREE WATER REGIMES IN THE 2004–2005 SEASON

Source of	Yield (t ha^{-1})		HI	Ash content (%) ‡					Δ (‰) ¶		CTD (^{o}C) #	
variation †	Grain	Biomass	(%)	m_aL_p	m_aL_b	m_aL_a	m_aL_m	m_aG_m	ΔL_a	ΔG_m	CTD_a	CTD_g
RSMS	2.34 a	6.59b	35.7a	16.07a	9.35a	9.77b	11.03b	1.41b	18.21b	15.81b	3.63	1.81
	± 0.20	± 0.45	± 2.3	± 0.86	± 1.27	± 1.29	± 1.44	± 0.19	± 0.30	± 0.35	± 0.59	± 0.51
PAWS	3.65 b	11.33a	32.4b	15.42a	10.34a	12.31a	12.74a	1.58a	20.09a	17.14a	2.83	5.49
	± 0.46	± 1.54	± 2.6	± 0.73	± 1.46	± 0.87	± 1.00	± 0.14	± 0.50	± 0.30	± 0.57	± 0.51
WW	4.58 c	12.87a	35.7a	12.54b	9.39a	12.58a	13.26a	1.73a	20.08a	16.56a	4.72	4.20
	± 0.72	± 1.21	± 4.8	± 0.73	± 1.53	± 0.86	± 1.26	± 0.14	± 0.28	± 0.44	± 0.51	± 0.66
F value	97***	160***	6.1**	116***	3.13ns	46***	17.6**	19.2***	168***	66 ***	57.6***	219***

†RSMS, residual soil moisture stress; PAWS, post anthesis water stress; WW, well watered
‡Ash content in plantlet (m_aL_p), flag leaf at boot stage (m_aL_b), at anthesis (m_aL_a) and at maturity (m_aL_m), and grain at maturity (m_aG_m)
¶ $\Delta^{13}C$ in flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m); values from pooled samples, analyzed at UAS Banglore; values from individual plots, analysed at IAEA Seibersdorf
#CTD, canopy temperature depression at anthesis (CTD_a) and grain filling (CTD_g)
±denotes standard deviation of the mean; mean values within a column followed by a common letter are not significantly different (P<0.05, Duncan test); **,***, significant at P<0.01 and 0.001, respectively; ns = not significant

3.1.3. 2005–2006 season

Genotypes differed significantly only in biomass, harvest index and ash content at the booting stage (Table 5). However all agronomic and physiological parameters differed significantly between water regimes, and the only non-significant G × T interaction was for biomass (Table 5).

TABLE 5. MEAN SQUARES OF COMBINED ANOVA FOR AGRONOMIC AND PHYSIOLOGICAL TRAITS OF WHEAT IN THE 2005–2006 SEASON

Source of	df	Grain	Biomass	HI	Ash content †				CID ‡	
variation		yield			m_aL_p	m_aL_b	m_aL_a	m_aG_m	ΔL_a	ΔG_m
Genotype (G)	19	10.1ns	37***	2.96***	4.5ns	1.85**	1.07 ns	0.05ns	7.8ns	3.8ns
Treatments (T)	2	217***	1902***	11.3***	928***	119***	168***	0.98***	18.0***	15***
G × T	38	7.6***	24ns	1.47**	5.4***	1.07***	1.25***	0.35***	7.8***	3.6***

†Ash content in plantlet (m_aL_p) and flag leaf at boot (m_aL_b) and anthesis (m_aL_a), and grain at maturity (m_aG_m).
‡ $\Delta^{13}C$ in flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m).
*, **, ***, significant at P<0.05, 0.01 and 0.001, respectively; ns = not significant.

Grain yield was significantly higher under WW than under PAWS and RSMS, while the opposite was true for harvest index (Table 6). Biomass showed a progressive and significant decline from WW to PAWS to RSMS. Leaf ash content between WW and PAWS was only significantly different at the boot stage, and the ash content of grain did not differ between water regimes. ΔL_a and ΔG_m under RSMS were always significantly less than under WW (Table 6). CTD differed among water regimes, but showed no consistent trend between boot and anthesis.

TABLE 6. AGRONOMIC AND PHYSIOLOGICAL TRAITS OF WHEAT UNDER THREE WATER REGIMES IN THE 2005–2006 SEASON

Source of variation †	Yield (t ha^{-1})		HI (%)	Ash content (%) ‡				CID (‰) ¶		CTD (^{o}C) #	
	Grain	Biomass		m_aL_p	m_aL_b	m_aL_a	m_aG_m	ΔL_a	ΔG_m	CTD_b	CTD_a
RSMS	3.37b	9.52c	35.6a	15.85a	9.82b	8.83b	1.31a	17.70b	16.18a	1.70c	2.88b
	± 0.45	± 0.95	± 2.8	± 0.86	± 0.68	± 0.66	± 0.07	± 0.28	± 0.35	± 0.83	± 0.83
PAWS	3.67b	11.61b	36.8a	15.43a	10.68b	11.19a	1.52a	18.84a	16.52a	2.67b	4.48a
	± 0.58	± 1.06	± 2.0	± 2.81	± 0.61	± 0.76	± 0.16	± 0.35	± 0.36	± 1.70	± 1.17
WW	4.59a	12.79a	30.5b	15.14a	12.57a	12.06a			17.59b	4.23a	2.82b
	± 0.48	± 0.88	± 1.8	± 1.34	± 0.70	± 0.51			± 0.28	± 1.24	± 0.99
F value	3.1***	6.7***	21***	183***	89***	141***	25***	70***	46***	19***	17***

†RSMS, residual soil moisture stress; PAWS, post-anthesis water stress; WW, well watered

‡Ash content in plantlet (m_aL_p), flag leaf at boot stage (m_aL_b) and at anthesis (m_aL_a), and grain at maturity (m_aG_m)

¶CID, carbon isotope discrimination (Δ^3C) in flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m); values from pooled samples, analyzed at UAS Banglore; values from individual plots, analysed at IAEA Seibersdorf

CTD, canopy temperature depression at boot (CTD_b) and at anthesis (CTD_a)

±denotes standard deviation of the mean; mean values within a column followed by the same letter are not significantly different (P<0.05, Duncan test); ***, significant at P<0.001

3.2. Relationships among agronomic and physiological traits

3.2.1. 2004-2005 and 2005-2006 seasons

The strongest positive correlations for both seasons and all water regimes were between yield and biomass and between yield and harvest index (Table 7). Strong negative correlations were observed between yield and m_aG_m for each water regime only in 2004–2005, but the relationships were significant across seasons under WW. Correlations between yield and ash content of plantlets and the flag leaves at different growth stages were inconsistent across years and water regimes. Yield was significantly and positively correlated with and ΔL_a and ΔG_m for each water regime only in 2005–06, but the relationships were significant under WW across seasons (Table 7).

Yield was significantly and negatively correlated with phenology under WW in 2004–2005 but not in 2005–06, and there were no significant relationships between yield and phenology under RSMS in both seasons (Table 8). Correlations between yield and 1000 grain weight and plant height were inconsistent between seasons and water regimes in terms of level of significance and slope (Table 8). Relative water content was not significantly correlated with yield. Several significant and negative correlations were observed between yield and canopy temperature depression, but the relationships were significant only under PAWS across seasons (Table 8).

TABLE 7. CORRELATIONS BETWEEN YIELD AND AGRONOMIC AND PHYSIOLOGICAL TRAITS OF WHEAT UNDER THREE WATER REGIMES OVER TWO SEASONS

Season	Water regime†	Biomass	HI	Ash content ‡					CID ¶	
				m_aL_p	m_aL_b	m_aL_a	m_aL_m	m_aG_m	ΔL_a	ΔG_m
2004	RSMS	0.445***	0.748***	0.090	0.182	0.235	0.210	–0.240***	0.229	0.067
–05	PAWS	0.764***	0.320**	–0.031	–0.160	0.256*	0.038	–0.428***	–0.201	0.268*
	WW	0.492***	0.772***	0.321**	–0.072	0.082	0.295*	–0.348**	–0.348**	0.352**
2005	RSMS	0.788***	0.530***	–0.179	0.129	0.149	–	–0.166	0.447***	0.568***
–06	PAWS	0.658***	0.836***	0.337**	0.448***	0.188	–	0.004	0.353**	0.435***
	WW	0.646***	0.510***	0.192	–0.178	–0.078	–	–0.250*	0.310*	0.549***

†RSMS, residual soil moisture stress; PAWS, post-anthesis water stress; WW, well watered.
‡Ash content in plantlet (m_aL_p), flag leaf at boot stage (m_aL_b), at anthesis (m_aL_a) and at maturity (m_aL_m,) and grain at maturity (m_aG_m).
¶CID, carbon isotope discrimination ($\Delta^{13}C$) in flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m); values from individual plots analysed at IAEA Seibersdorf.
*, **, ***, significant at P<0.05, 0.01 and 0.001, respectively.

TABLE 8. CORRELATIONS BETWEEN YIELD, PHENOLOGY AND PHYSIOLOGICAL PARAMETERS UNDER THREE WATER REGIMES OVER TWO SEASONS

Season	Water regime†	Days to flowering	Days to maturity	1000 grain weight	Plant height	RWC ‡	CTD_a #	CTD_g #
2004	RSMS	–0.147	0.063	–0.173	–0.052	–0.033	–0.097	0.028
–05	PAWS	0.176	0.373**	0.195	0.342**	0.011	–0.470***	–0.434***
	WW	–0.379**	–0.396**	0.260*	–0.196	0.185	–0.006	–0.072
2005	RSMS	–0.001	0.132	0.184	0.352**	–0.212	–0.539***	–0.066
–06	PAWS	–0.409**	–0.248	–0.239	0.383**	–0.044	–0.352**	–0.367**
	WW	0.086	0.124	0.185	0.031	–0.008	–0.238	–0.256*

†RSMS, residual soil moisture stress; PAWS, post-anthesis water stress; WW, well watered.
‡ RWC, relative water content.
#Canopy temperature depression at anthesis (CTD_a) and at grain filling (CTD_g).
*, **, ***, significant at P<0.05, 0.01 and 0.001, respectively.

Significant positive correlations were found between ΔG_m and ΔL_a for both seasons and all water regimes, with the only exception being PAWS in 2004–2005 (Table 9). Relationships between ΔG_m or ΔL_a with CTD were inconsistent, being either positive or negative and significant or non-significant across water regimes and seasons (Table 9). ΔL_a was significantly and positively correlated with m_aL_m under all water regimes in 2004–2005 (Table 9), but correlations between ΔG_m or ΔL_a and ash content of the flag leaf at other stages or the mature grain were inconsistent across seasons and water regimes (Table 9). ΔG_m and ΔL_a were consistently and significantly correlated with m_aL_a only under RSMS.

3.2.2. Parents and F_5 progenies

Very few significant correlations were observed under PAWS and WW regimes between yield, Δ and ash content (Table 10) for a set of semi-dwarf parents and 25 F_5 progenies derived from MACS 3125 (high yield, high Δ) × PDW 233 (low yield, medium-high Δ). Yield was only correlated with ΔG_m and m_aL_a under PAWS, and with m_aG_m under WW in the season. No significant correlations were found in the following 2005–06 season (data not shown).

TABLE 9. CORRELATIONS BETWEEN Δ AND PHYSIOLOGICAL TRAITS UNDER THREE WATER REGIMES OVER TWO SEASONS

Season	Water regime†	CID ‡	Ash content¶					CTD_a#	ΔL_a
			m_aL_p	m_aL_b	m_aL_a	m_aL_m	m_aG_m		
2004	RSMS	ΔG_m	–0.140	0.263*	0.386**	0.187	–0.187	–0.334**	0.386**
–05	PAWS		–0.089	0.213	0.194	–0.133	–0.434***	0.334**	–0.022
	WW		0.244	–0.020	0.157	0.450***	–0.250*	0.252*	0.387**
	RSMS	ΔL_a	0.103	0.161	0.303*	0.475***	–0.024	–0.357**	–
	PAWS		0.219	–0.079	0.206	0.291*	0.167	0.009	–
	WW		0.289*	0.096	0.188	0.310*	–0.092	–0.379**	–
2005	RSMS	ΔG_m	–0.244	0.243	0.437***	–	–0.015	–0.118	0.499***
–06	PAWS		0.278*	0.220	0.240	–	0.112	–0.326**	0.624***
	WW		–0.048	–0.112	0.003	–	–0.149	–0.379**	0.461***
	RSMS	ΔL_a	0.035	0.377**	0.311*	–	0.099	–0.024	–
	PAWS		0.364**	0.245*	0.282*	–	–0.053	–0.262*	–
	WW		–0.062	–0.040	–0.021	–	–0.087	–0.171	–

† RSMS, residual soil moisture stress; PAWS, post-anthesis water stress; WW, well watered.
‡ Carbon isotope discrimination ($\Delta^{13}C$) in flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m).
¶ Ash content in plantlet (m_aL_p), flag leaf at boot stage (m_aL_b), at anthesis (m_aL_a) and at maturity (m_aL_m), and grain at maturity (m_aG_m).
# Canopy temperature depression at anthesis (CTD_a).
*, **, ***, significant at $P<0.05$, 0.01 and 0.001, respectively.

TABLE 10. CORRELATIONS BETWEEN YIELD, $\Delta^{13}C$ AND ASH CONTENT IN PARENTS AND F5 GENERATION UNDER TWO WATER REGIMES IN 2004–2005

Water regime †	$\Delta^{13}C$ ‡		Ash content ¶	
	ΔL_a	ΔG_m	m_aL_a	m_aG_m
PAWS	0.394	0.425*	0.627**	0.313
WW	0.179	0.209	0.114	0.558**

†PAWS, post-anthesis water stress; WW, well watered.
‡In flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m).
¶ In flag leaf at anthesis (m_aL_a) and and grain at maturity (m_aG_m).
*, **, significant at $P<0.05$and $P<0.01$, respectively.

3.3. Quantitative trait loci in the RIL population

During the 2005–06 season, a major quantitative trait locus (QTL) responsible for around 15% of the variation in Δ was detected on chromosome 4B with a significantly high LOD score of 4.2.

During the 2006–2007 season, the same population was grown under two water regimes, limited irrigation and full irrigation. Under limited irrigation, a major QTL explaining 13% of the variation in CTD at LOD score 4.2 was identified on chromosome 2A, while under full irrigation, another QTL, accounting for 18% of the variation in CTD at LOD score 3.8, was identified on chromosome 6A. For ash content, a major QTL explaining 13% of the variation at LOD score 3.4 was detected on chromosome 5A under limited irrigation, whereas another QTL, explaining 12% of the variation at LOD score 4.2, was detected on chromosome 6A under full irrigation.

3.4. Estimation of ash content by Near Infrared Transmittance (NIT)

The ash contents of 103 wheat grain samples determined by the reference method were within the range of 1.2 – 2.3% on a dry weight basis, and were between 1.22 and 1.99% when estimated by NIT, the latter having a mean value of 1.495 and a standard deviation of 0.187. The relationship between the two methods was linear with a slope of 1.000 and an r^2 value of 0.961.

4. DISCUSSION

4.1. The use of $\Delta^{13}C$ to predict wheat yield under different water regimes

4.1.1. 2003–2004 season

Under the PAWS regime, conditions similar to the wheat mega-environment ME4A [21] or Mediterranean-type mega-environment, grain yield was found to be positively correlated with ΔG_m. Significant correlation was also found between ΔG_m and yield under limited irrigation in Mexico [22] and China (Xu, unpublished). Conversely, under RSMS the magnitude of the correlation was lower than under PAWS and was found to depend highly on the quantity of water stored in the soil at sowing, and on the stress intensity [22]. Ash content in the flag leaf at anthesis may be the best selection criterion in environments where Δ does not show any correlation with yield. CTD may also be used in selection under limited water stress conditions. The results highlighted the potential use of Δ and ash content as indirect selection criteria for wheat yield under the conditions of the Peninsular Zone of India [20].

4.1.2. 2004–2005 and 2005–06 seasons

The highest grain Δ was observed under WW and the lowest under RSMS during both 2004–2005 (Table 4) and 2005–2006 (Table 6) seasons which may be due to progressive stomatal closure from WW to RSMS due to the decreasing soil moisture under the prevailing higher temperatures during grain filling. Higher biomass accumulation during the favourable early environment and remobilization of soluble carbohydrate to the grain may have also contributed. A positive correlation between grain yield and biomass resulted in a positive correlation of grain yield with harvest index across the seasons and water regimes (Table 7). The higher biomass at anthesis resulted in more reserves, and hence a larger amount of stored assimilate can be translocated to the grain [23]. ΔL_a was equal under PAWS and WW and significantly higher than RSMS during both seasons.

4.2. Effect of water regime on grain yield and ash content

The highest m_aG_m and m_aL_a values were found under WW and PAWS regimes and the lowest in the RSMS treatment, indicating that rate of transpiration decreased in this order, reflecting the reduction in water availability. Yield in WW was also highest in both seasons suggesting that high yield was associated with lower transpiration efficiency. For m_aL_p, the highest values were recorded for RSMS followed by PAWS and WW suggesting a higher transpiration under RSMS at the plantlet stage. This could be due to the fact that growth reduction is a more immediate response to water stress than stomatal closure and reduction of transpiration**.** These results are in conformity with our experiment conducted during the 2003–2004 season [20].

4.3. Effect of water regime on relationships between grain yield, ash content and Δ

4.3.1. Residual soil moisture stress

Under RSMS higher values of Δ were recorded in leaves than in grain during both seasons. A non significant positive relationship was found in RSMS between grain yield and ΔL_a during 2004–05 while positive correlations were observed between grain yield and both ΔL_a and ΔG_m in 2005–2006. High-Δ genotypes tend to grow faster than low Δ genotypes [23]. By covering the ground more quickly, they are more successful in reducing soil evaporation. Having higher biomass at anthesis and more reserves, they consequently are able to translocate a larger amount of stored assimilate to fill the grain. High-Δ may also reflect higher stomatal conductance, particularly after anthesis, when soil moisture decreased and the stress became stronger due to an increase in temperature, resulting in minimal reduction in stomatal conductance and photosynthetic efficiency.

A lack of significant correlation between grain yield and m_aL_p, m_aL_b, m_aL_a and m_aL_m under RSMS was observed during both seasons. All genotypes under study were semi-dwarf varieties suggesting that differences in transpiration rate among genotypes became less marked and poorly explained in final yield differences. Significant correlations between ΔG_m and ΔL_a and between ΔG_m and m_aL_a during both 2004–2005 and 2005–2006 seasons suggest that m_aL_a may be used as a surrogate for ΔG_m where a significant correlation between grain yield and ΔG_m is not found.

4.3.2. Post–anthesis water stress

Correlation observed between grain yield and ΔG_m in both 2004–2005 and 2005–2006 seasons and between yield and ΔL_a in the 2005–2006 season in the hot environment of Peninsular India are consistent with results obtained under ME4A and Mediterranean conditions [24, 25]. Different hypothesis can explain the relationship between ΔG_m and grain yield under terminal drought conditions. Firstly, high ΔG_m and grain yield could reflect high Δ values at an early stage of growth. In this case high Δ may reflect a faster growth rate through crop development [23]. Secondly, high grain Δ could characterize genotypes with high leaf Δ that are more dependent on pre-anthesis vegetative reserves for grain filling. Under post-anthesis water stress, photosynthesis is generally more affected than translocation and plants mainly use assimilates from pre-anthesis reserves for grain filling [26]. The contribution of pre anthesis assimilates to grain filling may vary from 10 to 80% of final grain C [27]. Grain Δ is influenced by the proportion of reserves used for grain filling. It may be higher if a large proportion of starch originates from stem reserves that were accumulated when plants were less stressed and had high Δ values.

Canopy temperature depends on the quantity of water transpired by the leaves, being an integrative measure of a group of mechanism that range from radical absorption of water to the stomatal control of transpiration. When stomata close because of reduced water status, leaf temperature rises above ambient air temperature [28]. Under drought stress, those genotypes that present smaller canopy temperatures will use more available water in the soil, thus limiting the negative effect of water stress on grain yield [29].

Significant positive correlations were found between grain yield and m_aL_a during 2004–2005 and between grain yield and both m_aL_p and m_aL_b in 2005–2006. Negative correlations between grain yield and both m_aG_m and CTD_a were found during 2004–2005. Significant correlations between ΔG_m and ΔL_a were found during both seasons under all environments (except PAWS 2004–2005). Similarly, the positive correlation only under

RSMS was observed between ΔG_m and m_aL_a and the negative correlation between grain yield and both CTD_a and CTD_g (Table 7) during both seasons under PAWS, suggests that m_aL_a, CTD_a and CTD_g may be used, depending upon their correlation with Δ, as surrogates in a breeding programme under this environment.

4.3.3. Well watered

There is very little information concerning the relationship between grain yield and Δ under a favorable or irrigated environment. Positive correlations between yield and both ΔG_m and ΔL_a during both the seasons indicated that higher stomatal conductance made a significant contribution under this regime. Significant negative correlations between grain yield and m_aG_m were observed during both seasons under this regime. The wheat crop in this zone is continuously under heat stress after anthesis with about 120 days to maturity and the highest productivity per day in India.

Grain yield in irrigated cereals was found to be positively correlated with Δ in the peduncle [30] and grain [24, 31]. Our results indicated the usefulness of ΔG_m and ΔL_a for selecting high yielding genotypes under RSMS, PAWS and WW environments. Correlations between yield and m_aG_m were negative during both the years of the study, indicating that m_aG_m may be used as a criterion for selection for high yield. CTD_g showed a significant correlation with grain yield under WW only during the 2005–2006 season. CTD_a did not show a significant correlation with yield during either season, indicating that the ample availability of soil moisture under WW diluted the canopy temperature difference between genotypes, which makes this trait of no use for a breeding programme under such conditions.

4.4. Relationship between yield, Δ and ash content in segregating populations

The lack of consistent relationships between yield and ΔL_a, ΔG_m, m_aL_a and m_aG_m across years within PAWS and WW regimes provided no additional information compared with results obtained with the 20 semi-dwarf durum wheats (Table 7).

4.5. Near infrared transmittance

A wide range in ash concentrations in a large number of samples (minimum of 30–40) need to be analyzed in order to achieve a highly precise calibration of NIT against the standard procedure. NIT promises to be very useful analytical tool for analyzing large numbers of samples.

5. CONCLUSIONS

The results of the present study suggest that the highest wheat yields in the hot conditions of the Peninsular Zone of India can be attained by cultivars with high biomass production, high harvest index and rapid growth rates. Leaf-Δ at anthesis, grain Δ and grain ash content under WW, and CTD under PAWS, were consistent predictors of yield across seasons. There were no yield-predictors for wheat under RSMS across seasons. Analysis of ash content by precisely-calibrated NIT may be useful in breeding programmes due to its rapidity and low cost. The QTLs and their flanking markers identified in the present study for Δ, CTD and ash content may be used for tracking their flow in breeding material, which may lead to a better selection strategy for physiological traits associated with grain yield in durum wheat under water stress.

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GENETIC ASSOCIATION BETWEEN GRAIN YIELD OF BREAD WHEAT AND CARBON ISOTOPE DISCRIMINATION UNDER DROUGHT IN MOROCCO

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Abstract

The capacity of the wheat plant to discriminate against the carbon-13 isotope during photosynthesis, in favor of the lighter carbon-12 isotope, has been found to correlate with grain yield in many studies. This hypothesis was tested under four contrasting environments in Morocco during 2003–2006 using three sets of elite wheat genotypes. Significant positive correlations were found between carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) and grain yield, and were higher under more water stressed environments at Douyet and Jemaa Sahim, indicating that high Δ is associated with high yield under drought stress. Selection based on yield appeared to have indirectly acted on Δ as well, since the correlation was significant and remained relatively unchanged across years of selection. This indicates a possible genetic linkage between yield and Δ. Also, transgressive segregation occurred for most agronomic and physiological traits, including Δ, offering a potential genetic variation for selection. At the molecular level, CID was found to be linked to a molecular marker at the gwm095 region of chromosome 2A, and to a thousand kernel weight marker at the gwm071 region, distant by 20-30 cent Morgan (cM). A poor QTL associated with grain yield was detected at chromosome 5A, similar to the one detected at CIMMYT in Obregon (Mexico) and northern Australia. From these results, it can be concluded that CID may be used as a new selection criterion to enhance wheat grain yield potential under drought in Morocco.

1. INTRODUCTION

Early pot studies [1, 2] indicated the existence of a negative relationship between CID and transpiration efficiency (ratio of dry matter produced per unit of water transpired). Highly efficient genotypes are expected to discriminate less against ^{13}C, and thus should have low $\Delta^{13}C$. However, this relationship was not found to be consistent in field experiments. Phenotypic correlations between Δ and grain yield of wheat under different environments have been repeatedly reported [3, 4, 5, 6]. The negative relationship held as long as plants were sampled for Δ during early growth stages, mainly before anthesis [7]. Later samplings may lead to contradictory results. In Mediterranean type environments, where post-anthesis water is limited, high transpiration efficiency may not be associated with high yield. Reproductive rather than vegetative organs needed more water at grain filling, with higher efficiency of carbon partitioning to the kernel leading to higher grain yield. Consequently, it can be hypothesised that higher Δ should be associated with higher yield. This hypothesis was confirmed in Spain, where a significant positive correlation was found between yield and Δ when the data from all genotypes were pooled [6]. However, the correlation was affected by height, being positive for the old and intermediate cultivars, and negative for the modern ones, while unstable correlations were obtained in Algeria [4], ranging from negative to positive.

Nevertheless, whatever the relationship between Δ and yield is in any target environment, selection based on Δ may lead to improved yield under water stressed conditions. Long term selection for higher grain yield of wheat in Spain and Italy resulted in a simultaneous increase in yield by 51% and Δ by 6% [6]. Yield improvement in tall cultivars relied on early heading and long grain filling duration, while that of semi-dwarf cultivars relied on low Δ and increased height. In contrast, modern semi-dwarf cultivars tended to have high $\Delta^{13}C$ values compared to the old and tall local ones in Algeria [4]. The authors hypothesised that harvest index may play a role in a cultivar's ranking under water stress. Water input during grain filling was the variable most strongly correlated with grain Δ and grain yield across all environments in the Iberian peninsular [8]. It appears that under Mediterranean environments, the relationship between Δ and grain yield is still unstable, probably needing a more precise definition of the target environment [4].

Heritability studies have been carried out in order to verify the usefulness of this tool in selection [7]. High heritability values for $\Delta^{13}C$ under field conditions were commonly reported, as high as those for grain yield. However, the importance of sampling time for Δ measurements was stressed [7]. Heritability tended to be greater for plant material sampled early (before or during stem elongation) and lower for dry matter laid down near anthesis. In practice, Δ is commonly measured on mature grain.

The objective of the present study was to assess the relationship between grain yield and grain Δ for a set of elite wheat lines under contrasting environments in Morocco, and to explore the possibility of a genetic linkage between the two traits.

2. MATERIALS AND METHODS

2.1. Plant material

Three sets of 20 elite genotypes of bread wheat and 4 checks were evaluated between 2003 and 2006 for yield under different levels of naturally occurring drought. The elite lines underwent selection under varying stress conditions (4 to 6 sites over 4 years). Each set was tested for two years. Meanwhile, an existing double haploid population from Australia (Berkut × Krichauff), maintained and distributed by CIMMYT, consisting of 140 F_2 sister

lines and two parents, were evaluated under water stress in the field at Fes-Douyet for the 2007–2008 season.

2.2. Experimental

Trials were conducted at four experimental stations (Table 1). Jemaa Sahim is located in a semi-arid region of less than 350 mm of annual rainfall. Several major biotic and abiotic stresses occur, including Hessian fly, drought, terminal heat, leaf rust, and Septoria. Hessian fly is present yearly at this site at a potentially devastating level, and crop failures may result unless resistant cultivars are planted early to limit damage. Drought is the second next most important stress, followed by heat. Leaf rust is present almost every season, but damage on susceptible cultivars increases with increased moisture. *Septoria* occurs during moist seasons, which are not frequent. Fès-Douyet is a site located in the sub-humid area, between two chains of mountain, and is subject to the Sirocco, a dry and hot wind coming from the East. Stripe and leaf rusts are very common at this site, followed by *Septoria* in wet seasons, drought associated with heat, and Hessian fly in late sowings. Marchouch is a site located in the sub-humid region of about 400 mm of annual rainfall where *Septoria* and leaf rust are common, followed by Hessian fly, drought and heat. Tassaout is located in an arid region where wheat cannot be grown without irrigation. Heat is common, as well as stripe rust because of its proximity to the mountains, and leaf rust which is enhanced by moisture. Hessian fly can cause some damage to late sowings. Risk of drought is always present, as shortage of irrigation water is common, since it is distributed according to a schedule controlled by the irrigation service.

TABLE 1. SITES, LOCATION, AVERAGE ANNUAL RAINFALL AND SURFACE SOIL TEXTURE

Site	Latitude (^{o}N)	Longitude (^{o}W)	Rainfall (mm)	Soil texture
Fes-Douyet	30.04	–5.11	550	Clay, calcareous
Marchouch	33.56	–6.69	450	Clay
Jemaa Sahim	32.35	–8.83	350	Clay loam
Tassaout	31.83	–7.51	Irrigated	Loamy sand

The experiments were a Randomized Block Design with three replications and cultivars as treatments. The seeding rate was 15 g seed m^{-2}. Plots consisted of six rows, 5 m long and 30 cm apart. Grain was harvested at maturity, dried at 70^{o}C and weighed. Grain samples from different replicates were thoroughly mixed in equal weights and finely ground for $\delta^{13}C$ analyses.

2.3. Analytical

Carbon isotope composition ($\delta^{13}C$) was determined at CNESTEN on a sample containing 1 mg N using an isotope ratio mass spectrometer.

where $\delta\,^{13}C$ (‰) = $[(R_{sample}/R_{reference}) - 1] \times 1000$, and R = $^{13}C/^{12}C$ ratio

Δ was calculated according to the formula of Farquhar et al. [9]:

Δ (‰) = $[(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000$

where $\delta_p = \delta^{13}C$ of the sample and $\delta_a = \delta^{13}C$ of atmospheric CO_2, which has a deviation of approximately –8‰ measured against the PDB (Pee Dee Belemnite) International Primary Standard [9].

3. RESULTS AND DISCUSSION

3.1. Relationship between yield and $\Delta^{13}C$

Data on grain yield and grain $\Delta^{13}C$ were available for three sets of trials conducted at three different sites during four seasons. When yield was plotted against $\Delta^{13}C$, regardless of seasons, sites or trials, a positive correlation of 0.71** (P<0.01) was obtained (Fig. 1), indicating that for Moroccan environments the high yielding genotypes were those that had high $\Delta^{13}C$. Positive correlations were also obtained in Spain, but only for tall cultivars, and correlations were negative for semi-dwarf cultivars [6]. All cultivars used in these sets were semi-dwarf and yet the correlation was positive, contradicting the Spanish results. However, results from Algeria indicated that modern cultivars which are generally semi-dwarf showed high yields and high $\Delta^{13}C$, corroborating our results [4]. Positive correlations were also obtained in the limited irrigation areas of Northwest China [10], and under most water regimes in the peninsular zone of India [11].

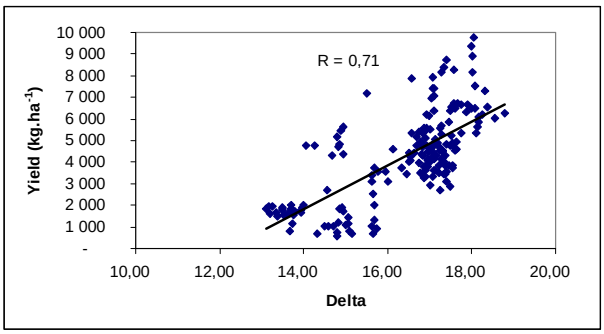

FIG. 1. Relationship between bread wheat yield and $\Delta^{13}C$ (‰) across all trials and sets of genotypes between 2003 and 2006.

The average $\Delta^{13}C$ and yield of each trial site across seasons was used as an environmental index to eliminate genotypic effects. A similar correlation coefficient was obtained (0.71**) between these averages, indicating that the higher the $\Delta^{13}C$ the higher the yield. Therefore, similar to genotypes, environments which produce high yields tend to exhibit high Δ as well. Average $\Delta^{13}C$ ranged from 13.62‰ in a low yielding dry site (Fes-Douyet) to 17.81‰ in a high yielding irrigated site (Tassaout). Since the elite lines used in this study have been selected for high and stable yield in these environments over the years, the similar correlation between Δ and yield for sets of genotypes and sets of environments indicates high environment effects on the genotype. It also indicates that the association between the two traits is genetic because direct selection of genotypes for high yield resulted in high Δ. This is a first indication that Δ and yield are genetically linked.

When computed for each environment, the significant correlations ranged from –0.45* to +0.78** (Table 2). The negative correlation was obtained in an environment with almost no stress during the season (Marchouch, 2003), consistent with our expectation based on early reports of the relationship between Δ and the yield-related trait of transpiration efficiency [1, 2]. In non-stressed environments transpiration efficiency appears to drive the relationship between Δ and grain yield. Hence, grain yield must have been determined by the extent of

biomass accumulation. However, such environments are not frequent, appearing once in 10 years [12].

In stressed environments like Jemaa Sahim and Fès-Douyet, correlations between Δ and grain yield were positive and highly significant (Table 2), implying selection for high Δ to improve yield under drought. In these environments, successful production of plump grain under extreme drought is more important than biomass production. The irrigated site, Tassaout, located in an arid environment where shortages of irrigation water and heat stress are frequent, behaved inconsistently, with mostly non-significant correlations between yield and Δ (Table 2). The overall results seem to indicate that high correlations were obtained under extreme situations, positive in dry environments and negative in favourable ones.

In most environments used in this study, drought occurs after anthesis. Based on this criterion, these environments can be classified as Mega-environment-4 (ME4) [13], although pre-anthesis drought can also occur quite frequently.

TABLE 2. AVERAGE CID AND YIELD OF WHEAT AND THEIR CORRELATION IN RELATION TO ENVIRONMENT

Environment †	Comment	$\Delta^{13}C$ (‰)	Yield (kg ha^{-1})	r ‡
03A1J	High stress	14.95	1307	0.71**
05A2F	High stress	13.62	1932	0.78**
06A2T	Irrigated with some stress	16.68	3595	0.20ns
03A1T	Irrigated with little stress	17.09	4432	–0.03ns
03A1M	No stress	17.08	4503	–0.45*
05A2T	Irrigated with stress	17.20	4736	0.43*
05A2M	Severe stress	14.69	4890	0.26ns
04A2T	Irrigated with little stress	17.81	6194	0.07ns
05A1T	Irrigated with stress	17.35	7582	0.37ns

†The environment is a combination of season, sets of elite lines and site; 03, 04, 05 and 06 denote the 2003, 2004, 2005 and 2006 cropping seasons, respectively; A1 and A2 denote different sets of elite lines; J, M, F and T denote Jemaa Sahom, Marchouch, Fes-Douyet and Tassaout sites, respectively.
‡ns, not significant; *, **, $P<0.05$, $P<0.01$, respectively.

When the correlations between Δ and yield of elite genotypes were plotted against the environmental index, represented by the average trial yield (Fig. 2) and the average trial $\Delta^{13}C$ (Fig. 3), a similar tendency was revealed, stressing once more the positive correlation between Δ and grain yield. The correlations change from highly positive values under stressed environments to highly negative values under non-stressed environments. The last four environments represent the arid zone site at Tassaout, irrigated, but often stressed by shortage of water or heat.

Similar results were obtained when average trial $\Delta^{13}C$ was used as an environmental index. Again the last four environments are from Tassaout, the irrigated arid site. These high correlations under severe stress are very encouraging, indicating that Δ can be used to select for drought tolerance.

Based on these results, sites where selection for Δ under drought can be applied include either Jemaa Sahim or Fès-Douyet. The Jemaa Sahim site is located in the semi-arid zone, with frequent droughts, while Fès-Douyet is located in the sub-humid zone with frequent drought and frequent desiccating winds (Sirocco or Chergui).

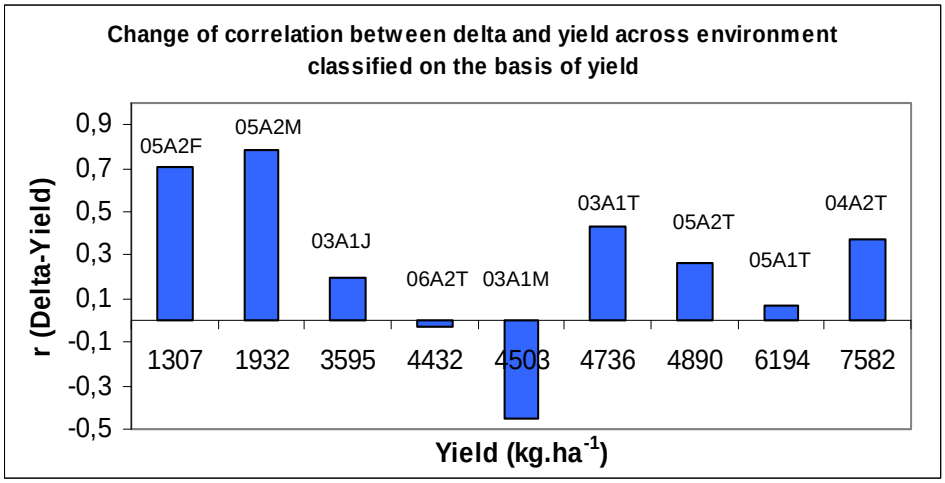

FIG. 2. Correlations between $\Delta^{13}C$ (‰) and yield (kg ha^{-1}) in ascending order of yield. For the meaning of trial labels see foot note in TABLE 2.

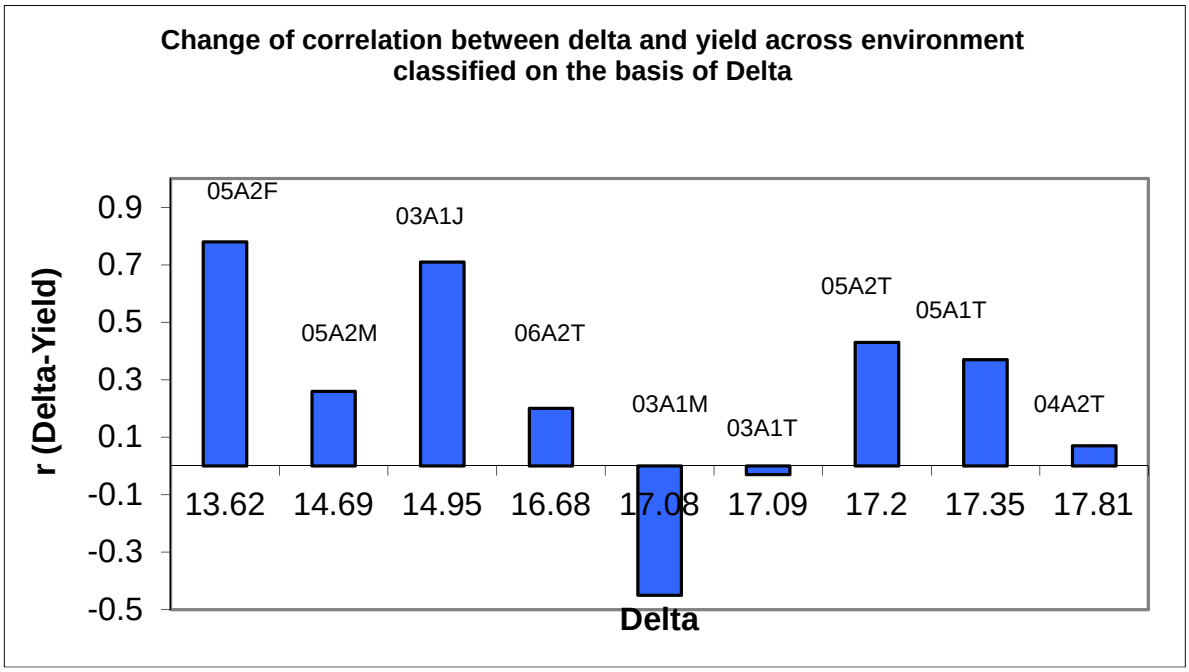

FIG. 3. Correlations between $\Delta^{13}C$ (‰) and yield (kg ha^{-1}) in ascending order of $\Delta^{13}C$. For the meaning of trial labels see foot note in TABLE 2.

3.2. Influence of selection on the relationship between yield and $\Delta^{13}C$

Elite Moroccan genotypes have undergone many cycles of selection under drought stress. Assuming that recent elite genotypes are more drought tolerant than antecedent ones, the availability of two sets of elite genotypes differing by two years of selection, offered the possibility to determine the effect of selection on the relationship between yield and $\Delta^{13}C$ (Fig. 4 and Fig. 5). The relationship between $\Delta^{13}C$ and yield remained relatively unchanged in the two sets of genetic material, despite two years of progress in genetic advancement. This may indicate that, while selecting for drought tolerance over generations, $\Delta^{13}C$ may have been indirectly selected for as well, indicating a possible genetic linkage between the two traits. However, the two sets of genetic material were available at the irrigated site where heat is frequent, in addition to periods of drought due to irregular delays in the release of dam water. This is an additional indication that $\Delta^{13}C$ and yield are genetically linked.

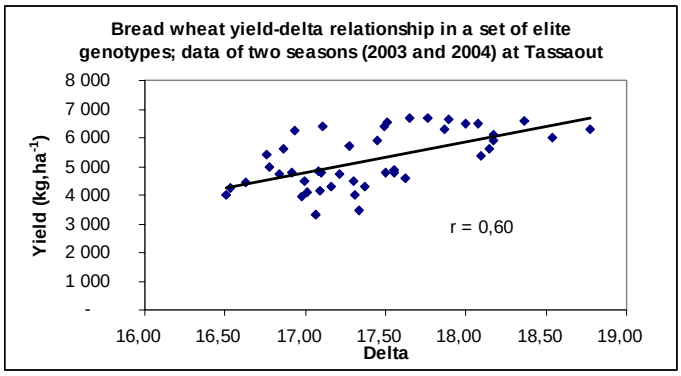

FIG. 4. Correlation between yield (kg ha^{-1}) and $\Delta^{13}C$ (‰) in an early set of elite genotypes of bread wheat at Tassaout irrigated site (two years' data pooled).

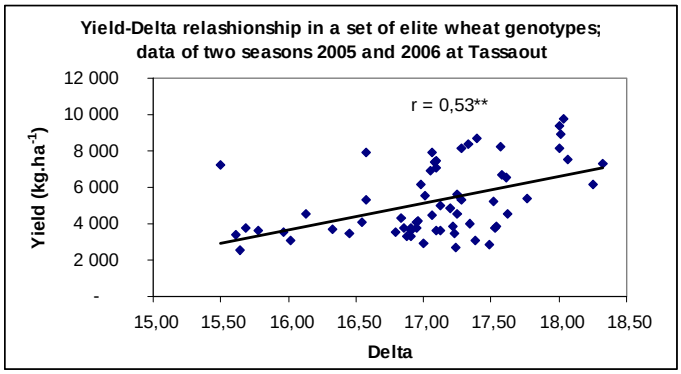

FIG. 5. Correlation between yield (kg ha^{-1}) and $\Delta^{13}C$ (‰) in a recent set of elite genotypes of bread wheat at Tassaout irrigated site (two years' data pooled).

3.3. Genetic segregation of $\Delta^{13}C$

The Berkut × Krichauff population was initially created for genetic analyses of drought tolerance. It involves two parents differing slightly in drought tolerance: Berkut is a CIMMYT line and Krichauff is Australian. The population was tested at Fès-Douyet during the 2007 cropping season which was particularly dry, with only 20% of the total rain (414 mm) falling during the crop cycle. Some phenotypic characteristics of this population are presented in Table 3. Height ranged from 31 to 72 cm, more than a two fold difference. Time to heading and maturity differed by 11 days from the earliest to the latest phenotype. Segregation in yield and its major components (number of grains m^{-2} and kernal weight) was very high, differences being 5, 20 and 90 fold for kernel weight, grain yield and grain number, respectively.

TABLE 3. PHENOTYPIC CHARACTERISTICS OF THE BERKUT X KRICHAUFF F_2 PROGENIES AND THEIR PARENTS

Parameter	Height (cm)	Heading (d)	Maturity (d)	Yield (kg ha^{-1})	Grains (m^{-2})	TKW † (g)	$\Delta^{13}C$ (‰)
Minimum	31	109	149	102	123	5.41	14.14
Mean	54	115	152	818	4785	14.75	14.90
Maximum	72	121	160	2175	10550	28.30	15.53
Berkut	53	118	152	313	2418	11.30	14.82
Krichauff	55	121	152	565	4190	12.80	14.72

†TKW, 1000 kernel weight.

A transgressive segregation took place for yield, with 74% of the population higher yielding than the best parent (Fig. 6). Δ also showed a transgressive segregation, with more than a half of the population (57%) above Δ levels of the parents (Fig. 7). Based on the phenotype, the two parents were similar with regard to yield and Δ. Based on the progenies' behaviour (genotype), they appear to be genetically different for the two traits because of segregation.

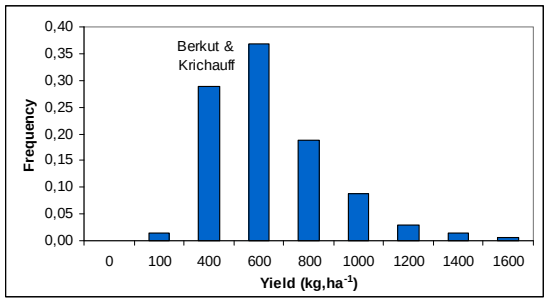

FIG. 6. Distribution of grain yield (kg ha^{-1}) in the Berkut × Krichauff F_2 segregating population.

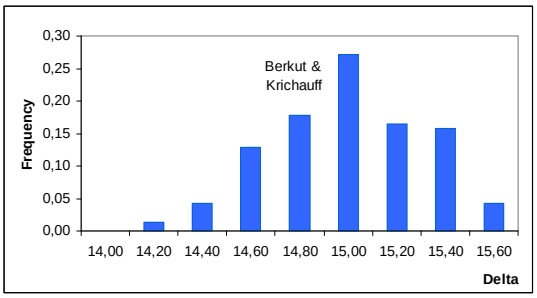

FIG. 7. Distribution of $\Delta^{13}C$ (‰) in the Berkut × Krichauff F_2 segregating population.

The correlation coefficient between Δ and yield in this segregating population was positive, with a value of 0.34**. The presence of a significant correlation despite segregation is another indication of a genetic linkage between Δ and grain yield. Total number of grains and kernel weight are the two major components that determined grain yield, with correlation coefficients of 0.89** and 0.61**, respectively (Table 4). Potential grain number is naturally set early in the season, before stem elongation. However, final grain number with plump seed, as well as kernel weight are determined during the grain filling period, which may endure more water deficit stress. Grain number and kernel weight were closely related with a correlation coefficient of 0.57**. Since yield was closely associated with grain number and kernel weight, similar correlation coefficients between these traits and Δ were obtained (Table 4).

TABLE 4. CORRELATION COEFFICIENTS AMONG YIELD, YIELD COMPONENTS AND $\Delta^{13}C$ IN THE BERKUT × KRICHAUFF F_2 PROGENIES TESTED AT FÈS-DOUYET DURING THE 2007 SEASON (N = 142)

Trait	Grain number	Kernel weight	Spike number	Tiller number
Grain yield	0.89**	0.61**	0.60**	0.26**
$\Delta^{13}C$	0.35**	0.31**	0.20**	–0.10ns
Grain number		0.57**	0.65**	0.25**
Kernel weight			0.34**	0.19*
Spike number				0.16ns

ns, not significant; *, **, $P<0.05$ and $P<0.01$, respectively.

3.4. Molecular markers associated with ^{13}C

Data on Δ of this population was sent to a genetic laboratory in Australia to look for a possible linkage with genetic markers. Given the transgressive segregation for most traits, markers were expected to be identified. At the molecular level, Δ was found to be linked to a molecular marker at the gwm095 region of chromosome 2A, and to a thousand kernel weight marker at the gwm071 region, distant by 20-30 cent Morgan (cM). A unit of cM corresponds to a 1% recombinant which diverges from the parents; high recombination means that the genes are distant. A poor QTL associated with grain yield was detected at chromosome 5A, similar to the one detected at CIMMYT in Obregon (Mexico) and northern Australia. Misra et al. (this IAEA-TECDOC) identified a major QTL marker closely linked to the dwarfing gene Rht-B1 on chromosome 4B in a durum cross. From these results, it can be concluded that $\Delta^{13}C$ may be used as a new selection criterion to enhance wheat grain yield potential under drought in Morocco and other parts of the world.

4. CONCLUSIONS

Significant relationships between Δ and yield under a Mediterranean environment were positive under severe water stress and negative under favourable conditions. Under drought, high Δ correlated with high yield, and in the absence of drought, low Δ correlated with high yield. Selection which was applied on yield appears to have acted indirectly on Δ as well, since the correlation was significant and remained relatively unchanged across selection pressure. This indicates a possible genetic linkage between yield and $\Delta^{13}C$. In an F_2 derived population, transgressive segregation occurred for most agronomic and physiological traits, including Δ, offering a potential genetic variation for selection. Hence the population might be suitable for studying the genetic relationship between yield and $\Delta^{13}C$ under drought. These correlations may offer the possibility of improving yield under drought through selection applied on the basis of Δ. The sites Jemaa Sahim and Fès-Douyet may be suitable for selection using this trait. At this moment, measurement of $\delta^{13}C$ is still expensive in Morocco. Molecular markers associated with this trait may offer a simple and reliable indirect selection criterion. Preliminary results indicate that such markers may be available. Further testing is needed for confirmation.

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SELECTION FOR GREATER AGRONOMIC WATER USE EFFICIENCY IN WHEAT USING CARBON ISOTOPE DISCRIMINATION IN ALGERIA

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Algeria

Abstract

A set of eight durum wheat genotypes was used to evaluate the relationship between carbon isotope discrimination (Δ) and grain yield under different rainfall regimes during the 2002-03 and 2003-04 seasons. The study included two old Algerian cultivars (Polonicum, Oued Zenati) and six advanced lines from the CIMMYT/ICARDA durum wheat breeding programme. The same set of genotypes was used in 2004-05 and 2005-06 to evaluate the influence of inter-annual climatic variation. These cultivars represent a wide range of agronomic characteristics. The Δ values allowed the selection of three parents to analyze the genetics of grain Δ: Waha and Mexicali (high Δ) and Oued Zenati (low Δ). In 2003-04, two crosses were made, Mexicali/Waha (high Δ/high Δ) and Oued Zenati/Mexicali (low Δ/high Δ). The parental cultivars and the F_2 and F_3 populations were then grown in 2004-05 and 2005-06 seasons. For the 2006-07 season, three categories of plants were chosen and sown from the F_4 population based on their similar heading date and plant height. Estimates of realized heritability were obtained, and the degree of correlation between Δ and grain yield was estimated. At the same time, two field experiments were carried out at Ain Abessa located in the north of the high plateau, and at Beni Fouda located in the east. Data generated by the water balance model 'Budget' were used to estimate the water use efficiency of the crops. The relative yield decline expected under specific levels of water stress at different growth stages was estimated using the FAO K_y approach.

1. INTRODUCTION

The high plateau of Algeria has a Mediterranean type climate and receives 250 to 500 mm rainfall, with about 70% occurring during the cold season from October to February. However, cereal crops suffer additional abiotic stresses such as winter-spring cold (due to altitude) and terminal heat (because of close proximity to the Saharan desert). Adoption of short cycle genotypes that can escape terminal drought stress was promoted, but in most years these genotypes were strongly affected by late frost. As a consequence, grain yield remains very low when compared to those obtained in the neighbouring regions or countries [1]. In addition, the inter-annual variation in yield is very high, due to variations in the amount and distribution of rainfall and the erratic occurrence and severity of frost and heat stress. The amount of rainfall received in spring can markedly affect crop yield [2].

The overall objective of this study was to investigate if carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) can assist in breeding wheat for drought tolerance under such conditions. Specific objectives included (i) to investigate the relationship between Δ and grain yield under contrasting climatic conditions with special reference to drought (ii) to utilize Δ for evaluating

different physiological components such as photosynthesis and translocation of assimilates and (iii) to identify lines varying in Δ in wheat breeding programmes in Algeria.

2. MATERIALS AND METHODS

2.1. Field experiments (2000-07)

2.1.1 Sites and durum wheat varieties

Field trials were established in 2000-01 and 2001-02 to evaluate a group of 10 CIMMYT lines (Table 2) in the field of the ITGC Sétif experimental station. Plant samples were analysed for $\delta^{13}C$ to identify lines presenting a large variation for Δ. Based on Δ analysis, six advanced lines from the CIMMYT/ICARDA durum wheat breeding programme (Sooty9/Rascon57, Dukem12/Rascon21, Altar, Kucuk, Mexicali, Waha) and two old Algerian cultivars (Polonicum, Oued Zenati) were selected (Table 3) and evaluated from 2002-03 to 2006-07 at the Sétif experimental station, at Kroub station in 2003-04 and at Ain Abessa and Beni Fouda in 2006-07 (Table 1). The soil type at Sétif is a Rendzin characterized by low organic matter content and shallow (0.6 m) depth. Surface soil pH is 8.2 and organic matter content is 13.5 g kg^{-1}.

TABLE 1. LOCATION AND CHARACTERISTICS OF THE FIELD EXPERIMENTS

Season	Site details				Seasonal rainfall (mm)	Cultivars/lines
	Location	Lat./Long.	Hasl† (m)	Soil		
2000-01	ITGC Station, Sétif	36° 9' N, 5° 21' W	1123	Rendzin; Mollisol, Calcixeroll (USDA)	217	10 CIMMYT lines (Table 2)
2001-02					162	
2002-03					526	2 local + 8 CIMMYT lines (Table 3)
2003-04					453	
	Khroub	36°25'N, 6°6'E	640	Alluvial clay	663	
2004-05	Sétif	As above for this location				
2005-06						
2006-07						
	Ain Abessa	36° 18'N, 5°18'E	1166	Steppic brown soil	397	
	Beni Fouda	36°10'N, 5°20'E	1180		356	

†Hasl, height above sea level.

TABLE 2. BRIEF DESCRIPTION OF TEN GENOTYPES GROWN IN SEASONS 2000-01 AND 2001-02 AT SÉTIF STATION

Cultivar No.	Name	Origin
1	Mexicali	CIMMYT cultivar, released in 1975
2	Sooty9/Rascon57	CIMMYT advanced line
3	Nacori	CIMMYT cultivar, released in 1997
4	Waha	CIMMYT/ICARDA line (Sham 1) released in Algeria in 1986
5	Tilo1/Lotus4	CIMMYT advanced line
6	Yavaros	CIMMYT cultivar, released in 1979
7	Altar	CIMMYT cultivar, released in 1984
8	Dukem12/Rascon21	CIMMYT advanced line
9	Kucuk	CIMMYT cultivar, released in 1984
10	Cado/Boomer33	CIMMYT advanced line

TABLE 3. BRIEF DESCRIPTION OF EIGHT GENOTYPES GROWN IN SEASON 2003-04 AT SÉTIF AND KHROUB STATIONS

Cultivar No.	Name	Origin
1	Mexicali	CIMMYT cultivar, released in 1975
2	Sooty9/Rascon57	CIMMYT advanced line
3	Waha	CIMMYT/ICARDA line (Sham 1) released in Algeria in 1986
4	Oued Zenati	Local variety
5	Altar	CIMMYT cultivar, released in 1984
6	Dukem12/Rascon21	CIMMYT advanced line
7	Kucuk	CIMMYT cultivar, released in 1984
8	Polonicum	Local variety

On the basis of Δ values obtained in these studies [3], three parents were chosen to analyze the genetics of Δ, Waha and Mexicali (high grain Δ) and Oued Zenati (low grain Δ). In 2003-04, two crosses were made, Mexicali/Waha (high Δ/high Δ) and Oued Zenati/Mexicali (low Δ/high Δ). The parental cultivars and the F_2 populations were grown in 2005-06. In 2005-06, the eight cultivars were sown on November 26 (D1) and on December 22 (D2), and the F_2 was sown on November 27.

2.1.2. Experimental conditions

The field experiments were conducted in a randomized complete block design with three replications. Plots were 10 m × 4 rows with 18-cm row spacing and an interplant spacing of 3 cm. Sowing density was 300 seeds m^{-2}. P (super phosphate at 100 kg ha^{-1}) and 100 kg ha^{-1} of potassium sulphate (48 %) were applied to all plots before sowing, while basal N (urea 150 kg ha^{-1}) was applied to all plots. Weeds were removed manually as and when required.

Weather data, monthly precipitation and monthly mean temperatures were obtained from an automatic weather station located at the experimental sites. Data recorded included grain yield, number of grains per spike, biomass, 1000-kernel weight (TKW). 20 mature spikes from the sets were randomly collected and threshed manually to obtain the number of grain per spike (NGS). Grain yield (GY) was determined from a 2.88 m^2 central area. Grain samples of the three parents and the F_2, F_3 and F_4 populations were analyzed for Δ.

2.2. Measurements

2.2.1. Carbon isotope analysis

Grain samples were ground to a fine powder and about 100 mg of every plant was prepared for $\delta^{13}C$ analysis. Carbon isotope composition was determined on 5- to 10-mg sub-samples with an isotope ratio mass spectrometer (Optima, VG Instruments, UK) at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. Results were expressed as:

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{reference}) - 1] \times 1000$$

with R being the $^{13}C/^{12}C$ ratio. A secondary standard calibrated against Pee Dee Belemnite (PDB) carbonate primary standard was used as the reference.

Δ was calculated using the following formula [4]:

$$\Delta\ (‰) = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000$$

where δ_p is the $\delta^{13}C$ of the plant sample and δ_a is the $\delta^{13}C$ of atmospheric CO_2. On the PDB scale, atmospheric CO_2 has a current deviation of approximately –8‰ [4].

2.2.2. Stability analysis

The stability regression coefficient (b-value) was calculated for each genotype [5] to determine the stability of grain yield and CID across different environments. In general, genotypes with b-values of <0.70 were considered unresponsive to different environments or had above average stability; between 0.70 and 1.30 had average stability and >1.30 were considered responsive to good environments or had below average stability [6]. Δ values on 15 and 20% selection intensity were calculated for the two crosses.

The same set of genotypes used in 2004-05 was maintained in 2005-06 to evaluate the influence of inter-annual climatic variation, and F_4 for the two crosses was harvested in June 2007. For the 2007-08 season, these three categories of plants were chosen and sown from the F_4 population based on their similar heading date and plant height. Estimates of realized heritability were obtained [7]:

$$h^2_R = \text{Mean } F_4 \text{ high} - F_4 \text{ low/Mean } F_3 \text{ high} - F_4 \text{ low}$$

where the mean of F_4 or F_3 is based on 15% selection intensity.

2.2.3. 'Budget' model and water use efficiency

The three crosses Mexicali/Waha, Oued Zenati/Mexicali1 and Oued Zenati/Mexicali2 were analyzed for Δ. Two experiments were carried out at two stations (Ain Abessa and Beni Fouda) during the 2006-07 growing season (Table 1). One of the purposes of these experiments was to use the water balance model 'Budget' [8], where the change of water stored in the root zone was determined on a daily basis by keeping track of incoming rainfall and irrigation, and outgoing evapo-transpiration and deep percolation water fluxes at its boundary. The relative yield decline that is expected under specific levels of water stress at different moments in the growing period is estimated by integrating the FAO K_y approach [9].

$$1 - (Y_a/Y_m) = K_y\ [1 - (Et_a/ET_c)]$$

where Y_a/Y_m is the relative yield, $(1 - [Y_a/Y_m])$ is the relative yield decrease, ET_a/ET_c is the relative evapo-transpiration and $[1 - (Et_a/ET_c)]$ is the water stress or relative evapo-transpiration deficit. The response of yield to water stress for a given environment is quantified through the yield response factor K_y.

Water use efficiency (WUE) was estimated for the Ain Abessa experiment [10]. Although transpiration and soil evaporation (ET) occur simultaneously in the field, they are difficult to measure as separate entities. However, the crop simulation model 'Budget' can reasonably estimate each component.

2.2.4. Statistics

Data were analysed using VISTA software (Visual Statistics System), v. 6.4 [11], and Sigma stat software for Windows, v. 3.5. The degree of association between Δ and grain yield

was estimated through simple correlation and linear regression analysis. Stepwise regression was used to explain the effects of different components on Δ.

3. RESULTS AND DISCUSSION

3.1. 2000-01 and 2001-02

3.1.1. Grain yield

Grain yield (GY) ranged from 0.38 to 1.66 t ha^{-1} during the first two seasons. Mean GY in season 2001-02 was significantly higher than in season 2000-01. Overall GY was not affected by genotypes but grain yield was significantly affected by season and season x genotype interaction. The genotype effect on GY was significant in season 2 but not in season 1. The highest GY was obtained by the cultivars Kucuk (0.99 t/ha^{-1}) and Yavaros (1.66 t/ha^{-1}) in season 1 and 2, respectively.

3.1.2. Carbon isotope discrimination

Overall highly significant effects of genotype, season and the genotype x season interaction were found for leaf and grain Δ. Leaf-Δ values in all genotypes were higher than grain Δ in both seasons. Mean leaf Δ and grain Δ were significantly higher in season 1 compared with season 2. In season 1 Dukem12/Rascon21 showed the highest leaf Δ (17.94‰) followed by Mexicali (17.78‰) and Kucuk (17.22‰). In season 2, Yavaros showed the highest leaf Δ (15.62‰), followed by Dukem12/Rascon21 (15.46‰) and Sooty9/Rascon57 (15.32‰). In season 1, Cado/Boomer33 showed the highest grain Δ (14.76‰) followed by Kucuk and Dukem12/Rascon21 (14.64 and 14.55‰, respectively). In season 2, the highest grain Δ (13.81‰) was shown by Waha followed by Tilo1/Lotus4 and Kucuk (13.57 and 13.37‰, respectively). Waha had a similar grain Δ in both seasons.

3.2. 2003-04

Rainfall was higher in Khroub Station (663 mm) than in Sétif Station (453 mm) in 2003-04 (Fig. 1). However, during the first experiments (2000-01 and 2001-02), the rainfall was 217 mm and 162 mm, respectively. During 2000-01, almost 80% of rainfall occurred during the first four months, while the post-anthesis period was characterized by very low rainfall and high temperatures. Conversely, during the second season (2001-02), the rainfall was evenly distributed during the growth cycle, with moderate terminal drought. Minimal spring temperatures were much lower during the first season, with negative (freezing) temperatures occurring during the heading period.

Mean GY in Khroub station was significantly higher than in Sétif station. Overall GY was affected significantly by genotype. Grain yield was also affected significantly by location and location x genotype interactions. Two local varieties with low Δ and a CIMMYT variety with a high Δ were identified from the 2001-02 and 2003-04 studies, and crosses were made. The F2 seeds were evaluated for divergent selection.

3.3. 2004-05 and 2005-06

3.3.1. CIMMYT lines and local varieties

In 2004-05, about 60% of the total rainfall occurred during the first 90 days of the cropping cycle, and was consequently characterized by an intensive water deficit during the

grain filling period. The mean grain yield for all genotype was 2.72 t ha^{-1} (Table 4). The genotype effect for grain yield was low but significant (P<0.018), while the genotype effect for grain Δ was highly significant (P<0.001) (Table 5). Grain yield and Δ values were slightly higher than those previously reported [12, 13] in the same region, suggesting that drought stress was less severe under the present conditions. The low Δ values of the old Algerian cultivars Oued Zenati and Polinicum have already been noted in previous studies [12, 13].

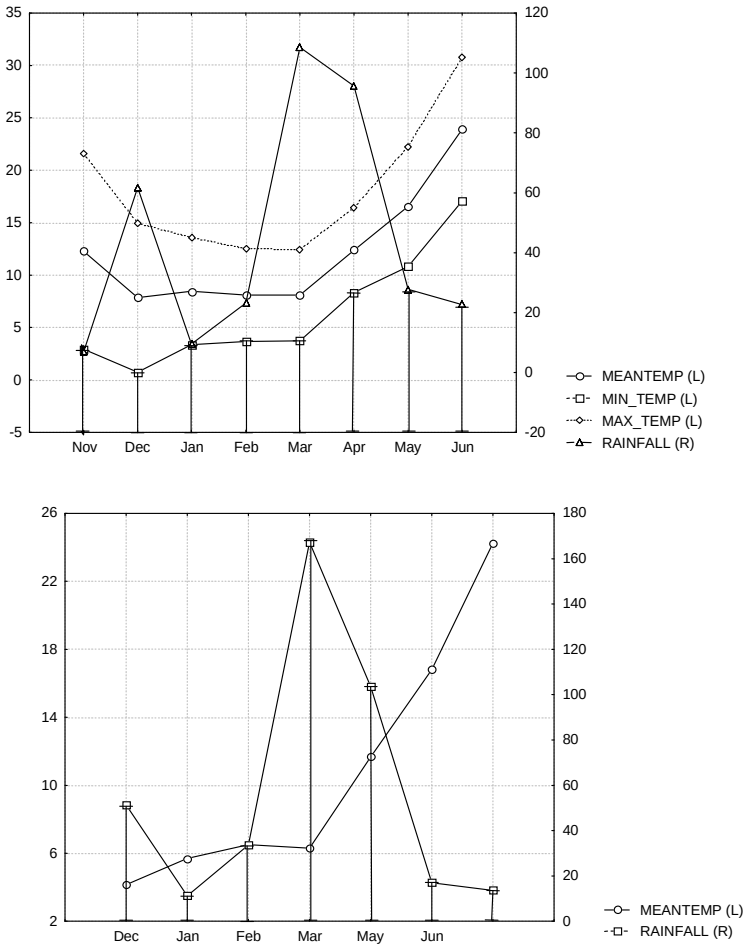

FIG. 1. Temperature (°C, L) and rainfall (mm, R) at Khroub Station (above) and in Sétif Station (below) during the 2003-04 season.

TABLE 4. GRAIN YIELD AND Δ OF CULTIVARS IN THE 2004-05 SEASON

Cultivar	Grain yield (t ha^{-1})	Grain–Δ (‰)
Oued Zenati	2.63	14.17
Altar	2.81	15.22
Sooty	2.99	15.24
Polinicum	2.39	14.62
Waha	2.73	15.44
Dukem	2.39	15.36
Mexicali	2.91	15.56
Kucuk	2.92	15.26
Mean	2.72	15.11

The mean grain yield for all genotypes was about 1.3 times higher in 2004-05 trial than the first trial in 2005-06 (Table 5). Significant differences among genotypes were observed for grain Δ. The greatest difference between extreme genotypes for grain Δ was observed in the 2004-05 season (1.39‰). The smallest range for grain Δ was observed in the second trial of 2005-06 (0.98‰). The effect of environment, understood as the combination of region and total rainfall, on grain yield and grain Δ was much higher than that of genotypic variability. The interaction between genotypes and trials for Δ was not significant (Table 5).

TABLE 5. MEAN, STANDARD DEVIATION (SD) AND VARIANCE (σ^2) FOR GRAIN YIELD AND GRAIN-Δ AT MATURITY FOR 2004-05 AND 2005-06 EXPERIMENTS

Experiments	Grain yield (tha $^{-1}$) †	Grain-Δ at maturity (‰) †
Trial 1 (2004-05)		
Mean	2.714a	15.11a
SD	0.44	0.03
σ^2 genotype	16.44*	0.66***
Trial 2 (2005-06, Date 1)		
Mean	2.093b	16.45a
SD	0.60	0.06
σ^2 genotype	39.03**	0.37**
Trial 3 (2005-06, Date 2)		
Mean	2.673a	17.26b
SD	0.47	0.07
σ^2 genotype	10.05ns	0.29*
All genotypes and trials		
σ^2 genotype	21.05***	0.51*
σ^2 trials	281.40***	24.92***
σ^2 genotype x trials	19.20***	0.36ns

†***, Significant at $P<0.001$;**, Significant at $P<0.01$;*, Significant at $P<0.05$; ns, not significant; mean values within the same column without a common letter are significantly different ($P<0.05$), Duncan comparison test.

The correlation between Δ and grain yield was not significant (r = 0.434) when all the cultivars were considered together. However, the correlation became significant (Fig. 2), by excluding the cultivars Oued Zenati (low Δ, low yield), an old cultivar having a very good adaptation to the high plateau conditions, and Waha (high Δ, high yield), and the earliest cultivar most susceptible to late frost. No significant correlation was observed between grain yield and grain Δ in 2005 and 2006. Conditions during the grain filling period were very dry and hot for the two seasons. Previous reports on barley (*Hordeum vulgare* L.) and durum wheat under Mediterranean conditions found a strong dependence of grain Δ on rainfall during the later growth stages, from heading and anthesis to maturity [14].

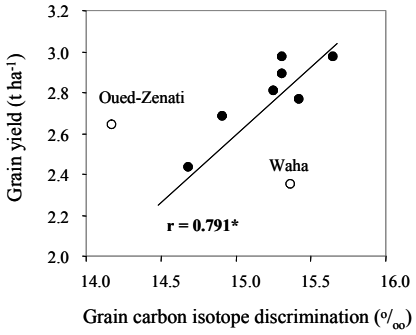

FIG. 2. Relationship between grain yield and Δ when Oued Zenati (low Δ, low yield) and Waha (high Δ, high yield) cultivars are excluded.

3.3.2. F_2 and F_3 populations

The Δ values for each of the three cultivars used as progenitors varied widely (Fig.3). This large variation illustrates the difficulty of selecting for Δ on individual F_2 plants. The Δ values within the F_2 populations Oued Zenati/Mexicali (low Δ/high Δ) and Mexicali/Waha (high Δ/high Δ) (Fig. 4 and Fig. 5) were highly dependent on the values of the parents, with trangressive effects noted in the case of the cross Mexicali/Waha (high Δ/high Δ). Carbon isotope discrimination variation in parents was more important than observed in the two crosses in F_2 (Fig. 6). Δ gain between F_2/F_3 was about 2‰ for the Oued Zenati/Mexicali cross and 1.75‰ for the Mexicali/Waha cross (Table 6).

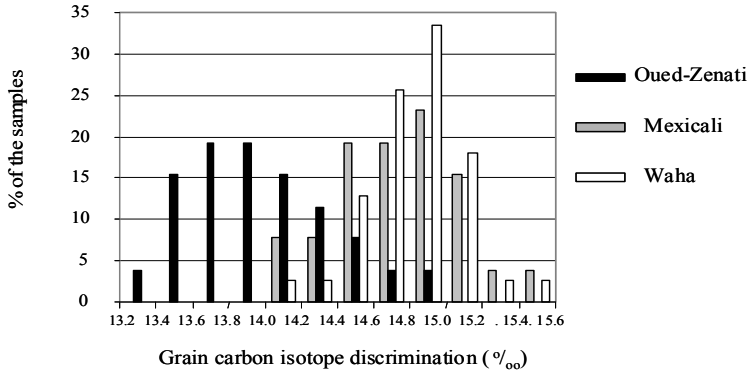

FIG. 3. The distribution of grain Δ for each of the three cultivars.

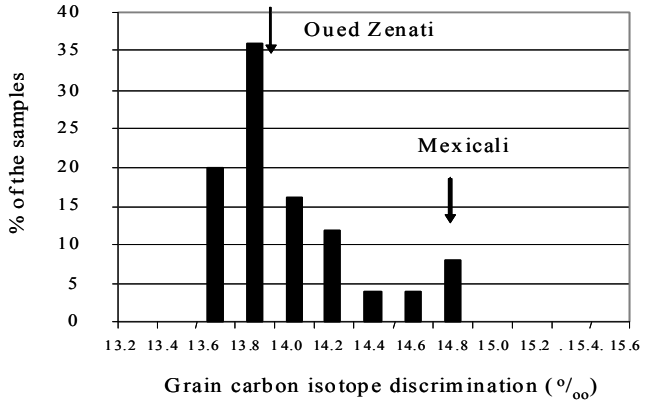

FIG. 4. Δ values within the F_2 populations of Oued Zenati/Mexicali.

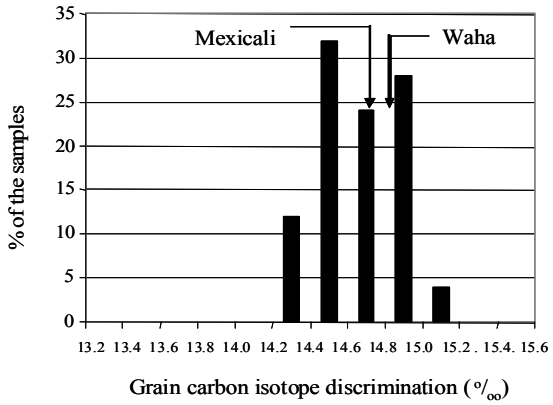

FIG. 5. Δ values within the F_2 populations of Mexicali/Waha.

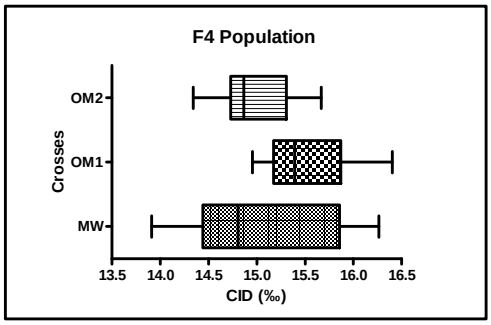

FIG. 6. Variation in Δ in Mexicali/Waha (MW), Oued Zenati/Mexicali1 (OM1) and Oued Zenati/Mexicali2 (OM2) crosses.

TABLE 6. MEAN Δ (‰) AT 15 AND 20 % INTENSITY SELECTION FOR THE TWO CROSSES IN F_2 AND F_3 POPULATIONS

Crosses	Mean of 15% High-Δ	Mean of 15% Low-Δ	Mean of 20% High-Δ	Mean of population
F_2 population				
Oued Zenati/Mexicali	14.64	13.24	14.62	14.07
Mexicali/Waha	14.98	14.34	14.92	14.65
F_3 population				
Oued Zenati/Mexicali	17.04	15.05	16.97	16.18
Mexicali/Waha	17.31	15.62	17.14	16.41

3.4. 2006-07

3.4.1. CIMMYT lines and local varieties

Seasonal rainfall in Beni Fouda and Ain Abessa was 356 and 397 mm, respectively. From tillering to heading, rainfall represented 70 and 65% of the total in Ain Abessa and Beni Fouda stations, respectively. Only 14% of total rainfall occurred between anthesis and grain filling in Beni Fouda. The Budget model simulated an intense water stress for this period (Table 7). The water stress factor was lower at heading compared with anthesis and grain filling, with more water stress for the local cultivars (Table 7).

TABLE 7. WATER STRESS FACTORS AT THREE PHENOLOGICAL STAGES (K_Y)

Cultivars	Heading date	Anthesis	Mid grain filling
CIMMYT/ICARDA	0.015	0.318	0.743
Local	0.310	0.662	0.870

Significant differences among genotypes were observed for grain yield, biomass, spikes m^{-2}, kernels $spike^{-1}$ and 1000-kernel weight (Table 8). The difference between the two sites was significant (P<0.001) for all components cited above except for 1000-kernel weight. The environmental effect on grain yield was significant and more favourable in Ain Abessa (Table 8). The mean grain yield for all genotypes was 5.93 t ha^{-1} in Ain Abessa and 5.12 t ha^{-1} in Beni Fouda. The Δ values were significantly different at Beni Fouda and ranged from 17.01 to 15.25‰ for Waha and Oued Zenati, respectively. As already mentioned, the lowest Δ was registered by local landraces.

TABLE 8. MEANS FOR GRAIN YIELD, BIOMASS AND YIELD COMPONENTS AND VARIANCE ANALYSIS FOR TWO SITES

Site/variance	Biomass ($g\ m^{-2}$)	Grain yield ($t\ ha^{-1}$)	Kernels ($spike^{-1}$)	Spikes (m^{-2})	1000-kernel weight (g)
Ain Abessa	1851.1a	5.93a	40.2a	492.6a	36.2a
Beni Fouda	1583.8b	5.12b	30.1b	263.2b	36.3a
σ^2 genotypes (G)	ns	***	***	***	***
σ^2 sites (S)	***	***	***	***	ns
σ^2 G x S	ns	***	ns	ns	ns

***, significant at $P<0.001$; ns, not significant; mean values within a column without a common letter are significantly different.

The factor having most influence on the performance of the best Mediterranean typical landraces is undoubtedly the higher production of aerial biomass. In drought-prone environments, this characteristic may arise either from a greater ability to extract water from the soil or better WUE, the latter determined by higher transpiration efficiency and/or a faster growing canopy that reduces the relative proportion of water lost by evaporation. The correlation between Δ and grain yield was significant at $P<0.080$.

3.4.2. F_4 population

In the F_4 population, significant differences were observed between crosses ($P<0.0003$) and between planting dates ($P<0.0004$) for harvest index (Table 9). Harvest index ranged from 0.28 to 0.35 for the two crosses. The Oued Zenati/Mexicali cross gave tall plants. No significant difference was observed between crosses and dates for grain yield. In this region, grain yield could be reduced also by frost stress that coincided with anthesis. No significant difference was observed between crosses by date, but crosses were significantly difference with 33.1 and 29.3 grains $spike^{-1}$ for (high Δ/high Δ) and (low Δ/high Δ) crosses, respectively.

TABLE 9. GRAIN YIELD, GRAINS PER SPIKE AND HARVEST INDEX IN F_4 POPULATION OF TWO CROSSES (C1, C2) AND TWO PLANTING DATES (D1, D2)

Main effects and interactions	Grain yield ($g\ m^{-2}$)			Grains $spike^{-1}$			Harvest index		
	W x M†	OZ x M†	Signif.	W x M	OZ x M	Signif.	W x M	OZ x M	Signif.
Dates (D1, D2)	591.1	543.5	$P<0.28$	32.1	30.2	$P<0.19$	0.28	0.35	$P<0.0004$
Crosses (C1, C2)	571.5	563.1	$P<0.84$	33.1	29.3	$P<0.02$	0.28	0.35	$P<0.0003$
C1 x D1, CI x D2	589.5	592.8	*, *	35.0	29.3	*, *	0.21	0.35	*, *
C2 x D1, C2 x D2	553.6	533.4	*, *	31.2	29.3	*, *	0.35	0.36	*, *

†W X M, Waha x Mexicali cross = C1; OZ x M, Oued Zenata x Mexicali cross = C2.

Analysis of variance showed a highly significant variation in Δ in the F_4 population among the two crosses Waha x Mexicali/Oued Zenata x Mexicali (D1) ($P<0.02$) and Oued Zenata x Mexicali (D1)/Oued Zenata x Mexicali (D2) ($P<0.000003$). Δ ranged in the F_4 population ranged from 13.91 to 16.27‰ for the Waha x Mexicali cross, while Oued Zenata x Mexicali (D1) and Oued Zenata x Mexicali (D2) ranged from 14.96 to 16.4‰ and from 14.34 to15.67‰, respectively (Fig. 6).

Realized heritability estimates, based on 15% selection intensity were intermediate to high, with $h^2 = 0.5708$, $h^2 = 0.6644$ and $h^2 = 0.7771$ for Oued Zenata x Mexicali (D1), Oued Zenata x Mexicali (D2) and Waha x Mexicali crosses, respectively. The high heritability of Δ

indicates that good gains from selection can be expected in the earliest parental materials with similar flowering dates.

3.5. All trials with CIMMYT lines and local varieties

The relationships between the mean grain yield and the mean Δ across all trials were significant (Fig.7).

The level of stability of the genotypes using grain yield and the corresponding value of Δ for each genotype is given in Table 10 [5].

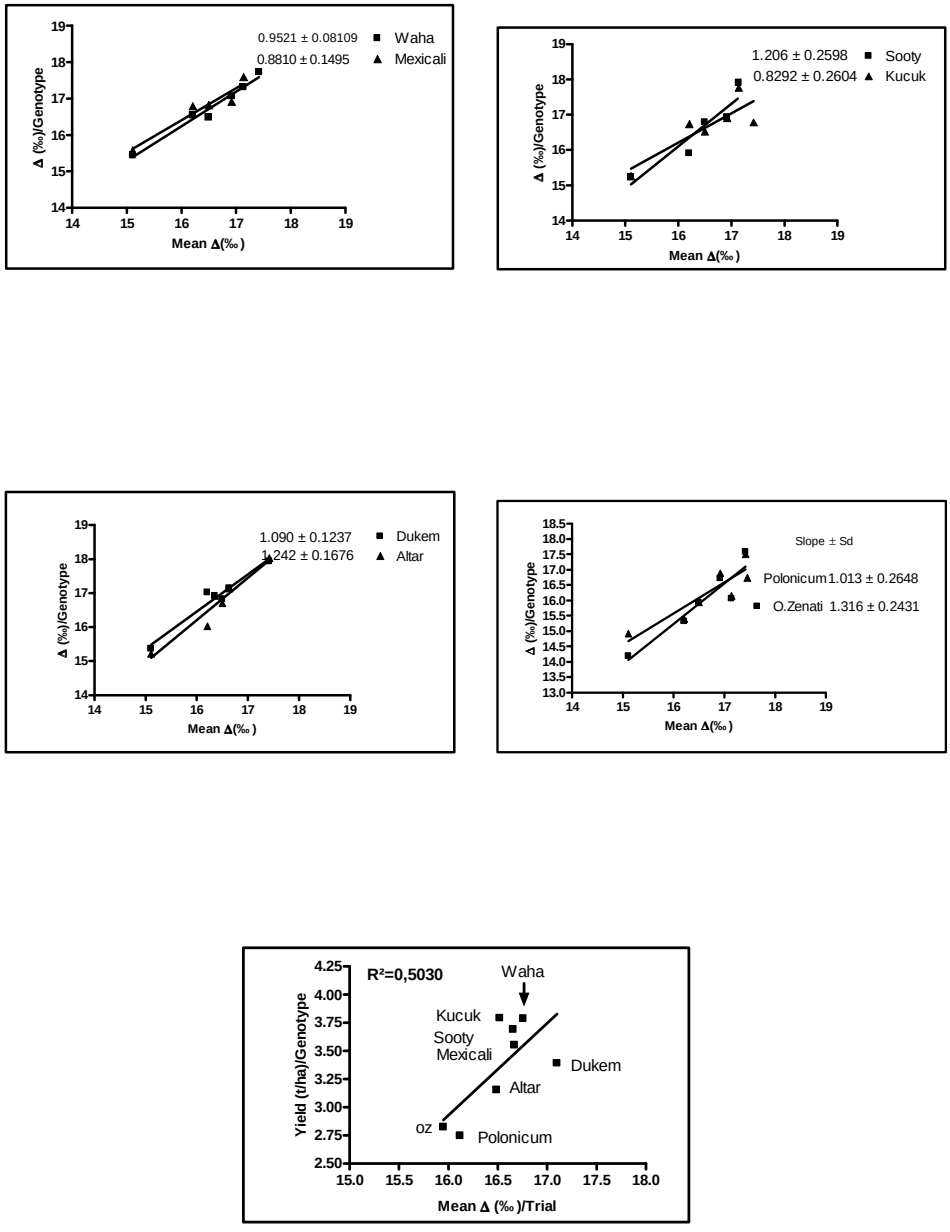

FIG. 7. Stability of CID across environments.

TABLE 10. STABILITY PARAMETERS, RANGE AND MEAN OF GRAIN YIELD AND Δ FROM ALL TRIALS

Genotypes	Grain yield (slope ± SD)†	Grain yield range (t ha^{-1})	Mean yield (t ha^{-1})	Δ (slope ± SD)†	Δ range (‰)	Mean Δ (‰)
Altar	0.766 ± 0.099	1.67 – 5.05	3.15	1.242 ± 0.168	15.22 – 18.03	16.55
Dukem	0.915 ± 0.203	2.16 – 5.51	3.49	1.090 ± 0.124	15.36 – 18.05	17.16
Kucuk	1.309 ± 0.033	2.09 – 7.22	3.69	0.829 ± 0.260	15.26 – 17.77	16.71
Mexicali	1.038 ± 0.110	2.24 – 6.34	3.55	0.881 ± 0.150	15.57 – 17.59	16.69
Oued Zenati	0.610 ± 0.121	1.71 – 4.44	2.82	1.316 ± 0.243	14.18 – 17.57	16.04
Polonicum	0.609 ± 0.036	1.86 – 4.28	2.73	1.013 ± 0.265	14.62 – 17.21	15.80
Sooty	1.317 ± 0.204	2.53 – 7.82	3.78	1.206 ± 0.260	15.24 – 17.91	16.83
Waha	1.437 ± 0.137	2.06 – 7.87	3.74	0.952 ± 0.081	15.44 – 17.40	16.76

†SD = standard deviation.

It appears that the subset of modern cultivars obtained from the CIMMYT/ICARDA durum wheat breeding programme had on average, a higher Δ value (16.78‰) than the subset of local cultivars (15.82‰). This result is in good agreement with previous results obtained in the same type of environment. It can be explained by a lower stomatal conductance, or more likely, by less effective re-mobilization efficiency, reflected in their lower harvest index [15]. The local cultivars, and particularly Oued Zenati, yielded well under the adverse environmental conditions of the high plateau, mainly because of a better phenological adaptation. The results of the present experiments, as well as those of previous studies [3, 13] suggest that Δ has a limited application as a yield predictor in this type of environment. The lack of correlation between Δ and grain yield is likely to be due to a strong contribution of pre-anthesis assimilates to yield together with a sink limitation of yield, breaking the association observed between Δ and yield [12]. Heading coincided with strong drought and frost (particularly in the first season) that markedly reduced potential grain number.

The results confirmed that the relationship between Δ and grain yield reported under a Mediterranean climate is unstable under our conditions [15, 16], and emphasizes the need for a precise definition of environment. This relationship is not stable under very severe stress, particularly when sink capacity is affected. The results suggest that breeding programmes in the Algerian high plateau may be based on crosses between local cultivars, maintaining a high sink capacity under stress conditions, and modern cultivars, having high Δ values, with further selection during the first cycles on the number of grains per spike (sink capacity) and on Δ values in F_3 or F_4 families having the higher sink capacity.

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CARBON ISOTOPE DISCRIMINATION AS A SELECTION TOOL FOR HIGH YIELDING WHEAT GENOTYPES FOR WATER LIMITED ENVIRONMENTS

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Abstract

130 bread wheat (*Triticum aestivum* L.) lines obtained from CIMMYT and local sources were grown to maturity during the 2004–2005 and 2005–2006 seasons under irrigated conditions without water stress. Carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) was determined on early leaf and grain samples. 111 genotypes were well adapted. Grain yield was positively and significantly correlated with harvest index. Six genotypes with leaf Δ values 18–18.99‰ were ranked low, 55 with Δ values 19–20.99‰ were ranked medium, 10 genotypes with Δ values 22–22.99‰ were ranked high, and one with a Δ value of 23.01‰ was ranked very high. Due to insufficient variability in Δ among these genotypes, a crossing and generation programme was initiated to evolve new genotypes with a larger variation in Δ. The advancement phase is in progress. Meanwhile, eight genotypes were selected from the 111 adapted varieties based upon grain yield and variation in Δ, for testing under four soil moisture regimes, including well-, medium-, low watered and stored soil moisture conditions. The aim was to identify promising lines for target environments and to establish relationships between grain yield and Δ, water use efficiency (WUE) and other plant parameters. All 8 genotypes exhibited higher leaf than grain Δ under the four water regimes, with a variation of 1.35 and 0.91‰, respectively. Water stress reduced both leaf and grain Δ, which were significantly and positively correlated. An increase in soil moisture stress decreased grain yield in all genotypes. Grain yield and grain WUE were positively correlated with leaf and grain Δ, harvest index, spike length and 1000 grain weight, and were negatively correlated with number of tillers and time to heading. Sitta and FD-83 genotypes had high yields with greater increases in WUE under water stress. e.g. Sitta produced the highest grain yield of 4.4 t ha^{-1} with the highest grain WUE of 16.99 kg ha^{-1} mm^{-1}, and can be exploited to obtain high grain yield in rain fed and water- limited environments. Grain and biomass yields were significantly and inversely correlated with leaf and grain Δ in 19 CIMMYT lines grown under irrigated conditions, and were strongly correlated with leaf ash content, which was itself negativity correlated with leaf and grain Δ. Grain ash content was positively correlated with grain Δ, but not with leaf Δ. Under irrigated and high evaporative demand conditions, leaf stomatal conductance appears to be the main yield-limiting factor, as shown by the significant correlation between yield and both Δ and ash content. These results suggest that leaf ash content is a useful indirect selection criterion for yield in irrigated environments, and might be considered as a surrogate for Δ and grain yield.

1. INTRODUCTION

Wheat is the most important crop in Pakistan, being grown country-wide under irrigation as well as in rain fed areas. Pakistan is among the top ten wheat producing countries of the world and ranks as 9th in the area under wheat cultivation. During the last 8 years wheat was grown on 8.25 Mha including 1.112 Mha (13.5%) non-irrigated and 7.138 Mha (86.5%) irrigated. During this time the weighted average yield remained at 2.422 t ha^{-1}, with 2.632 t ha^{-1} irrigated and 1.067 t ha^{-1} rain fed, which are among the lowest in the world [1].

A major part of Pakistan's climate is semi-arid to arid, with average annual precipitation of 250 mm with a range of 100 to 760 mm. It is estimated that 66.7% of the area receives rainfall less than 254 mm, 24.2% between 254 and 508mm, 5.4% between 508 and 762 mm and only 3.7% more than 762 mm. Rainfall occurs mainly in March–April and July–August. The precipitation during the wheat growing season is inadequate, and varies greatly both within and between seasons.

Higher crop performance may be achieved through improvements in water use, WUE and harvest index [2, 3]. Water use is relevant when soil water remains available at maturity or when deep-rooted genotypes access water deep in the soil profile that is not normally available. The other two factors become more significant when all available water is used up by the end of the season. The single most important attribute under water stress tackled so far has been phenology, matching crop development and seasonal rainfall patterns [4, 5], which may affect either water use or WUE.

Selection of plants for high grain yield or WUE is desirable to improve crop production in water limited environments. Therefore, screening for high grain yield or water use efficient wheat genotypes is a logical approach to enhance wheat yields under environments where water plays a crucial role. However, information on screening for high grain yield or WUE is limited, due mainly to the lack of fast screening techniques.

The objective of the present study was to evaluate the potential of CID as an indirect selection criterion for grain yield in bread wheat under different water regimes. Several research initiatives were undertaken at NIAB, using germplasm obtained from national yield trials at different locations and CIMMYT. These initiatives included (i) screening of wheat genotypes (local and exotic) for agronomic water use efficiency, Δ and yield components (ii) initiation of new crosses in case of insufficient variability in Δ among tested genotypes, including low Δ x low Δ and low Δ x high Δ genotypes (iii) generation advancement (F_1, F_2, F_3, …..) of hybrid material and selection of plants having desirable physio-morphological traits and (iv) selection of elite wheat lines based on Δ and grain yield for evaluation in target environments. Objective (iii) is currently on-going.

2. MATERIALS AND METHODS

2.1. Experimental

2.1.1. Screening wheat genotypes under irrigation

130 bread wheat (*Triticum aestivum* L.) genotypes collected from 5 different sources were screened during the 2004–2005 and 2005–2006 seasons. Seventy one (71) genotypes were grown during 2004–2005, and fifty nine (59) during 2005–2006, the latter including nineteen genotypes provided by CIMMYT. These genotypes were grown under irrigated conditions without water stress. Δ was determined for early leaf (4 weeks after emergence) and grain samples at maturity. Plant vigour at tillering, leaf color, days to 50% flowering, days to 100% maturity and plant height were recorded. Plants were harvested at physiological maturity, and spike length, number of grains spike^{-1}, grain weight spike^{-1}, biomass yield, grain yield and harvest index were determined. Grain and early leaf samples were analyzed for ash content.

2.1.2. Performance of wheat genotypes under different water regimes

A set of eight top-yielding wheat genotypes (Sarsabz, NR-234, Nesser, Bhittai, NR-241, FD-83, Sitta and Pfau/vee#9//ures) screened previously for variation in Δ and higher grain yield were grown under four water regimes: well-watered (WW), medium-watered (MW), low watered (LW) and stored soil moisture (SSM) conditions. Plants were grown in concrete lysimeters (5 m × 5 m × 1 m) in a sandy clay loam (45% sand, 33% silt and 22% clay) that originated from the NIAB experimental field. The soil had an average bulk density of 1.4 g cm^{-3}, an electrical conductivity (EC) of 1.5 dS m^{-1}, a pH of 7.6 and a sodium adsorption ratio (SAR) of 1.36. In four lysimeters all genotypes were sown in subplots (1 m × 1 m) randomly in three replicates. Three seeds per hill were sown in 5 rows with a 20-cm row spacing and an interplant spacing of 10 cm, at an adjusted seeding rate of 150 seeds m^{-2}. The sowing date was 23rd November 2006, and plants were harvested in the 3rd week of April, 2007.

Each lysimeter contained three PVC access tubes installed to 1 m depth for soil water measurement using a neutron moisture meter (NMM). The soil water during the whole experiment was estimated with the neutron moisture meter (NMM) (503 Model, CPN, USA), with readings taken at depths of 15, 25, 50 and 75 cm as and when required. One pre-sowing irrigation was applied to each lysimeter. Lysimeters were randomly selected to impose the 4 water treatments. In WW, the soil was kept at 100% of total available water (TAW), at 75% of TAW under MW and at 50% of TAW in the LW treatment. TAW was defined as the difference between soil moisture at field capacity and soil moisture at the permanent wilting point. Field capacity and soil moisture at the permanent wilting point were determined by placing the soil samples in plastic rings in a pressure membrane apparatus, and measuring water retained by the soils at –0.03 MPa and –1.5 MPa, respectively. The required volume of water for each lysimeter was added through a locally-fabricated irrigation system including a water pump, fixed pipes, water flow meters and taps, etc. Total water consumed was determined by adding water applied by irrigation, and rainfall recorded during the course of study. The irrigation water had an electrical conductivity of 0.76 dS m^{-1}, a pH of 7.5 and a SAR of 2. Urea was applied at 120 kg N ha^{-1} to all treatments.

Early leaf (4th leaf stage) and grain samples collected at maturity were analysed for Δ. Phenotypic characters including number of tillers, plant height, time to heading and maturity were recorded. Spike length, number of grains $spike^{-1}$, 1000 grain weight, biomass and grain yields were recorded at harvest. The harvest index (%) was determined as the ratio of grain yield to above ground biomass multiplied by 100. Grain and biomass WUE were determined by dividing the respective yield by the quantity of water consumed during the growth period.

2.2. Analytical

Early leaf and grain samples were collected before water stress and at maturity respectively. The samples were dried at 60 °C for 48 h and were then ground to fine powder. The carbon isotopic ratios ($R = {}^{13}C/{}^{12}C$) of the samples (R_{sample}) and a standard ($R_{standard}$) were determined using an isotope ratio mass spectrometer (GD 150, MAT, Germany). R values were converted to $\delta^{13}C$ using the relationship:

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

The primary standard is CO_2 obtained from the Pee Dee Belemnite (PDB) limestone formation in South Carolina, USA. The $\delta^{13}C$ values were converted to Δ values using the relationship [6]:

$$\Delta\ (‰) = [(\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p)] \times 1000$$

where a and p represent air and plant, respectively. A value of –8.00‰ for $\delta^{13}C_a$ was used [7]. CID in leaf, straw and grain are hereafter referred to as ΔL, ΔS and ΔG, respectively. Ash content was determined using the AACC official method [8]. Approximately 1.5 g of dry material was incinerated at 575 ^oC for 16 h (until light grey ash was obtained). Ash content (%) was expressed on dry mass basis as the mean of two replicate samples.

2.3. Statistical analysis

The data were subjected to an analysis of variance (ANOVA), using SAS version 8.1. [9]. The F test was used to identify treatment main effects and interactions, followed by Duncan's multiple range test at the 0.05 probability level.

3. RESULTS

3.1. Screening wheat genotypes under well-watered conditions

3.1.1. Pakistani and CIMMYT lines

Out of 130 genotypes, 111 showed good adaptation (Table 1). Grain yield varied from 1325 to 4759 kg ha^{-1} (Table 1). Pfau produced the highest grain yield of 4759 kg ha^{-1} followed by 4732 and 4644 kg ha^{-1} for Sitta and Sarsabz, respectively. Δ varied from 18.52 to 23.01‰. Genotype 5039 showed the highest Δ value 23.01‰ followed by 22.98 and 22.24‰ for Bau/Opat and TC-4881 genotypes, respectively. The lowest Δ (18.52‰) was shown by ELITE-73 followed by NR-234 (18.63‰) and LU-26-S (18.76‰). The harvest index varied from 16.1 to 43.5%. Sitta showed the highest harvest index of 43.5% followed by 40.1 and 38.5% for Weebill-1 and NR-231, respectively. Harvest index was significantly and positively correlated (r = 0.756**) with grain yield (Fig. 1).

Plant height, spike length, number of grains $spike^{-1}$, grain weight $spike^{-1}$ and days taken to 50% flowering and to physiological maturity varied greatly among the genotypes. Grain yield was significantly correlated with number of grains $spike^{-1}$, spike length and grain weight $spike^{-1}$. Plant height and heading days were negatively and significantly correlated with GY (Table 2). Six genotypes with leaf Δ values 18.00–18.99‰ were ranked low Δ genotypes, 55 with Δ values 19.00–20.99‰ were ranked medium-Δ, ten genotypes with Δ values 22.00–22.99‰ were ranked high Δ genotypes, and one with a markedly high Δ value of 23.01‰ was ranked very high Δ.

3.1.2. CIMMYT elite lines

A total of 262 mm water was applied to maturity, including 52 mm of rainfall recorded during the growth cycle. The temperature remained mild during the growth period (Fig. 2). Grain yield varied from 3516 (Bau/Opat) to 4759 kg ha^{-1} (Pfau/vee#9//ures) (Fig. 3). All genotypes showed higher leaf Δ than grain Δ under irrigated conditions (Fig. 4). Leaf-Δ ranged from 21.06 to 22.98‰ and grain Δ varied from 19.01 to 20.7‰. There were negative correlations between grain yield and leaf Δ (r = –0.735**) and grain Δ (r = –0.619**) (Fig. 5a and Fig. 6a). Grain WUE was also negatively correlated with leaf and grain Δ (Table 3).

TABLE 1. ORIGIN, GRAIN YIELD (GY, KG HA^{-1}) AND LEAF-Δ (‰) OF WHEAT GENOTYPES GROWN UNDER WELL-WATERED CONDITIONS

No.	Genotype	GY	Δ	No.	Genotype	GY	Δ	No.	Genotype	GY	Δ
Centro Internacional de Mejoramiento de Maize y Trigo (CIMMYT), Mexico											
1	Elite 37	3083	20.95	12	Sitta	4732	21.06	23	Or791432/Vee#3	4065	21.61
2	Elite 34	1325	21.20	13	Nesser	4206	19.38	24	Irena	4406	21.63
3	Elite 33	2354	21.43	14	Weebill-I	4106	20.46	25	Chil/Prl	4270	21.95
4	Elite 8	1970	21.94	15	Dharway Dry	2825	20.09	26	Pfau/Vee#9//Ures	4759	21.12
5	Elite 83	1700	19.75	16	Baviacora M 92	3776	21.91	27	BUC/CHRC/	3550	22.07
6	Elite 73	1744	18.52	17	Galvez S 87	4519	21.65	28	Bjy/Coc//Prl/Bow	3582	22.04
7	Elite 56	1618	19.67	18	Firetail	4436	21.83	29	Chil/Bomb	4472	21.59
8	Elite 39	1588	19.36	19	Anb/Buc	3706	21.79	30	Gim/Lira	3879	21.78
9	Elite 90	2052	18.94	20	Pfau/Bow/	3883	21.57	31	Ures/Jun//Kauz	3919	22.01
10	Elite 88	3145	19.90	21	Attila	4133	22.07	32	Fedwing	3853	21.88
11	Opata	2760	20.99	22	Bau/Opat	3516	22.98	33	RL6010/4*Inia66/	3888	22.09
University of Agriculture Faisalabad (UAF), Pakistan											
34	FD-83	3983	21.42	41	9244	3698	22.22	48	Rawal-87	2952	19.29
35	TC4881	2189	22.24	42	6500	2604	20.33	49	LU-26-S	2063	18.76
36	9316	3737	20.77	43	TC 4884	2543	19.98	50	5039	2329	23.01
37	FD-85	2915	21.13	44	PAK-81	2517	22.17	51	TC-4928	2029	20.48
38	9021	2617	19.50	45	9258	2736	20.35	52	6529-II	2269	19.55
39	Kohistan-97	3200	20.40	46	6544-6	2630	21.82				
40	Barani-83	2147	20.39	47	9250	2610	20.88				
Nuclear Institute for Agriculture (NIA), Hyderabad, Pakistan											
53	C-228	3124	20.23	58	Soghat-90	4110	19.88	63	Bhittai	4100	22.19
54	Marvi-2000	3067	19.41	59	Kiran-95	3025	21.34	64	QM-4934	3202	20.64
55	Sarc-1	4317	20.82	60	Chakwal-86	3761	19.37	65	C-591	2439	20.47
56	CM-24/87	3827	20.62	61	Sarsabz	4644	21.20				
57	Abadgar	4044	20.12	62	Sindh-81	2526	21.37				
Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan											
66	02013	4277	20.85	75	Sarc-6	2560	20.88	84	03138	4070	21.10
67	02134	3163	19.29	76	Sarc-5	2533	20.41	85	03158	4164	21.00
68	02005	3290	20.83	77	03156	2848	20.28	86	04179	4241	21.30
69	03153	3265	21.17	78	01078	4235	20.70	87	AS-02	4113	21.50
70	02006	3515	20.44	79	02192	4290	21.50	88	04188	4153	20.90
71	02156	4109	20.90	80	03007	4280	21.50	89	04189	4078	21.30
72	03158	3613	21.09	81	03056	4072	21.20	90	UQAB	4178	20.60
73	Sarc-2	3748	20.18	82	03079	4176	21.40	91	Pasban-90	4042	21.05
74	Sarc-3	2778	20.51	83	03094	4184	21.60	92	Inqlab-91	4255	18.99
National Agricultural Research Center (NARC), Islamabad, Pakistan											
93	GA-02	3024	20.50	100	NR-254	3445	20.50	107	L.C	4190	20.50
94	CH-97	4335	19.58	101	NR-267	4294	20.90	108	Wafaq-01	3027	20.46
95	NR-240	3775	20.83	102	NR-268	4174	20.80	109	Suleman-96	2672	20.36
96	NR-241	4059	18.87	103	NR-269	4326	20.90	110	Margalla-99	2765	19.44
97	NR-231	4301	20.19	104	NR-271	3796	21.00	111	Marwat-5-01	2802	20.53
98	NR-234	4602	18.63	105	NR-273	4221	21.50				
99	NR-252	3858	21.30	106	NR-274	3669	21.00				

TABLE 2. CORRELATION COEFFICIENTS BETWEEN PHENOTYPIC CHARACTERISTICS AND GRAIN YIELD, BIOMASS YIELD AND HARVEST INDEX

Parameter	Grain yield	Biomass yield	Harvest index
Leaf-Δ	0.300**	–0.181ns	0.483**
Grain-Δ	0.417**	0.200*	0.346**
Plant height	–0.429**	0.135ns	–0.565**
Spike length	0.233*	0.311**	0.055ns
Number of grains spike^{-1}	0.436**	0.220*	0.403**
Grain weight spike^{-1}	0.543**	0.296**	0.447**
Heading days	–0.404**	0.163ns	–0.621**
Maturity days	–0.362**	0.362**	–0.679**

*, $P<0.05$; **, $P<0.01$; ns, not significant.

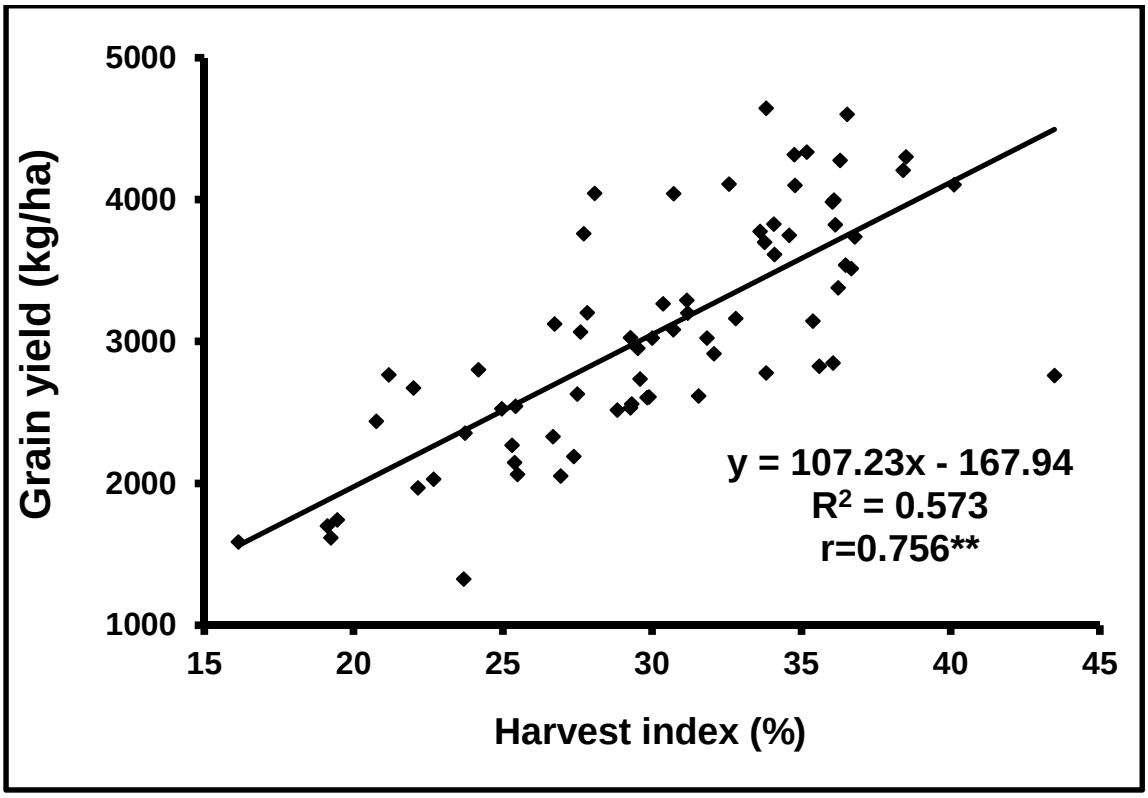

*FIG. 1. Relationship between grain yield and harvest index in wheat genotypes grown under irrigated condition with no water limitation. ** = P<0.01.*

Grain yield strongly correlated with the plant vigour (r = 0.671**) and leaf ash content (r = 0.815**) (Fig. 7a). Biomass yield varied from 7690 to 10810 kg ha^{-1}. There was a positive correlation of yield with plant vigour (r = 0.671**) and leaf ash content (r = 0.751**) (Fig. 7b). However, the correlation between biomass yield and grain ash content was not significant (Table 3). A negative correlation between biomass yield and leaf Δ (r = –0.714**) and grain Δ (r = –0.658**) (Fig. 5b and 6b) was observed. Similarly, the biomass WUE was also negatively correlated with leaf and grain Δ (Table 3). Ash content of the leaf was negativity correlated with leaf Δ (r = –0.604**) and grain Δ (r = –0.754**). However, the percent ash content of grain was positively correlated with grain Δ (r = 0.527*) but not with leaf Δ. The percent leaf ash content also correlated positively with biomass (r = 0.755**) and grain WUE (r = 0.815**) and with plant vigour (r = 0.607**).

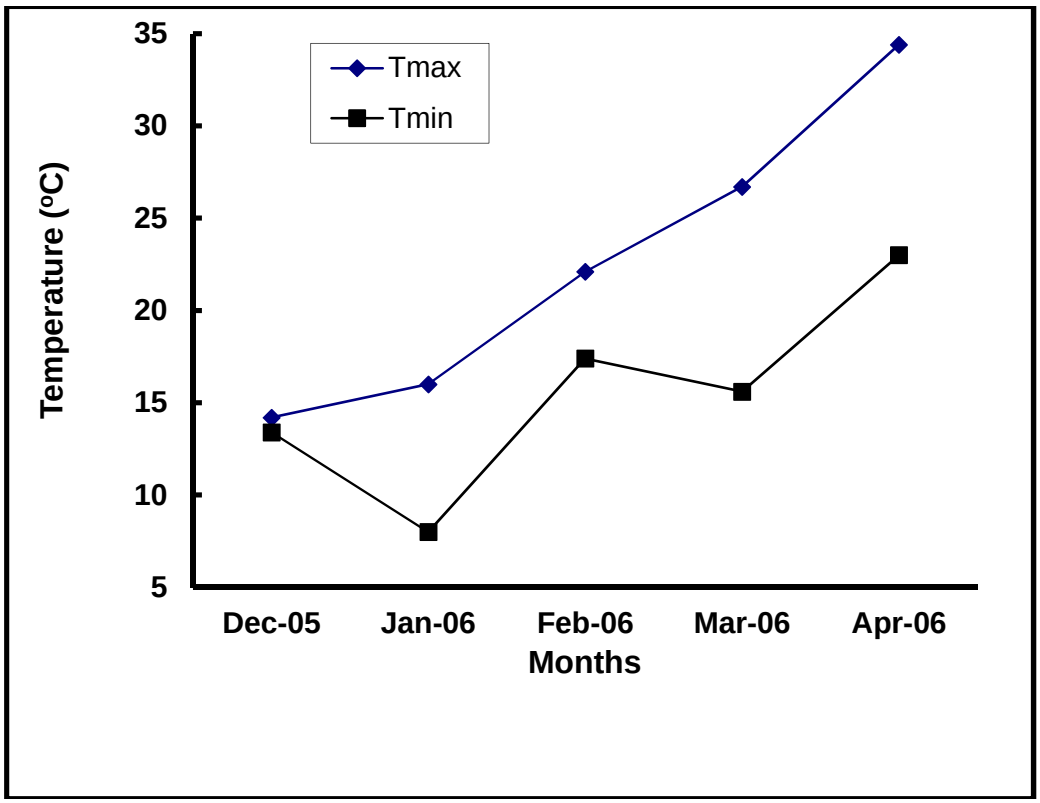

FIG. 2. Average monthly minimum and maximum air temperatures.

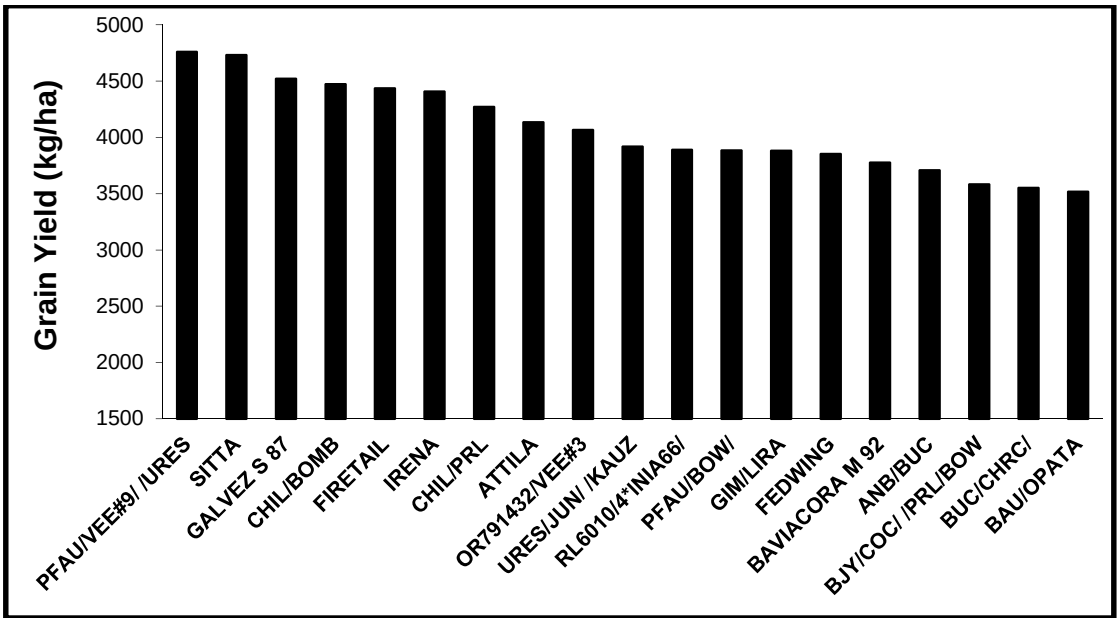

FIG. 3. Variation in grain yield in 19 CIMMYT genotypes grown under irrigated conditions.

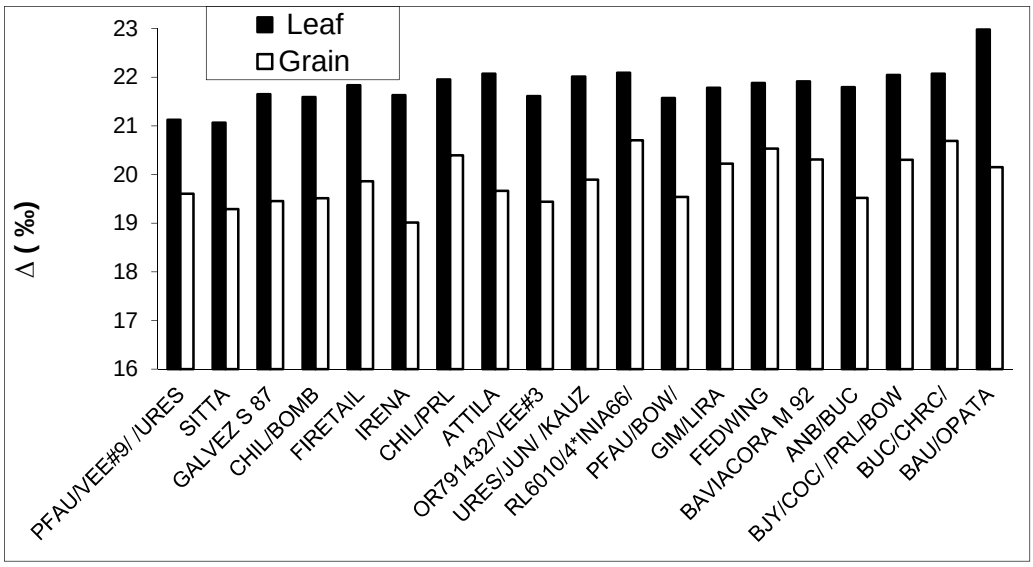

FIG. 4. Variation in leaf and grain Δ in 19 CIMMYT genotypes grown under irrigated conditions.

TABLE 3. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF 19 CIMMYT WHEAT GENOTYPES

Parameter	Leaf-Δ	Grain-Δ	Leaf ash	Grain ash
Grain yield	–0.735**	–0.619**	0.815**	–0.239ns
Biomass yield	–0.714**	–0.658**	0.755**	–0.142ns
Harvest index	–0.019ns	0.125ns	0.143ns	–0.252ns
Grain WUE	–0.735**	–0.619**	0.815**	–0.239ns
Biomass WUE	–0.714**	–0.658**	0.755**	–0.124ns
Leaf ash	–0.604**	–0.754**		–0.245ns
Grain ash	0.065ns	0.527*		

*, $P<0.05$;**, $P<0.01$; ns, non-significant.

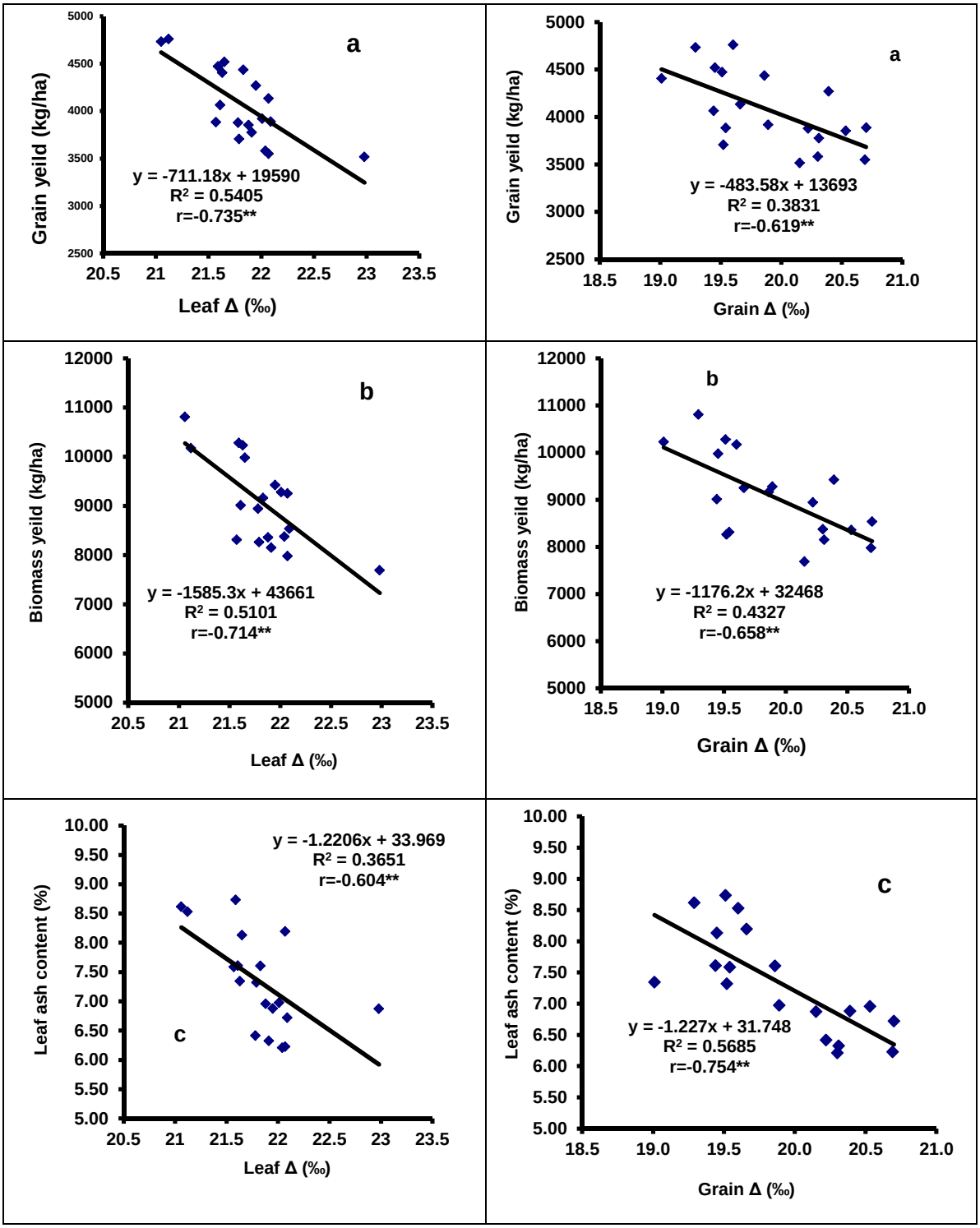

*FIG. 5. Relationships of leaf Δ with (a) grain yield (b) biomass yield and (c) leaf ash content in 19 CIMMYT wheat genotypes under irrigated conditions. ** = P<0.01.*

*FIG. 6. Relationships of grain Δ with (a) grain yield (b) biomass yield and (c) leaf ash content in 19 CIMMYT wheat genotypes under irrigated conditions. ** = P<0.01.*

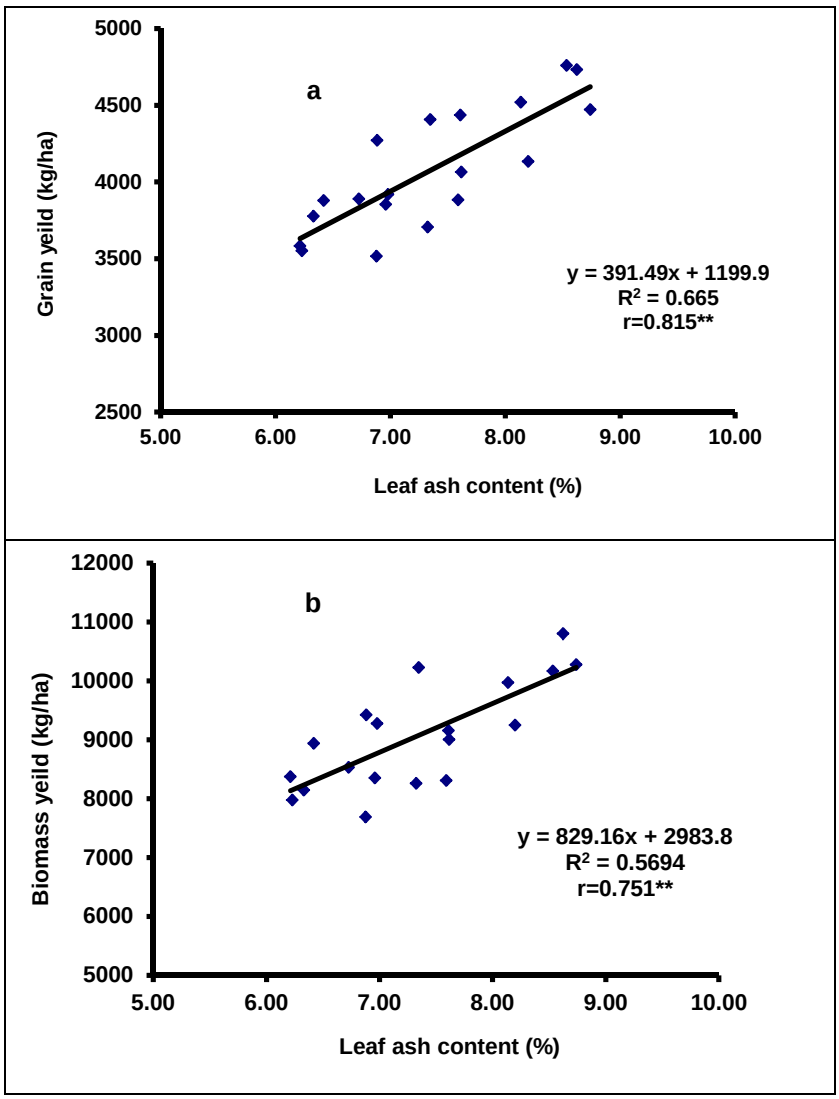

*FIG. 7. Relationships of leaf ash content (%) with (a) grain and (b) biomass yields in 19 CIMMYT wheat genotypes under irrigated conditions. ** = P<0.01.*

3.2. Influence of water regime

3.2.1. Characteristics of 8 wheat genotypes

Eight genotypes were selected from the initial screening (Table 1), based upon grain yield and variation in Δ under well-watered conditions (Table 4). These eight genotypes were tested under four soil moisture regimes to gauge their suitability for different target environments.

Grain yield, biomass, WUE of grain and biomass, harvest index, plant height, leaf and grain Δ, days to heading and maturity and 1000 grain weight differed significantly between 8 genotypes when averaged over water regimes (Table 5). The genotype Sitta had the highest grain yield (4.40 t ha^{-1}) while Nesser exhibited the lowest (3.12 t ha^{-1}). Biomass ranged from 13.47 t ha^{-1} (Bhittai) to 10.91 t ha^{-1} (Pfau/vee#9//ures) (Table 5). There was a large variation among genotypes for time to heading (15 d), and time to maturity varied by 5-d from 143 to 138 days (Table 5).

TABLE 4. VARIATION IN GRAIN YIELD AND Δ AMONG EIGHT WHEAT GENOTYPES UNDER WELL-WATERED CONDITIONS

Genotypes	Source†	Grain yield (t ha^{-1})	Leaf-Δ (‰)	Grain-Δ (‰)
1. Sarsabz	NIA	4.644	21.20	19.46
2. NR-234	NARC	4.602	18.63	19.68
3. Nesser	CIMMYT	4.206	19.38	19.45
4. Bhittai	NIA	4.100	22.19	20.28
5. NR-241	NARC	4.059	18.87	19.57
6. FD-83	UAF	3.983	21.42	18.84
7. Sitta	CIMMYT	4.732	21.06	19.29
8. Pfau	CIMMYT	4.759	21.57	19.60

†See Table 1.

TABLE 5. MEAN VALUES OF PHENOLOGICAL TRAITS OF EIGHT WHEAT GENOTYPES ACROSS FOUR WATER REGIMES

Genotypes	Yield (t ha^{-1})		WUE (kg ha^{-1} mm^{-1})		HI (%)	Height (cm)	Δ (‰)		Time (d) to		TGW† (g)
	Grain	Biomass	Grain	Biomass			Leaf	Grain	Heading	Maturity	
Sitta	4.40a	11.74b	16.99a	45.26b	37.5a	91e	20.03d	19.28f	89d	140cd	43b
FD-83	4.37a	11.44b	16.80b	43.74b	38.5a	97c	20.18a	19.71a	84e	138e	46a
NR-234	4.30a	11.59b	16.49bc	43.64b	37.3a	90e	19.72e	19.50c	93c	139d	47a
Bhittai	3.89b	13.47a	14.93cd	51.80a	28.9d	103a	19.74e	19.64b	98a	143a	38c
Sarsabz	3.86b	13.24a	15.04cd	49.50a	30.0d	99b	20.16a	19.40d	94bc	140bc	39c
Pfau	3.80b	10.91b	14.69d	42.14b	34.7b	92d	20.00c	19.26g	99a	141b	35d
NR-241	3.71b	11.59b	14.09de	43.38b	32.3c	85f	19.37f	19.33e	96ab	141b	38c
Nesser	3.12c	11.58b	12.51e	44.00b	27.7d	80g	18.88g	18.80h	97a	141b	33e

†TGW, 1000 grain weight; Means within a column followed by different letters are significantly different (P<0.05).

When averaged over water regimes, the genotype Sitta had significantly higher grain WUE (16.99 kg ha^{-1} mm^{-1}) followed by FD-83 (16.80 kg ha^{-1} mm^{-1}) due to their higher grain yield driven by a relatively higher harvest index compared with the other genotypes. Bhittai exhibited significantly higher biomass WUE due to the higher accumulation of biomass, and had relatively lower grain yield or grain WUE because of the comparatively lower harvest index (Table 5).

Water stress resulted in a marked decrease in grain yield, and the extent of the decrease was dependent on genotype and the degree of water stress (Table 6). The highest grain yields generally occurred under WW conditions, and the lowest were in the SSM treatment. There was also a large reduction in the number of tillers (11%) and plant height (63%) under SSM compared with WW conditions (data not shown). The highest water use efficiencies for grain were consistently in the SSM treatment, while the lowest were under WW conditions.

Both leaf and grain Δ varied significantly with genotype and water regime (Table 6). All genotypes tended to have higher Δ in leaf than in grain. Lower Δ was noted in grain than in leaf samples in WW (0.82‰), MW (0.33‰), LW (0.19‰) and SSM (0.29‰) averaged across the genotypes. Water stress reduced Δ in both leaf and grain, and the difference between leaf and grain Δ decreased in general with increasing water stress and were genotype-dependent. The genotype FD-83 showed the highest leaf Δ (20.93‰) in WW, and Nesser the lowest leaf Δ (18.63‰) in SSM with the highest water stress (Table 6). The

genotype Bhittai exhibited the highest grain Δ (20.21‰) in WW and Nesser the lowest grain Δ (18.23‰) under SSM (Table 6). Genotype x water regime interactions were significant for harvest index, plant height, spike length, 1000 grain weight, and days to heading and maturity, but were non-significant for other traits (data not shown).

TABLE 6. GRAIN YIELD, WUE OF GRAIN AND Δ IN LEAF AND GRAIN IN EIGHT WHEAT GENOTYPES UNDER FOUR WATER REGIMES†

Genotypes	Grain yield (t ha^{-1})				WUE (kg ha^{-1} mm^{-1})				Leaf-Δ (‰)				Grain-Δ (‰)			
	WW	MW	LW	SSM	WW	MW	LW	SSM	WW	MW	LW	SSM	WW	MW	LW	SSM
Sitta	5.18	5.06	4.17	3.22	12.75	15.35	16.50	23.35	20.56	19.75	19.92	19.90	19.50	19.37	19.25	18.98
FD-83	5.09	5.08	4.24	3.10	12.55	15.45	16.80	22.40	20.93	20.70	19.55	19.53	19.84	19.75	19.59	19.64
NR-234	5.43	4.64	4.04	3.10	13.40	14.10	16.00	22.45	20.75	19.52	19.31	19.28	19.68	19.34	19.56	19.43
Bhittai	4.93	4.25	3.59	2.82	12.15	12.95	14.20	20.40	20.87	18.68	19.72	19.69	20.21	19.67	19.34	19.32
Sarsabz	4.34	4.48	3.73	2.90	10.70	13.60	14.80	21.05	20.52	19.97	20.08	20.05	19.46	19.33	19.45	19.35
Pfau	4.14	4.78	3.54	2.75	10.25	14.55	14.05	19.90	20.53	19.86	19.81	19.79	19.60	19.32	19.11	19.02
NR-241	5.01	4.01	3.21	2.62	12.40	12.20	12.70	19.05	20.21	20.04	18.64	18.64	19.57	19.35	19.27	19.11
Nesser	3.73	3.14	2.83	2.77	9.20	9.55	11.25	20.05	19.53	18.68	18.66	18.63	19.45	18.95	18.56	18.23
LSD	0.36				1.75				0.03				0.03			

†WW, well-watered; MW, medium-watered; LW, low watered; SSM, stored soil moisture

3.2.2. Correlations among phenotypic characters

Biomass showed non-significant correlations with most of the traits studied, but had a highly significant correlation with biomass WUE (Table 7). Biomass WUE was not correlated with any other parameter. Grain yield and grain WUE were highly positively correlated (r = 0.994**), and each showed significant positive correlations with harvest index, leaf and grain Δ, spike length and 1000 grain weight, and highly significant negative correlations with number of tillers $plant^{-1}$ and days to heading (Table 7), but neither were correlated with days to maturity.

TABLE 7. CORRELATION COEFFICIENTS AMONG PHENOTYPIC CHARACTERISTICS OF EIGHT WHEAT GENOTYPES ACROSS FOUR WATER REGIMES

Parameter	Biomass yield	Grain yield	Biomass WUE	Grain WUE	Harvest index
Harvest index	–0.557ns	0.845**	–0.526ns	0.846**	–
Biomass WUE	0.984**	0.004ns	–	–0.526ns	–
Grain WUE	–0.036ns	0.994**	0.003ns	–	–
Leaf-Δ	0.157ns	0.779*	0.198ns	0.783*	0.572ns
Grain-Δ	0.319ns	0.753*	0.335ns	0.707*	0.451ns
Tillers $plant^{-1}$	–0.224ns	–0.854**	–0.250ns	–0.814*	–0.571ns
Spike length	0.533ns	0.779*	0.545ns	0.784*	0.373ns
1000-GW	–0.016ns	0.899**	–0.036ns	0.892**	0.766*
Days to heading	0.152ns	–0.708*	0.142ns	–0.743*	–0.694ns
Days to maturity	0.532ns	–0.592ns	0.547ns	–0.635ns	–0.809*

*, $P<0.05$; **, $P<0.01$; ns, not significant.

Leaf-Δ showed a significant positive linear correlation (0.946**) with grain Δ averaged across water regimes (Fig. 8). The slopes of the regression equations and the r values increased with an increase in water stress (Fig. 8). Grain WUE was significantly and positively correlated with leaf and grain Δ (Fig. 9). Grain yield was positively and linearly

correlated with leaf Δ (r = 0.779*) and grain Δ (r = 0.753*) averaged across water regimes (Fig. 10a). Grain yield showed a significant positive linear correlation with leaf Δ under WW (r = 0.697*) and LW (0.666*) regimes, but under MW and SSM conditions the correlations were non-significant (Fig. 10b). Grain yield and grain Δ showed significant positive linear relationships under MW and LW regimes, and non-significant correlations under WW and SSM conditions (Fig. 10c).

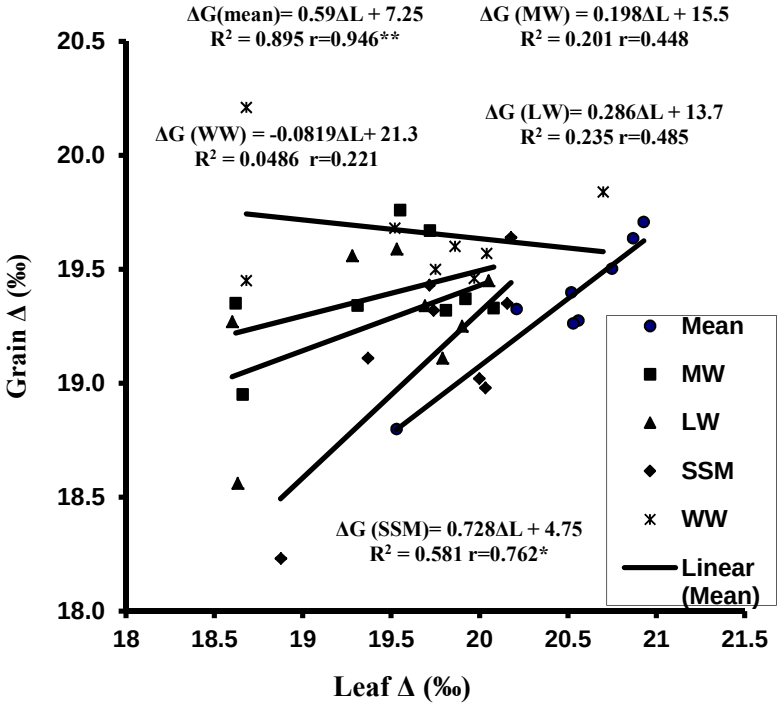

*FIG. 8. Relationships between CID of leaf (ΔL) and grain (ΔG) of eight wheat genotypes under each of four and mean of four water regimes. * = P<0.05, ** = P<0.01.*

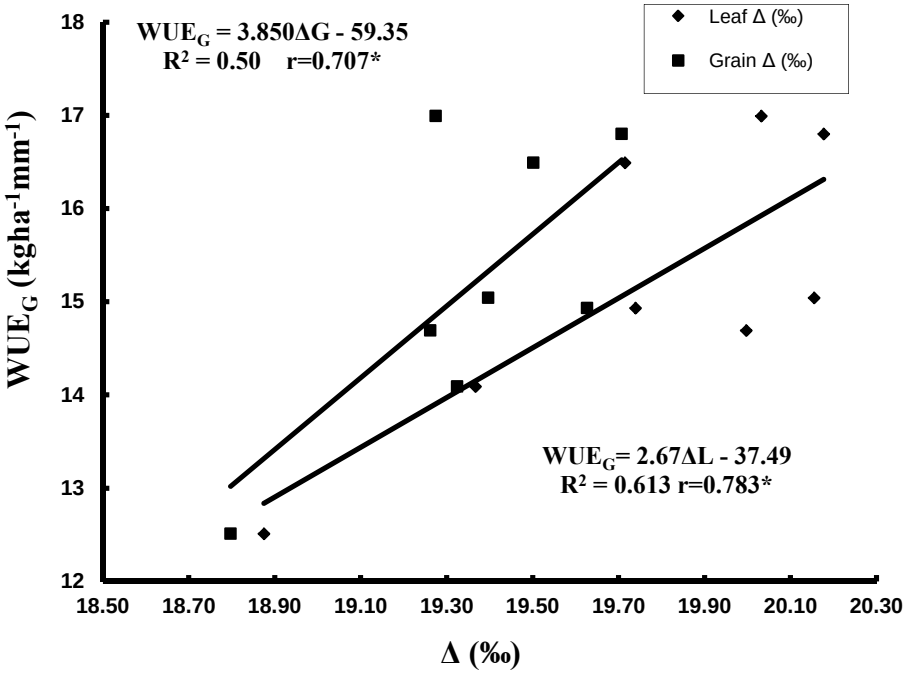

*FIG. 9. Relationships between water-use efficiency of grain (WUE_G) and CID of leaf (ΔL) and grain (ΔG) of eight wheat genotypes averaged over four water regimes. * = P<0.05.*

4. DISCUSSION

4.1. CIMMYT elite lines

Both leaf Δ and grain Δ were negatively correlated with grain yield, which may be attributed to mild temperatures and consequently low evapo-transpiration during the year compared with earlier years. Under conditions of irrigation and low evaporative demand, stomatal conductance is likely to be high [10]. Increased photosynthetic capacity would decrease C_i, leading to lower Δ values, which are expected to correlate negatively with grain yield. Grain yield and Δ provide an estimation of photosynthetic capacity [11]. Significant correlations between grain yield and grain Δ were noted under a wide range of climatic conditions [11, 12, 13, 14, 15] while correlations between grain yield and leaf Δ were reported mainly under early drought [12, 15]. The negative correlation between grain yield and Δ was due to the decrease in Δ between anthesis and maturity, even under mild water stress, as a consequence of increasing evaporative demand, and an increase in Δ under optimal irrigation during early crop growth [15]. Significant correlations under drought conditions were previously reported between leaf and grain Δ [14, 16, 17]. Under drought stress, stomatal conductance and Δ are reduced much earlier in the growth cycle. In addition, grain filling depends more on pre-anthesis reserves accumulated during periods of reduced stress [18]. As a consequence, the leaf Δ isotopic imprint on the final isotope composition of the grain is stronger than under more favourable conditions [19].

Grain yield and leaf ash content depend highly on the transpiration rate. In the present study, grain yield was strongly correlated with leaf ash content. Significant positive correlations between grain yield and leaf ash content, both at anthesis and maturity, have been reported in durum and bread wheats grown under full irrigation [15]. Considering that higher transpiration increases the concentration of passively transported minerals in the leaves [20], it can be concluded that yield was directly related to the capacity of the genotypes to maintain a high transpiration rate. High leaf ash content could also reflect more efficient translocation of remobilized C products during grain filling [21].

The positive correlation of grain yield with leaf ash content and the negative correlation with grain Δ suggest that under optimal irrigation, yield is more strongly influenced by the physiological process controlling mineral concentration in the leaf [20] than with Δ values resulting from the combined effects of stomatal conductance and photosynthetic capacity [22]. Significant correlations between Δ and ash content of the flag leaf have also been reported under severe drought stress [14, 23], indicating that genotypes that transpire more per unit of dry matter produced (high Δ, low transpiration efficiency) have higher concentrations of minerals in biomass.

The significant correlations between grain yield and leaf and grain Δ and leaf ash content under irrigated and low evaporative demand conditions in this study, and studies under terminal stress conducted elsewhere, suggest that leaf ash content is a useful indirect selection criterion for yield in environments ranging from full irrigated to strong terminal water stress conditions. Leaf ash content might be considered as a surrogate for CID as previously suggested [21, 23]. A reliable and faster estimation of ash content in plant tissues would favour the integration of this criterion into breeding programmes.

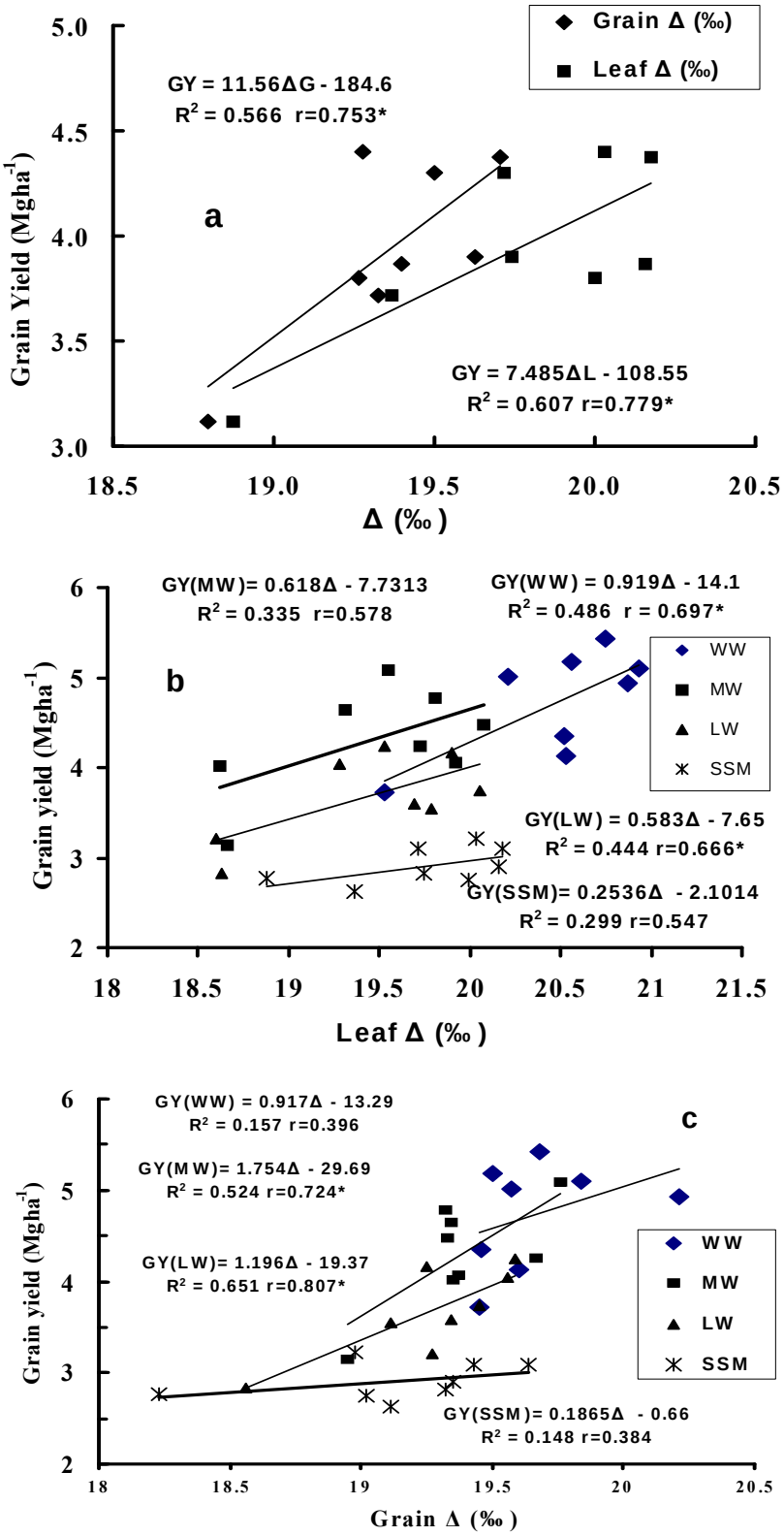

*FIG. 10. Relationships between grain yield and CID (Δ) of eight wheat genotypes for (a) average leaf and grain (b) leaf and (c) grain under four water regimes. * = P<0.05.*

4.2. Water regime

Grain and biomass yields decreased in all genotypes with increasing water stress and were accompanied by decreases in the number of tillers plant^{-1}, plant height, number of grains spike^{-1} and 1000 grain weight. The highest grain yield obtained by Sitta was coupled with a relatively higher harvest index and the highest 1000 grain weight compared with the other genotypes. The lowest grain yield obtained by the genotype Nesser, despite the relatively high biomass yield with the highest number of tillers, may be attributed to the lowest harvest index, spike length, and 1000 grain weight. The weak correlation between leaf and grain Δ was found under optimal moisture conditions, and the correlation improved with an increase in water stress. Under drought conditions, significant correlations were reported between leaf and grain Δ [14, 16, 17]. Stomatal conductance and Δ are reduced much earlier in the growth cycle under drought conditions, and grain filling depends more on pre-anthesis reserves accumulated during periods of reduced stress [18]. As a consequence, the leaf Δ isotopic imprint on the final isotope composition of the grain is stronger than under more favourable conditions [19]. The significant decrease in leaf Δ and the less pronounced increase in grain Δ with increasing water stress in the present study is in agreement with previous reports on cowpea [24, 25], wheat [26], barley [27] and Russian wild rye [28], indicating the consistent water stress effects on Δ. It was claimed that Δ of structural material represents a long term integration of C_i/C_a over the entire C assimilation period [29].

The genotype effect on leaf and grain Δ under different water regimes was found to be consistent. Lower Δ values under WW conditions indicated that Nesser assimilated more ^{13}C, while FD-83 and Bhittai discriminated against the heavier isotope to a greater degree. Genotypic variation in the response of stomata to soil water depletion was reported to have caused variation in Δ in wheat, and variation in photosynthetic capacity may also lead to cultivar variation in Δ [30]. Greater photosynthetic capacity, lower stomatal conductance, or both, may result in lower values of C_i/C_a. This means that greater photosynthetic capacity should be reflected in lower values of Δ unless stomatal conductance also increases to balance the change in photosynthetic capacity and maintain C_i constant.

Grain yield was positively correlated with both leaf and grain Δ. The grain Δ correlations were significant under moderate water stress (MW and LW), but were non-significant under optimal conditions (WW) and severe stress (SSM). Positive correlation between grain yield and grain Δ were found under a wide range of climatic conditions [11, 12, 13, 15], while correlations between grain yield and leaf Δ were reported mainly under early drought conditions [14, 31]. Wheat grain yield was positively correlated with grain Δ under post anthesis water stress [32]. Under Mediterranean conditions, a significant positive correlation was repeatedly found between grain yield and grain Δ [14, 33], and between grain yield and flag leaf Δ under severe stress [23, 34].

Conversely, under residual soil moisture (out-of-season rainfall) conditions, genotypes with low Δ values in seedlings were found to be more productive [22], while the relationship between grain Δ and yield varied greatly with total rainfall and its distribution [22, 32, 35]. A positive correlation between Δ and the production of grain and total biomass was reported in wheat under controlled conditions [36]. Positive correlations between grain yield and both grain and leaf Δ under terminal water stress at anthesis, strong correlations under residual soil moisture stress and no correlation under optimal irrigation were reported in China [37], indicating the potential utility of Δ as an indirect selection criterion for grain yield, and as a phenotyping tool under limited irrigation.

Grain WUE was positively correlated with both leaf and grain Δ and with harvest index, but was inversely correlated with number of tillers and time to heading and maturity. An increase in grain WUE can be obtained by enhancing either the quantity of water transpired by the crop, the transpiration efficiency, or the harvest index [2], and the relative contribution of each component to grain WUE is likely to depend upon their variation within the evaluated germplasm. Sitta and FD-83 gave high grain yields with relatively greater increases in grain WUE under increasing water stress, suggesting that both genotypes may be very useful for drought and water limited environments. The increase in grain or biomass WUE with increasing water stress may be because of a higher reduction in stomatal conductance than a reduction in assimilation. Extended drought can increase WUE substantially [38, 39] although that effect may not always be found [40]. A significant negative correlation between Δ and WUE was found in *Eucalyptus camaldulensis* and a significant positive correlation in *Acacia ampliceps* subjected to different water regimes [41].

An increase in WUE appears to be an alternative strategy for improving performance, where additional water is not available to the crop (i.e. all the available water is exhausted during the crop cycle). Therefore, lines producing greater biomass and yield due to superior WUE will have lower $\Delta^{13}C$ and perform better, in contrast to situations where lines perform better because of increased access to water [4]. The lowest leaf and grain Δ values were under SSM, where the most severe water stress corresponded to the lowest grain yield. Enrichment in ^{13}C in grain may be related to progressive soil drying and stomatal closure [30]. In the present study, water use efficiency and grain yield were mainly driven by harvest index, as previously reported [42, 43], due to the wide variation of this trait among the tested genotypes.

The present study confirms significant positive correlations between Δ (leaf and grain) and grain yield or grain WUE in bread wheat genotypes, indicating that Δ is a candidate for indirect selection for high grain yield and crop WUE under different (WW, MW, LW and SSM) water regimes. However, for selection or identification purposes, early leaf Δ would be preferred over grain Δ as it is less time consuming. Genotypic variation in Δ and its relationship with grain WUE or agronomic WUE can be exploited for increasing crop productivity by selecting water-use efficient genotypes for target environments.

5. CONCLUSIONS

Due to insufficient variability in Δ among the 130 genotypes screened during the 2004–2005 and 2005–2006 seasons, a crossing and generation programme was initiated to evolve new genotypes with a larger Δ variation. Crosses were made among selected combinations of high yielding and contrasting Δ wheat lines in all possible combinations in a diallel fashion during two seasons (2006 and 2007). From the 1st year crosses 2718 F_0 seeds were harvested and were sown during the 2007 season for advancement of generation (F_1). Out of 1500 F_1 plants 35872 F_2 plants were grown during 2008, and 417 plants with desirable traits were selected. These plants will be grown as F_3 in the 2008–2009 season. The seeds from the 2nd phase crosses were grown in 2008 for advancement of generation F_1. From F_1, 2855 plants were harvested at maturity and will be grown for F_2 in the 2008–2009 season.

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USE OF CARBON ISOTOPE DISCRIMINATION AS A SELECTION TOOL IN WHEAT BREEDING FOR DROUGHT TOLERANCE IN YEMEN

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Abstract

The objectives of the present investigation was to determine the potential of carbon isotope discrimination (CID, Δ) as an indirect selection criterion for the tolerance of wheat to drought, heat and low soil fertility under Yemeni field conditions. From the results of kernel-Δ of 63 wheat accessions (local and improved lines) obtained in the first year of the project, several parents were selected for a crossing programme. More than 120 crossbreds were obtained and planted in the field as the F_1 generation (July–November 2005). The harvested plants were planted in the F_2 population during the season December 2005–April 2006. Spike-Δ was analyzed for parents and 328 F_2 individual plants of the cross between the local *Triticum dicoccum* var. Arabi47 and the advanced line N°. 4 issued from the cross *T. polonicum x T. durum*. Results obtained for spike-Δ permitted heritability in the F_2 population to be calculated (0.70), and high variability for this trait was observed (14.03–18.91‰). Relationships between drought tolerance traits such as Δ, plant development or morphological attributes such as plant height, tiller number, number of spikes per plant and number of days from sowing to heading and maturity were evaluated. The seeds of 287 individual plants in the F_2 population were planted in F_3 rows under field conditions during the 2006 season (July–November). Phenological and morphological traits and grain yield were measured, and the correlations between Δ and these parameters were determined. During the December 2006–May 2007 season, 83 F_4 lines divergent for Δ were evaluated under field conditions and grain was analyzed for Δ. There was no correlation between spike-Δ in the F_2 plants and grain Δ in F_4. F_5 adapted lines were selected and planted in the July–November 2007 season at three sites for production and adaptation measurements. Twenty F_6 lines were evaluated at several rain fed sites in Yemen.

1. INTRODUCTION

1.1. Location and areas

Yemen is located in the south-west of Arabia and is the most fertile and densely populated country in the Arabian Peninsula. It lies between 12 and 19° 20′ North and 45 to 54°20′ East. The country is bordered by the Arabian Sea in the South, Saudi Arabia in the North, the Sultanate of Oman in the East, and the Red Sea in the West. Yemen has a total land area of about 55.5 million ha. However, the cultivated area has been estimated at about 3%. Because of a shortage in rainfall, the cultivated land ranges from 1.4–1.6 million ha. There are about 16 million ha classified as range lands and 4.2 million ha as woodland.

Based on 2005 statistics, it has been estimated that 82% of the cultivated area is utilized for cereal production, grain legumes and forage crops. Vegetables and fruits cover only 12%, and cash crops are planted on 6% of the total area. According to the 2004 census, the total population of the country is estimated at 21.5 million, the annual growth rate at 3.7% and the population density at 28 km^{-2}.

1.2. Climate

Agro-climatic conditions in Yemen are classified as semi-arid, arid, and extreme arid. The aridity depends on topography; more rain, luxuriant vegetation and rich bio-diversity are related to the altitude, aspect, edifice and biotic factors. Agriculture is still subsistence oriented and heavily dependent on climate. Altitude is an important factor determining vegetation; it ranges from sea level to well over 3600 m in the western mountains. Rainfall and temperature correlate with altitude. This led to the diversity of the natural vegetation, which can be classified according to topography to six zones (coastal, southern, northern, western, highland and eastern plateaux).

Water resources are rainfall, Wadi flood-water and underground water. There is great variation in the annual rainfall; in the coastal area the annual rainfall is about 50–100 mm while in the high lands it is about 400–800 mm. The temperature differs according to the altitude, aspect and the green cover of the land. It reaches 38–40 ºC during summer in the coastal region, and in the highlands it drops to zero in winter, and reaches 32 ºC during summer.

However, the contribution of the coastal-area to total agricultural production is approximately 39%. It has been reported that rain fed agriculture has decreased by 40%. Consequently, the use of groundwater has increased to include 30 000 wells, most of which are used for irrigation. The annual decline of aquifer levels in most water basins averages 1–8 m. Country-wide, current quantities of pumped water are estimated at 138% (2.8 billion m^3) of the annual renewal estimated at around 2.1 billion m^3. This means that Yemen consumes 700 million m^3 over and above its renewable water resource.

Finally, the agriculture sector plays an important role in the country's economy, and contributes 20% to the national income. There is a shortage of agricultural products to meet the national demand.

1.3. Wheat production

Cereals and cereals products rank highest among Yemini agricultural imports (more then 2 million tons a^{-1}). Wheat is mainly grown in the eastern plateau (Mareb, Al-jawf and Hadhramout), in the highland regions, Northern mountains (Hajah, Al Mahwit, Amran and Saadah), highland plains (Sana'a, Dhamar and Al Baydah) and Southern highlands (Ibb and Taiz).

In the highlands wheat is grown twice a year (once under rain fed conditions during July–October and then under irrigation or reserved soil water in December–April). However in the eastern region wheat is grown completely under irrigated conditions during October–March. In 2005 it occupied about 14% of the total area of cereals grown and 21% of the total cereal production, ranking second among cereal crops after sorghum [1].

The agricultural productivity of Yemen is greatly affected by drought and limited water resources. Plant breeding for resistance to biotic and abiotic stresses was considered a good strategy for the improvement of productivity [1]. The area of wheat sown during the period 1981–1998 was around 100,000 hectare (Fig.1); production increased during this period, due to increases in both planted area and yield ha^{-1}, but with marked fluctuations in 1983, 1984, 1991 and 1997. It is seem that in this case the impact of environmental conditions is more than biological or social factors, with the average yield not exceeding 1.5 t ha^{-1}. This is mainly due to highly erratic and low rainfall as well as degraded soils deficient in plant nutrients. However, most of the wheat has been intensively cultivated in mountainous areas using traditional methods. Until 1980, the wheat area was populated by tetraploid species;

then the hexaploid (*Triticum aestivum*) varieties were introduced and replaced progressively these local forms, but until 2000, the local tetraploids still covered about 75% of the wheat-cultivated area. Among these tetraploid species can be found *Triticum dicoccum* as Bouni and Alass landraces, as well as some forms belonging to *T. durum* as Samra cultivars. The *dicoccum* wheat, known as *emmer* wheat, has been cultivated in many parts of the world, but it is very much appreciated in Yemen, due to the local diet preference, its adaptability to local conditions and suitability for making traditional breads.

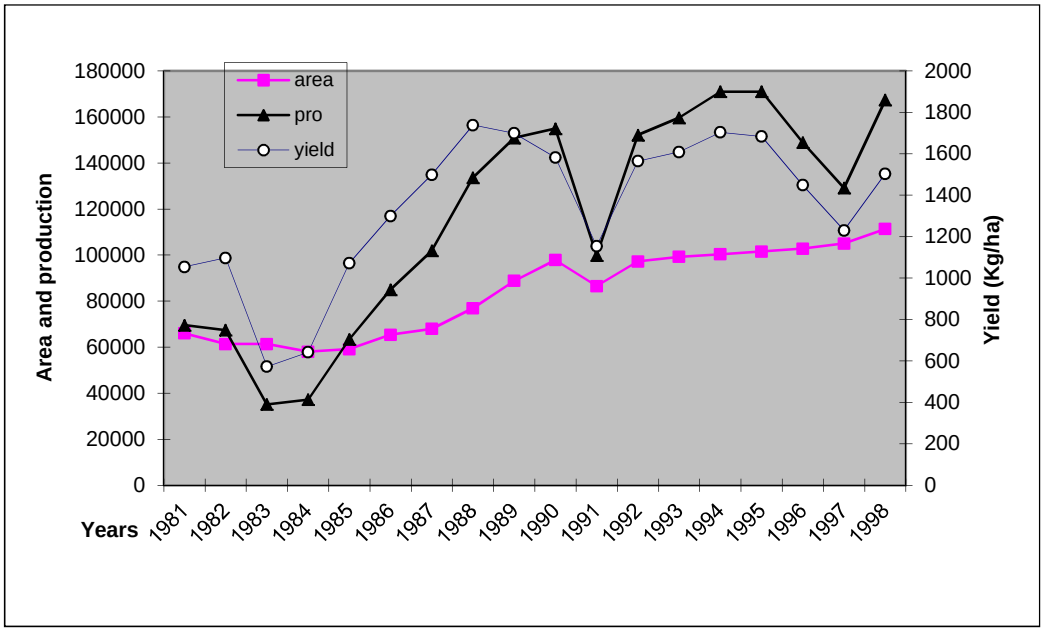

FIG. 1. Areas (ha), production (ton) and yield (kg hā[1]) of wheat in Yemen for the period 1981–1998.

As in many countries of the region, cereal production in Yemen has been strongly affected by drought. In this regard, two approaches could be adopted to address this problem. Irrigated areas could be increased, but salinization (e.g. Marib and Hadramawt areas), increased susceptibility to foliar diseases and accelerated depletion of water resources are known negative outcomes. The second approach is the improvement of water use efficiency and drought tolerance of wheat cultivars. This long term strategy, which is a part of a general approach to enhance the sustainability of farming systems, is the basis of the present study.

Several mechanisms of drought tolerance are involved in dehydration tolerance, such as tolerance of the photosynthetic apparatus with better transpiration efficiency, tolerance of photosystem II, better storage, translocation and partitioning of plant assimilate. Plants ensure the maintenance of turgor essentially through three mechanisms: water absorption, reduction of water loss and osmotic adjustment.

1.4. Carbon isotopic discrimination (CID, $\Delta^{13}C$, Δ) and water use efficiency (WUE)

Previous reports and theoretical considerations showed that variations in WUE should be associated with differences in the ability of plants to discriminate against ^{13}C compared with ^{12}C during stomatal CO_2 diffusion and enzymatic fixation [2, 3, 4]. Genetic variations in Δ have been observed in cultivated [5, 6] and tetraploid [7] wheats.

In water limited environments, providing all genotype use the same amount of water for transpiration, then the relationship between Δ and transpiration efficiency (TE) in the field

should translate to low genotypes having greater biomass and hence the potential for higher yields [8]. The major physiological attributes that support net C gain under drought can be approached by the relationship [9]:

$$YE = T \times WUE \times HI$$

where YE is economic yield, T is transpiration, WUE is crop water use efficiency (defined at the whole plant level as the ratio of total plant dry matter accumulated per total water consumed, and at the leaf level, as the ratio of photosynthetic C gain to transpirational water loss) and HI is harvest index.

Increasing attention has focused on WUE during the last twenty years. When the stomata open wide, thus increasing the internal pressure of CO_2, the discrimination by RuBisCO becomes greater as compared to discrimination by diffusion, increasing the overall Δ values. As a consequence, Δ values in plant dry matter were noted to be positively correlated with the ratio of the intercellular and the atmospheric partial pressures of CO_2 (C_i/C_a) and negatively correlated with WUE [5, 10]. The determination of Δ has been facilitated by the introduction of an automatic N_2 sample preparation apparatus linked to a dual inlet isotope ratio mass spectrometer for the analysis of $\delta^{13}C$ [11, 12].

A successful breeding programme for improving WUE in wheat based on selection for low values of Δ requires a better knowledge of the gene action in field grown wheat [6]. Several studies have shown negative correlations between Δ and total biomass production for plants grown under field conditions [4, 5]. Other authors have observed positive correlations [3, 13, 14]. These contradictory results could be related to the correlation observed in several species between Δ and earliness [11, 13, 15, 16, 17, 18], since earliness is a well known escape mechanism. This relationship should be taken into account in the use of Δ in breeding programmes, particularly in environments where terminal water stress is frequent. This relationship could be explained by the fact that early genotypes can fix much of their C in a relatively drought-stress-free period that could engender high C_i/C_a values [16].

Multi-location evaluation for a given trait could be a good way to answer all of these questions. It would help the cereal breeders to verify the potential usefulness of the trait under field conditions, by providing fundamental genetic information *(h^2b, h^2n, h^2r* values), and permitting definition of the relationships between the selected trait and other morpho-physiological traits and yield components [1, 7, 19, 20].

The objectives of the present research were to determine the potential of indirect selection for CID to improving drought tolerance of wheat (grain and biomass yield under drought) under Yemeni conditions, and to select lines adapted to Yemeni conditions (drought, heat and low soil fertility).

2. EXPERIMENTAL METHODS

The cross between the local landrace Arabi (YGRC 47) and line derived from *Triticum polonicum*/*T. durum* (N^o. 4) [7] was selected for this study. Parents used in this research were selected from a large range of material (including Yemeni local *T. dicoccum* and *T. durum* landraces, advanced lines derived from *dicoccum/durum, and polonicum/durum* crosses, and some *T. aestivum* checks) evaluated for yield and several morpho-physiological traits including $\Delta^{13}C$, under rainfed conditions in 2004. The overall scheme of the research is shown in Fig. 2.

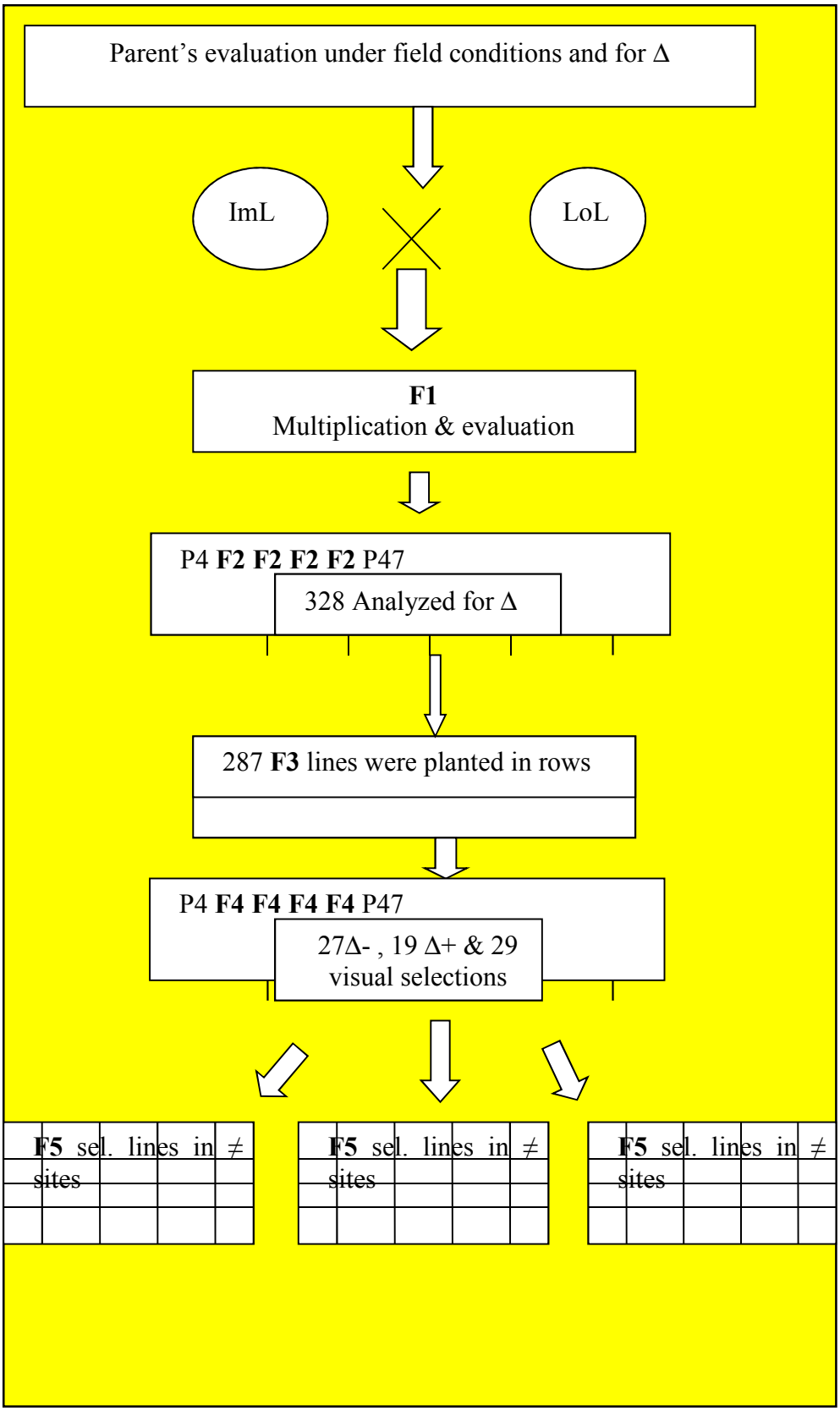

FIG. 2. Experimental schematic.

2.1. F_2 evaluation (Dec. 2005–May 2006)

Experiments were established in the Faculty of Agriculture experimental field with a winter sowing (December 2005); more than 6 crosses corresponding to local varieties and advanced lines were planted. Parents of these crosses showed wide variations in phenology, morphological traits and yield components as well as in kernel-Δ. Eight flood irrigations were applied during the growing season. This experiment was designed to test if the parental crosses were successful in producing F_2 plants, to select adapted lines from these crosses on the basis of spikelet-Δ, and to estimate the broad sense heritability of this trait.

Heritability was calculated according to the formula $[vF_2 – (vp1 + vp2)/2]/vF_2$, where (vP) is the variance of the parents, and (vF2) is the variance in F_2 segregation population [7, 19]. In addition, the effect of drought on the plants that were selected for the next F_3 generation was evaluated under rain fed conditions (July 2006).

2.2. F_3 evaluation (July–Nov. 2006)

287 plants harvested in F_2 were selected for their adaptability to the local conditions. Seeds of F_3 lines were planted in a screened nursery under rain fed conditions, in rows 1.5 m in length. Fertilizer was added as an N: P: K mix (60: 60: 0 kg ha^{-1}) during land preparation and parents were planted after 10 lines. This experiment was to multiply seed, evaluate these materials, study the effect of selection on the basis of Δ, and to effect selection on the basis of morphological and agronomical traits related to drought tolerance under water stress in F_3 lines.

Phenology (heading date and date of maturity) was recorded, and morphological traits including growth vigour (GV), form of plant spike (form), plant colour (PC), plant height (PH) and grain production in 10 plants were measured.

2.3. F_4 evaluation (Dec. 2006–May 2007)

75 F_4 lines selected in F_3 on the basis of field evaluation and divergent values for Δ in F_2 were planted under field conditions; each entry planted on a 1 m^2 plot with an interval of parents after 10 entries. The objective of this experiment was to evaluate biomass and grain production of the selected lines for their high or low Δ values, and visual selection on the basis of morphological or agronomic traits.

Traits evaluated in the field included phenology, morphological traits including growth vigour (GV), form of plant spike (form), plant colour (PC), plant height (PH), peduncle length (PDL), biomass production (BP), grain yield (GP), harvest index (HI), 1000-kernel weight (1000KW).

2.4. F_5 evaluation (July–Nov. 2007)

About 53 F_5 lines selected in the basis of field evaluation and Δ results were planted under three different conditions for grain and biomass production, as well as farmer impression. The three sites were Sana'a (farm of the Faculty of Agriculture) under rain fed conditions and under supplemental irrigations applied at planting and heading. The second site was at Al Irafa (Al Sadah district) characterized by high rainfall, and the third site was at Masiab (Bani-Matar district) characterized by low rainfall.

2.5. Analytical

The isotope ratio analysis of plant samples for $\delta^{13}C$ was carried out at the Soil Science Unit, Agriculture and Biotechnology Laboratory, Seibersdorf, Austria.

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{reference}) - 1] \times 1000$$

where R is the isotope ratio of $^{13}C/^{12}C$. A secondary standard, calibrated against the primary standard, Pee Dee Belemnite (PDB) fossil carbonate, was used as the reference.

$\Delta^{13}C$ in the plant samples was calculated according to [12] as:

$$\Delta^{13}C\ (‰) = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000$$

where $\delta_p = \delta^{13}C$ of the plant sample and $\delta_a = \delta^{13}C$ of atmospheric $CO_2 = –8‰$.

3. RESULTS AND DISCUSSION

Evaluation of a large range of material under rain fed (summer season) and irrigated (winter season) conditions for yield and several morpho-physiological traits including $\Delta^{13}C$, facilitated the crossing of the best adapted and most divergent parents for $\Delta^{13}C$. More than 120 seeds obtained from this cross were planted in the summer season (July–Nov. 2005) under field conditions, and the derived F_1 plants were evaluated in the field for producing F_2 populations.

3.1. F_2 and F_3 generations

About 328 individual plants in the F_2 segregating population were identified and harvested in April 2006. The individual spikelet parts (awns, glumes and rachises) from each F_2 plant and the parents were prepared for $\delta^{13}C$ analysis.

The F_2 segregating population and parents showed wide variations in phenological and morphological traits and yield components (Table 1). Individual plants in F_2 were highly segregated for most of these characters. Plant height in F_2 varied from 66 to 90 cm and time to heading varied from 77 to 95 days.

TABLE 1. MEAN AND STANDARD DEVIATION FOR MORPHOLOGICAL AND PHENOLOGICAL TRAITS ASSESSED IN THE F_2 POPULATION AND PARENTS

Generations	No. of plants	Plant height (cm)	Days to heading	Tillers plant $^{-1}$	Spikes plant $^{-1}$	Plant vigour†
T. dicoccum Arabi (local N°. 47)	15	70 ± 5	80 ± 4	8.0 ± 2	6.0 ± 2.0	3.0
Polonicum/durum advance line (N°. 4)	13	100 ± 4	95 ± 5	6.0 ± 2	4.0 ± 2.0	2.0
F_2 generation	328	78 ± 11	87 ± 9	7.2 ± 3	5.1 ± 2.8	2.6

†1 = poor to 3 = very good.

Several F_2 plants reached heading earlier or at the same time as the local parents under optimum irrigation. Early heading wheat genotypes are very advantageous under Yemeni conditions due to severe terminal drought in September-October and high temperatures in April-May, which corresponds to the grain filling period [1].

Plant height of selected crosses remained high, as well as resistance to lodging. This character under water stress, allowed high biomass production as well as more grain filling [9]. Analyses of variance illustrated a high variation among plants from the F_2 population.

Plants with poor vigour in early stages had reduced plant vigour in later stages. However, the local landraces had very good vigour in the early stages and intermediate vigour at heading. Rapid and vigorous early growth is important in dry environments, particularly on soils with poor water holding capacities [8]. Under such conditions, it is important for seedlings to develop sufficient biomass earlier when moisture is available. One reason for the improved yield with early growth may be a reduction in soil evaporation resulting from the greater ground cover. Under water stress, considerable variation existed for early growth, particularly in rates of tiller production and ground cover. We considered that selection for this trait would be pronounced in F_3 lines.

Parents also showed variation in the number of tillers and spikes per plant, and their F_2 population hybrids also showed height variations (Table 1), and the values of Δ for 328 F_2 plants were more variable than the parents, with a range of 14.03–18.91‰ and a mean of 16.75‰ (Table 2).

Broad sense heritability (h^2) of Δ in F_2 shows that a moderately large heritability (0.70) controls the transmission of this trait from parents to generations, in accordance with previous observations [6].

TABLE 2. RANGE, MEAN AND VARIATION OF Δ (‰) IN PARENTS AND F_2 GENERATION AND HERITABILITY

Material	N°. of plants	Range	Mean	Variation	Heritability (h^2)
T. dicoccum Arabi (local N°. 47)	15	16.50 – 18.50	17.60	0.36	
Polonicum/durum advance line (N°. 4)	13	16.00 – 17.50	16.59	0.19	
F_2 generation	328	14.03 – 18.91	16.75	0.92	0.70

A significant correlation (P<0.05) was found between Δ and grain yield in F_3 (Fig. 3), and between Δ and spikelet number per plant in F_2 (Table 3). Crosses in F_3 showed high variations for morphological and phenological traits and yield components.

Leaf colour during vegetative stages varied considerably among F_3 lines, and this trait can be considered as a tool for selection under Yemeni drought conditions. Leaves with lighter colour were observed more in local landraces than in tetraploid lines [1]. Plant height is inherited from tetraploid parents, and the higher plants in F_2 remained the higher plants in F_3, as well as retaining resistance to lodging. The high numbers of lines selected were from lines exhibiting stable characteristics related to drought tolerance and yield components.

Correlations between Δ and drought tolerance traits such as plant development or morphological attributes such as plant height, leaf orientation, tiller number and production under drought (yield and yield components) were confirmed with the results in F_4. From the evaluation in F_3 and the results of Δ in F_2 more than 89 lines were selected by the end of 2006, within which 43 has been selected on the basis of breeder impression (visual selection

of morphological aspects); 27 lines were selected for low values of spike-Δ and 19 lines for high values of spike-Δ. This evaluation identified lines exhibiting stable characteristics related to drought tolerance and yield components as well as grain Δ. Analysis of Δ in the F_4 population allowed confirmation of the effectiveness of selecting in the early F_2 generation as a tool in breeding for drought tolerance under Yemeni conditions. From this evaluation we identified drought tolerant lines to be used in selection programmes or for breeding to incorporate new characters into the local forms.

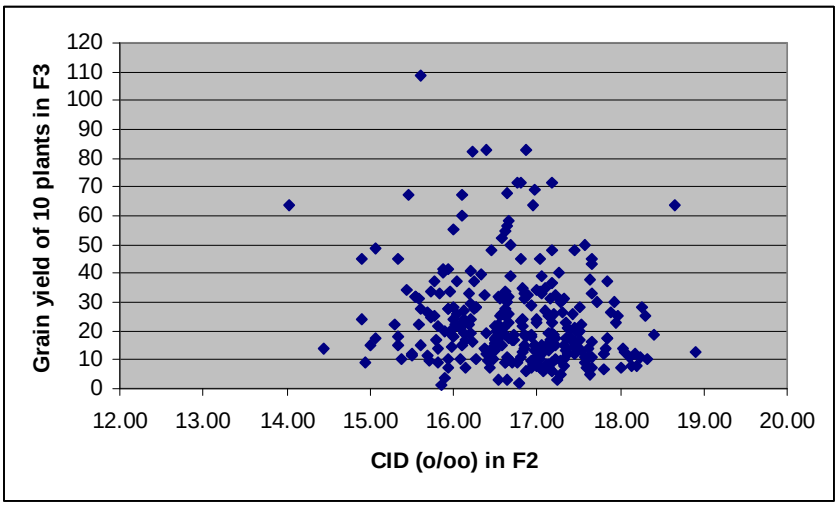

FIG. 3. Relationship between CID (‰) and grain yield (g 10-plants^{-1}) in F_3.

TABLE 3. CORRELATION COEFFICIENTS AMONG F_2-Δ AND MORPHOLOGICAL TRAITS IN F_2 AND F_3 LINES

Trait †	F	F_2-Δ	% C	F_2 lines			F_3 lines				
				PLH	TN/p	Sp/p	Gv	Form	Dh	PLH	DM
% C		0.191									
Prob. F_2		0.00									
PLH	F_2	0.026	0.079								
Prob.		0.656	0.181								
TN/p		0.174	0.107	0.315							
Prob.		0.003	0.071	0.00							
Sp/p		0.078	0.091	0.339	0.580						
Prob.		0.188	0.127	0.00	0.00						
Gv	F_3	0.203	0.028	0.179	0.117	0.115					
Prob.		0.001	0.641	0.002	0.050	0.053					
Form		0.053	0.006	0.049	–0.005	–0.024	0.431				
Prob.		0.309	0.925	0.406	0.927	0.690	0.018				
Dh		0.046	–0.017	0.005	0.026	–0.011	–0.078	–0.083			
Prob.		0.438	0.775	0.927	0.666	0.857	0.192	0.162			
PLH		–0.047	0.039	–0.023	0.116	0.007	0.006	0.015	0.252		
Prob.		0.024	0.475	0.693	0.051	0.907	0.921	0.805	0.00		
Dm		0.046	–0.048	0.133	0.080	0.066	0.397	0.212	0.015	0.021	
Prob.		0.43	0.417	0.024	0.508	0.179	0.00	0.00	0.805	0.92	
Y/10p		–0.15	0.043	–0.003	0.039	–0.760	0.287	0.157	0.207	0.132	0.120
Prob.		0.010	0.472	0.957	0.515	0.199	0.00	0.00	0.00	0.025	0.026

†Prob. = probability; % C= total carbon; PLH = plant height; TN/p = tillers plant^{-1}; Sp/p = spikelets plant^{-1}; Gv = growth vigour; form = form of plants; Dh = days to heading; Dm = days to maturity; Y/10p = yield 10-plants^{-1}

3.2. Evaluation of F_4

Parents and the 83 selected F_3 lines were evaluated for their adaptation under field conditions in the winter season (minimum irrigations) for morphological and agronomical traits and grain Δ. The minimum, maximum, average and standard deviation of Δ, yield and morphological and phenological traits in parents and F_4 lines are shown in Table 4. Large variations were observed for Δ in the F_4 segregating populations, as well as for production and morphological traits compared with the parents.

The correlation between spikelet-Δ in F_2 and grain Δ in F_4 was not significant (r = –0.11). Significant negative correlations (P<0.05) were observed between Δ and biomass production, plant colour at early and late stages and late plant vigour (Table 5).

3.3. Evaluation of F_5

Results of 53 F_5 lines selected and evaluated under rain fed conditions in three locations show that several lines had an earlier heading date than local landraces, and exhibited plant height, biomass production and grain yield greater than local landraces. Evaluation of the selected adapted lines in the farmer's field allowed farmers to declare their preferences for grain colour, form of grains and form of spikes.

TABLE 4. STATISTICAL PARAMETERS FOR Δ, YIELD AND MORPHOLOGICAL TRAITS FOR THE SELECTED F_4 LINES AND PARENTS (P4 AND P47)

Lines†	Δ	Yield (t ha^{-1})		Length (cm)‡				DH#	Plant colour		Plant vigour	
	(‰)	Grain	Biomass	AWL	SL	PDL	PLH	(d)	Late	Early	Late	Early
P4												
Ave.	15.84	1676	5730	13.7	7.1	59.6	98.3	77.0	1.8	2.0	1.8	1.8
SD	0.06	104	701	0.4	0.8	2.8	6.9	1.1	0.4	0.9	0.7	0.8
Min.	15.78	1560	4560	13.0	6.0	55.0	86.7	75.0	1.0	1.0	1.0	1.0
Max.	15.93	1800	6500	14.0	8.0	63.0	106.0	78.0	2.0	3.0	3.0	3.0
F_4												
Ave.	15.70	1399	3778	11.7	6.1	48.3	81.7	66.7	1.9	1.9	2.5	2.5
SD	0.57	421	1221	1.8	0.9	6.6	9.9	4.4	0.7	0.7	0.6	0.7
Min.	14.09	500	1812	5.0	4.0	35.0	45.0	53.0	1.0	1.0	1.0	1.0
Max.	16.81	2260	6342	15.0	8.5	70.0	103.0	76.0	3.0	3.0	3.0	3.0
P47												
Ave.	16.36	1284	5306	11.4	5.3	39.8	63.5	60.2	2.0	2.4	2.8	3.0
SD	0.27	187	669	1.3	0.7	4.3	9.5	3.8	0.6	0.5	0.4	0.0
Min.	16.04	1120	4320	9.0	4.3	33.3	49.3	56.0	1.0	2.0	2.0	3.0
Max.	16.81	1560	6342	12.7	6.3	47.0	72.7	66.0	3.0	3.0	3.0	3.0

†Ave. = average; SD = standard deviation; Min. = minimum; Max. = maximum.
‡AWL = awn; SL = spikelet; PDL = peduncle; PLH = plant height.
#DH, days to heading.

TABLE 5. CORRELATION COEFFICIENTS† BETWEEN CID, YIELD AND MORPHOLOGICAL AND PHENOLOGICAL TRAITS IN F4 LINES

Trait	Δ	Biomass	Grain yield
Grain yield	–0.05 ns	0.27*	
Awn length	0.30*	0.08ns	–0.02ns
Spikelet length	–0.17 ns	0.28*	–0.03ns
Peduncle length	0.18 ns	–0.01ns	0.03ns
Plant height	0.08ns	0.14 ns	0.15 ns
Days to heading	0.03ns	0.13ns	0.11ns
Late plant colour	–0.34*	0.28ns	0.15ns
Early plant colour	–0.32*	0.09ns	0.08ns
Late plant vigour	–0.22*	0.08ns	0.24*
Early plant vigour	0.01ns	0.09ns	0.17 ns
Δ		–0.27*	–0.05

†ns, not significant; *, $P<0.05$.

Correlations between Δ in F_2 and F_4 lines with grain and biomass yields and harvest index in F_5 lines grown at three sites were only significant at the Sana'a site (Table 6). Significant negative correlations were seen between F_2-Δ and F_5 biomass and grain yields, and between F_4-Δ and F_5 grain yield (Table 6). A significant positive correlation was obtained between F_4-Δ and F_5 harvest index. The negative correlations between Δ and yield in the dry site show that breeding for yield should involve selecting for low Δ.

TABLE 6. CORRELATIONS BETWEEN Δ IN F_2 AND F_4 GENERATIONS WITH GRAIN YIELD (GY), BIOMASS PRODUCTION (BP) AND HARVEST INDEX (HI) OF F_5 LINES IN THREE SITES

Δ	Sana'a			Al Irafa			Masiab		
	BP	GY	HI	BP	GY	HI	BP	GY	HI
F_2	–0.27*	–0.22*	–0.05ns	0.10ns	0.16ns	0.15ns	0.06ns	0.07ns	0.16 ns
F_4	–0.15ns	–0.24*	0.26*	0.07ns	0.02ns	–0.06ns	–0.17ns	–0.04ns	0.18 ns

†ns, not significant; *, $P<0.05$

There was genotypic variation in the productivities of these advanced F_5 lines, which were selected on the basis of low and high Δ values in the early F_2 generation (Table 7). These results show that selecting for Δ in early generations to be a potentially valuable tool in breeding programmes for improving water use efficiency (WUE) and productivity under Yemeni conditions.

TABLE 7. STATISTICAL PARAMETERS FOR BIOMASS PRODUCTION (BP), GRAIN YIELD (GY) AND HARVEST INDEX (HI) IN THREE SITES

Statistical parameters	Sana'a			Al Irafa			Masiab		
	BP ($g\ m^{-2}$)	GY ($g\ m^{-2}$)	HI (%)	BP ($g\ m^{-2}$)	GY ($g\ m^{-2}$)	HI (%)	BP ($g\ m^{-2}$)	GY ($g\ m^{-2}$)	HI (%)
Average	246	103	42	781	202	26	335	136	41
Std. dev.	84	46	10	489	124	7	96	45	7.6
Maximum	560	226	65	2200	510	60	574	264	57
Minimum	95	36	20	180	50	18	166	58	23

At the end of these evaluations more then 20 advanced F_6 wheat lines were selected for their adaptation to rain fed cultivation in the high altitude area of Yemen, and this material was integrated into the national wheat breeding programme through Ph. D student research and staff involvement from the Agriculture Research Authority of Yemen during the period of 2007–2010.

5. CONCLUSIONS

Research conducted in the first and second years (2005–2006) of the study produced crosses on the basis of Δ. Heritability of Δ in the F_2 segregating population was estimated, and the F_3 hybrids were characterised on the basis of morphological, phenological and yield components under field conditions, which were then correlated with Δ values. About 89 promising lines were selected and planted in the F_4 generation for preliminary yield evaluation and for characterization of low and high values of Δ, which formed the basis of visual selection (morphological and breeder observations) under field conditions. Significant correlations between Δ and yield, yield components and morpho-phenological traits were obtained in F_4 lines and at one site in F_5. Selected materials were under evaluation post-2007 in several high altitude regions of Yemen for selecting tolerant lines adapted to drought conditions.

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RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION ($\Delta^{13}C$) AND WATER USE EFFICIENCY OF DURUM WHEAT IN THE SYRIAN ARAB REPUBLIC. 1. FIELD EVALUATION

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Abstract

Field experiments were conducted over three seasons (2004–2005, 2005–2006 and 2006–2007) using six durum wheat genotypes, similar in phenology, to minimize the genotype x environment (G*E) interaction, but varying in grain carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ). The four field sites at Tel Hadya, Breda, Muslmieh and Yahmoul were fully characterized according to environment and soil classification. Plant growth and development were monitored throughout the growing season. Δ was measured for every plot using grain or the 4th fully-expanded leaf. Also, ash and protein contents of grain were analyzed. Radiation use efficiency was measured at Tel Hadya only. Stomatal conductance and resistant as well as canopy temperature was also measured for the different sites and years. Soil water content throughout the growing season was measured at some sites to calculate water use. Variability between sites was mostly related to rainfall, with variability within a site being related to season. Δ was lower at the drier sites and higher at the wettest site, ranging from 12.88 to 16.62‰ for grain, and 19.97 to 22.57‰ for the 4th fully-expanded leaf. Positive correlations were found between grain Δ and yields of grain and biomass. The stronger correlations were at the drier sites, whilst at the wettest site (Yahmoul) there was no significant relationship. Δ was also related to ash and protein content. The genotypic variation in Δ (grain and leaf) was substantially independent of season or site.

1. INTRODUCTION

The primary challenge in wheat improvement programmes in Mediterranean areas is to improve drought tolerance, yield stability and water use efficiency [1]. Wheat breeders at the International Centre for Agricultural Research in the Dry Areas (ICARDA) have conducted more than two decades of research to develop, select and test genotypic performance against different biotic and abiotic stresses, in addition to improving yield stability and grain quality [2].

Several breeding methods and selection approaches/tools are used. e.g. individual selection, crossing, backcrossing, stress physiology and molecular markers (simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLP) and restriction fragment length polymorphisms (RFLP)). Genetic maps for durum wheat are developed to determine the responsible genome regions linked to traits of interest [3]. However, this research requires a long period of time, and results are sometimes contradictory due to G*E interactions.

An alternative selection strategy is the use of the $\Delta^{13}C$ technique, which could shorten the period of the traditional breeding programme [4]. Many researchers suggested the use of this technique as an indirect tool for selection of high transpiration efficiency [5-11].

Research at ICARDA aims to improve the water use efficiency (WUE) of crops [12]. The measurement of WUE or transpiration efficiency (TE) is difficult, but crop productivity and morpho-physiological traits can provide qualitative information on TE, e.g. grain and biomass yields, leaf area, canopy temperature, stomatal conductivity and chlorophyll content

[13]. It is also possible to use Δ as an index of TE since Δ and TE are positively related [14]. Differences in Δ values between varieties at the one site reflect their different CO_2 assimilation and transpiration efficiencies. In this case, plant breeders can choose the variety that has high transpiration efficiency without the need for many years of testing [3].

Positive relationships between $\Delta^{13}C$ and yield were observed in many studies in Mediterranean environments [4, 12, 15, 16, 17, 18] and this relationship was also found to be positive in durum wheat varieties at two locations and over several seasons in the northern Syrian Arab Republic [2, 12].

Global climatic change is predicted to have a negative effect on the amount of rainfall in Mediterranean regions, increasing the incidence, duration and severity of drought. Plant breeders are therefore searching for genotypes that use water more efficiently. However, traditional breeding methods need a long period of testing, and the results are confounded by G*E interactions. Therefore CID was suggested as an alternate selection criterion based on its positive correlation with TE, which has the potential to compact the breeding programme. Nevertheless $\delta^{13}C$ isotope ratio analysis is expensive, and therefore surrogates for Δ are also being investigated.

Moreover, it is difficult to test genotypic performance to adequately cover the extreme year-to-year variability at a given location. Models could be used to simulate genotypic performance over a realistic period, following calibration and testing for robustness, using long term weather data.

The objectives of the present investigation were (i) to study water relations and morpho-physiological traits related to the optimum use of water for a range of durum wheat genotypes (ii) to determine the relationships between grain and 3-4 leaf Δ and yields of grain and biomass, and their stability across time and space in a dry, rain fed Mediterranean-type environment and (iii) to use the field experimental data for the validation of a simulation model, and then to run the model using long term weather data to determine crop performance for a full weather cycle of 35/a for a given location.

2. MATERIALS AND METHODS

Field experiments were conducted over three seasons at four sites (Table 1).

2.1. Site characterization

2.1.1. Climate

Long term annual rainfall data show Breda as the driest site, Yahmoul the wettest and Tel Hadya and Muslmieh as similar and intermediate (Table 1).

At Breda, the average maximum air temperature ranged between 23.1 and 26.4 ^{o}C while the minimum air temperature ranged between 8.5 and 11.7 ^{o}C. Solar radiation ranged between 17.7 and 24.4 MJ m^{-2} d^{-1} and total rainfall was as low as 183 mm and as high as 414 mm $year^{-1}$ (Table 2). At Tel Hadya, the average maximum air temperature ranged between 23.3 and 26.4 ^{o}C while the minimum air temperature ranged between 8.7 and 11.2 ^{o}C. Solar radiation ranged between 14.2 and 19 MJ m^{-2} d^{-1} and total rainfall was as low as 225.4 and as high as 517.3 mm $year^{-1}$ (Table 2).

TABLE 1. SITE CHARACTERISTICS

Site	Seasons†	Latitude (^{o}N)	Longitude (^{o}E)	Hasl (m) ‡	Rainfall (mm year^{-1}) #
Tel Hadya (TH)	1st, 2nd, 3rd	36.01	36.56	284	325
Breda (BR)	2nd, 3rd	35.56	37.10	300	225
Muslmieh (MO)	3rd	36.20	37.13	450	350
Yahmoul (YH)	3rd	35.36	37.03	510	420

†1st, 2004-05; 2nd, 2005-06; 3rd, 2006-07
‡Hasl, height above sea level
#Long term average

TABLE 2. AVERAGE MAXIMUM AND MINIMUM AIR TEMPERATURE, SOLAR RADIATION AND TOTAL RAINFALL AT BREDA AND TEL HADYA (1980–2007)

Year (Oct. 1–Sept. 30)	Max. temp. (^{o}C)		Min. temp. (^{o}C)		Sol. rad. (MJ m^{-2} d^{-1})		Precipitation (mm)	
	Breda	Tel Hadya	Breda	Tel Hadya	Breda	Tel Hadya	Breda	Tel Hadya
1980–1981	25.3	25.0	10.0	10.6	19.0	16.0	293.4	362.9
1981–1982	23.5	24.4	9.2	9.8	19.4	15.4	331.0	351.0
1982–1983	23.1	23.3	8.5	9.3	19.4	14.2	277.7	333.3
1983–1984	25.0	24.6	9.8	10.5	20.3	18.4	204.0	243.9
1984–1985	23.9	24.3	9.4	10.3	19.1	18.2	276.6	353.4
1985–1986	24.8	25.3	10.5	10.7	19.3	18.2	218.3	284.4
1986–1987	24.5	24.9	9.6	9.9	19.5	18.4	244.6	346.9
1987–1988	24.5	24.6	10.8	11.0	19.9	17.7	414.0	517.3
1988–1989	25.6	25.6	9.8	10.1	19.5	19.0	194.8	225.4
1989–1990	24.9	25.0	10.0	9.8	22.4	18.6	183.2	232.5
1990–1991	24.8	25.1	10.7	10.8	21.9	18.1	241.3	336.3
1991–1992	23.3	23.5	8.9	9.1	19.5	17.6	274.42	347.0
1992–1993	23.7	24.5	9.1	9.3	18.4	18.1	271.81	283.7
1993–1994	23.9	25.9	9.9	11.1	18.3	18.3	303.4	389.5
1994–1995	23.8	25.2	10.3	11.0	17.9	17.9	232.0	314.3
1995–1996	23.2	24.7	10.2	10.6	17.8	17.7	365.2	410.5
1996–1997	23.3	24.4	9.5	9.9	18.2	18.2	243.2	401.9
1997–1998	24.8	25.7	11.2	11.0	18.0	17.3	211.0	408.8
1998–1999	25.6	26.4	11.1	11.2	18.6	18.4	197.8	331.6
1999–2000	26.4	26.1	11.1	9.9	18.8	18.6	231.2	303.1
2000–2001	25.7	25.6	11.7	11.1	18.3	17.9	367.4	415.3
2001–2002	24.4	25.1	10.7	10.7	18.8	18.1	340.2	410.4
2002–2003	24.4	24.6	11.0	10.8	18.2	17.8	395.6	475.2
2003–2004	24.7	25.0	10.8	10.9	18.9	17.3	294.2	431.1
2004–2005	24.3	25.0	10.4	10.8	19.0	17.6	267.8	327.4
2005–2006	25.2	25.7	10.0	11.0	17.7	17.9	239.1	321.3
2006–2007	23.4	23.9	9.4	8.7	18.3	17.2	264.6	312.8

The hottest months at Breda were July and August, reaching about 37 ^{o}C, and the coldest month was December, reaching about 1.8 ^{o}C. On the other hand, the wettest months were January and February (about 50 mm) and decreased during anthesis and grain filling to 24 and 16.8 mm, respectively (Fig. 1). The hottest months at Tel Hadya were July and August reaching about 37 ^{o}C, and the coldest month was January reaching about 1.3 ^{o}C. The wettest month was January reaching about 69 mm and decreased at anthesis and grain filling periods to 32.7 and 16.8 mm, respectively (Fig. 2).

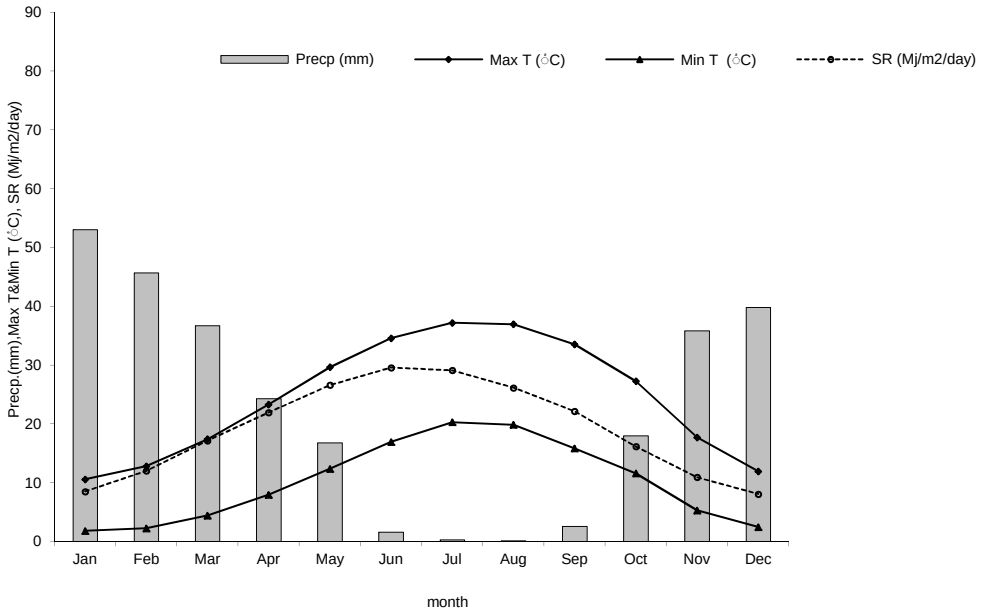

FIG. 1. Average monthly maximum and minimum air temperature, solar radiation and total rainfall at Breda (1980–2007).

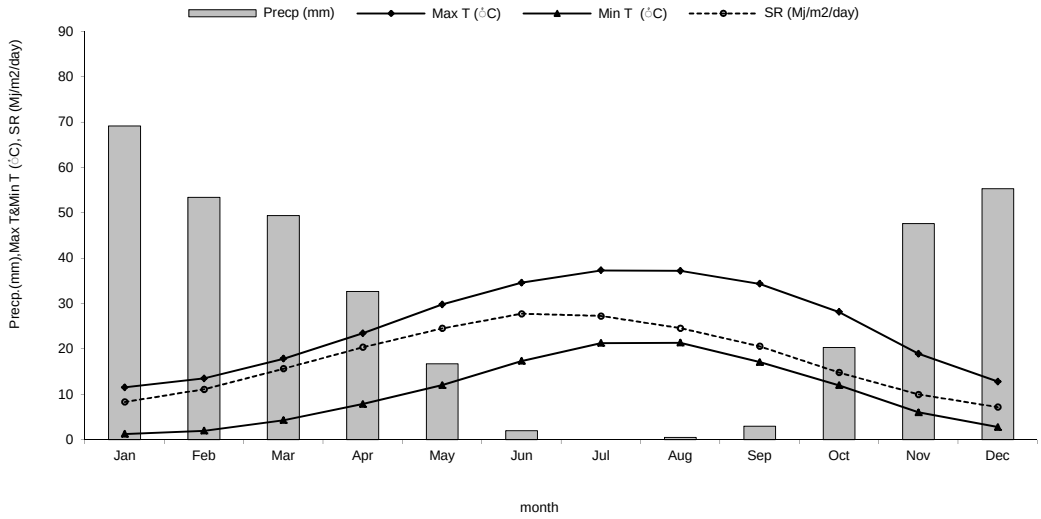

FIG. 2. Average monthly maximum and minimum air temperature, solar radiation and total rainfall at Tel Hadya (1980–2007).

At Muslmieh, the average maximum air temperature ranged between 23.1 and 26.9^{o}C, while the minimum air temperature ranged between 8.2 and 12.7^{o}C. Solar radiation ranged between 17.8 and 18.8 MJ m^{-2} d^{-1} and total rainfall was as low as 133 mm year^{-1} and as high as 460 mm year^{-1} (Table 3). At Yahmoul, the average maximum air temperature ranged between 19.2 and 24.6 ^{o}C while the minimum air temperature ranged between 6.8 and 13.6^{o}C. Solar radiation ranged between 11.3 and 18.4 MJ m^{-2} d^{-1} and total rainfall was as low as 355 mm year^{-1} and as high as 740 mm year^{-1} year (Table 3).

The hottest month at Muslmieh was July reaching approximately 37.5 ^{o}C while the coldest month was February reaching about 1.0 ^{o}C. On the other hand, the wettest month was

January with about 49 mm of rainfall and decreased at anthesis and grain filling periods to 31.2 and 22.5 mm, respectively (Fig. 3). The hottest month at Yahmoul was August reaching about 35.5 ^{o}C and the coldest month was December reaching 2.1 ^{o}C. The wettest month was December reaching about 90 mm and decreased at anthesis and grain filling periods to 55.9 and 18.7 mm, respectively (Fig. 4).

2.1.2. Soils

Physical and chemical properties of the distinct horizons at each site are given in Table 4. Additional properties are described by Ryan [19] and Shaaban [20].

All soils were alkaline (pH range 7.5 to 8.5) with large amounts of free carbonate present at Breda, Tel Hadya and Muslmieh. The Yahmoul soil had less carbonate and a lower pH compared with the other sites. Tel Hadya was saline throughout the profile, with salinity increasing with depth. Muslmieh and Yahmoul had moderate to low salinity, respectively, and Breda was non-saline (Table 4). All sites were low in total N and organic matter, and Olsen-P was moderate to low. Soil porosity was good at Breda, Tel Hadya and Muslmieh and moderate at Yahmoul.

2.2. Experimental

Six durum wheat genotypes were selected on the basis of contrasting Δ values, but with similar dates for the different phenological stages to ensure minimum G*E interaction effects. The genotypes, supplied by ICARDA, were Omruf-2 (Δ = 14.78‰), Brachoua (Δ = 14.88‰), Korifla (Cham$_1$) (Δ = 14.95‰), Waha (Cham$_3$) (Δ = 15.43‰), Massara-1 (Δ = 15.71‰) and Omrabi-14 (Δ = 15.88‰). The first three genotypes were classed as low Δ and the other three as high Δ.

TABLE 3. AVERAGE MAXIMUM AND MINIMUM AIR TEMPERATURE, SOLAR RADIATION AND TOTAL RAINFALL AT MUSLMIEH AND YAHMOUL (1992–2007)

Year (Oct. 1–Sept. 30)	Max. temp. (^{o}C)		Min. temp. (^{o}C)		Sol. rad. (MJ m^{-2} d^{-1})		Precipitation (mm)	
	Muslmieh	Yahmoul	Muslmieh	Yahmoul	Muslmieh	Yahmoul	Muslmieh	Yahmoul
1992–1993	23.3	na	8.4	na	18.4	na	248.1	na
1993–1994	24.8	19.7	10.1	13.6	18.4	11.3	453.3	435.6
1994–1995	23.9	19.2	10.5	12.5	17.8	12.3	324.7	416.4
1995–1996	23.4	21.5	9.3	11.6	18.0	15.2	459.9	740.5
1996–1997	23.1	21.6	9.1	11.4	17.9	15.4	396.5	667.2
1997–1998	24.2	23.0	10.1	10.9	18.0	16.9	334.6	570.9
1998–1999	25.5	24.2	10.3	11.3	18.6	17.5	230.4	382.0
1999–2000	24.9	24.4	9.4	10.1	18.8	18.4	214.3	392.1
2000–2001	24.8	24.4	10.7	10.6	17.8	18.1	361.1	390.8
2001–2002	26.5	24.0	12.4	9.8	17.9	18.4	226.4	534.3
2002–2003	26.9	24.6	12.7	10.5	18.0	18.3	133.1	555.6
2003–2004	26.9	24.2	12.7	10.6	18.1	18.1	152.5	461.0
2004–2005	na	23.9	na	10.7	na	17.8	na	406.8
2006–2007†	23.9	20.0	8.2	6.8	18.8	16.3	236.1	354.6

†Nov.–Aug., Muslmieh; Oct.–June, Yahmoul; na, not available; (2005–2006) not available at both sites.

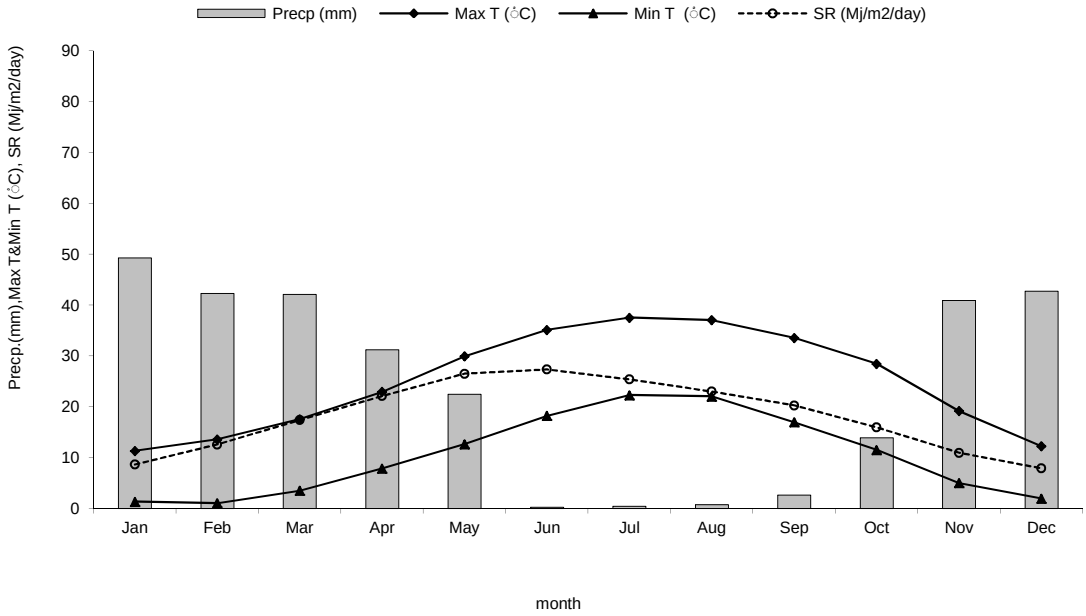

FIG. 3. Average monthly maximum and minimum air temperatures, solar radiation and total rainfall at Muslmieh (1992–2007).

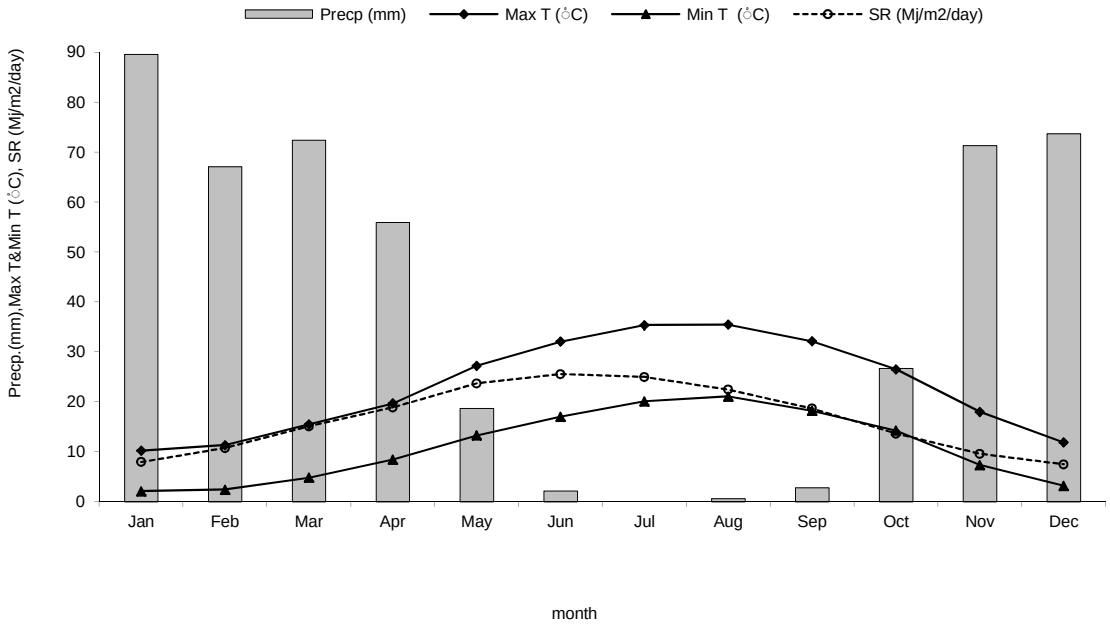

FIG. 4. Average monthly maximum and minimum air temperatures, solar radiation and total rainfall at Yahmoul (1993–2007).

TABLE 4. PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL HORIZONS AT THE FOUR SITES

Site	Depth	Particle size (g kg^{-1})			pH	Total N†	O.M	$CaCO_3$	Olsen-P	Elec. cond.
	(cm)	Sand	Silt	Clay		(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(μg g^{-1})	(mS cm^{-1})
Breda	0–20	470	250	280	7.8	0.70	7.0	340	10	0.42
(2006–2007)	20–70	430	250	320	7.9	0.42	3.4	452	15	0.47
	70–110	230	240	530	8.0	0.30	2.9	340	17	0.16
Tel Hadya	0–20	230	320	450	8.1	0.92	11.1	240	5.5	273
(2005–2006)	20–50	270	150	580	8.0	0.61	10.8	220	9.0	242
	50–90	170	230	600	8.3	0.54	7.9	250	3.0	359
	90–120	90	290	620	8.3	0.21	2.6	235	6.5	434
	120–150	70	300	630	8.5	0.19	0.5	230	2.5	484
Muslmieh	0–20	330	220	450	7.9	–	12.0	275	–	155
(2006–2007)	20–60	270	170	560	7.9	–	5.6	320	–	162
	60–90	270	170	560	7.9	–	4.4	385	–	173
	90–150	130	250	620	7.9	–	2.4	490	–	183
Yahmoul	0–30	130	230	640	7.5	–	6.1	30	–	49
(2006–2007)	30–70	170	290	540	7.6	–	4.4	5	–	36

†Source of data [19].

The experimental design was randomized complete block (RCB) with three replicates. A fallow plot was also included in the design. Plot size was between 4 and 32 m^2. Seeds were planted using an Oyjord planter with a seed rate of 120 kg ha^{-1}. Optimum agronomic practices were used including cultivation, fertilization and weed control. Daily weather data was recorded at each site.

2.3. Measurements

2.3.1. Soil moisture

Soil water content was monitored for every plot at Tel Hadya (2005–2006 and 2006–2007) using the Diviner 2000, a capacitance sensor manufactured by Sentek Sensor technologies in Australia. PVC access tubes were manually installed to a depth of about 160 cm. Measurements were taken throughout the season at 10 cm intervals. The sensor was calibrated at the end of the season when the soil was very dry. The water used per plot (evapo-transpiration) was calculated from the sensor measurements.

2.3.2. Plant measurements

Plant samples were taken from every plot to monitor plant growth throughout the season. The samples were taken according to site, season, plant developmental stage and area as shown in Table 5. Plants were cut at ground level, counted, sub-sampled for area measurements (e.g. individual leaf, stems, etc.), dried in an oven at 70 ^{o}C for 48 h and weighed for dry matter.

Plant development was recorded using the Zadock scale [21] throughout the growing season. A 2 m row plot^{-1}, representative of average growth, was identified early in the season. Two plants were marked and regular counts of leaf number were made. Phylochron was calculated for every cultivar and every location using a base temperature of 0 ^{o}C. Phyllochron was calculated as a reverse slope of the relationship between leaf number and thermal units needed (mean air temperature minus base temperature). Spikes (10 each) were sampled from anthesis till maturity every 3–4 days to monitor the grain accumulation during the grain filling stage.

TABLE 5. PLOT AREA (m^2) SAMPLED AT DIFFERENT GROWTH STAGES AT FOUR SITES BETWEEN 2004–2005 AND 2006–2007

Site	Season	3–4 leaf	Tillering	Stem elong.	Anthesis	Maturity
Breda	2005–2006	0.4	0.4	0.2	0.2	1.0
	2006–2007	0.8	0.2	–	0.2	1.0
Tel Hadya	2004–2005	–	–	–	–	0.2
	2005–2006	0.8	0.6	0.2	0.2	1.0
	2006–2007	0.8	0.2	–	0.2	1.0
Muslmieh	2006–2007	0.7	0.175	–	0.175	0.875
Yahmoul	2006–2007	0.8	0.2	–	0.2	0.8

The 4th fully-expanded leaf was sampled for each replicate for the six genotypes in 2005–2006 and for two replicates in 2006–2007, and physiologically-mature grain was taken from every plot. Leaf and grain samples were dried at 70 °C and finely ground for $\delta^{13}C$ analysis at the IAEA Laboratories, Seibersdorf, Austria. $\Delta^{13}C$ was calculated from the $\delta^{13}C$ data [22].

Solar radiation at ground level was monitored at Tel Hadya (2006–2007 season) for two genotypes (Omruf-2 and Omrabi-14) the two most divergent in Δ, using solar meter tubes (Delta–T Devices Ltd., Cambridge, UK) between the planted rows and in the fallow plot. Solar radiation and the total production were used to calculate the radiation use efficiency (units of dry matter production per unit of solar radiation and unit of area) at different times of the growing season.

Stomatal conductance and resistance measurements were made at Tel Hadya in 2005–2006 and at all locations in 2006–2007 at Zadock 75 using a porometer (AP4 AT, Delta-T devices Ltd. Cambridge, UK). Canopy temperature was measured using an Infrared gun (Model 110.2L micro computer–Everest USA) at weekly or two-weekly intervals at all locations (2006–2007). Grain protein and ash content were determined using near infrared spectroscopy (Foss-NIR Systems Europe, Raamsdonksveer, Holland) at all sites in 2005–2006 and 2006–2007. Results were statistically analyzed using Genstat V 7.0, SPSS 15.0 and GGE biplot [23] software packages.

3. RESULTS AND DISCUSSION

3.1. Seasonal dry matter accumulation

3.1.1. 2005–2006

The season at Tel Hadya started wet with 13, 72, 40.5 and 76 mm of rainfall in Dec., Jan., Feb. and March, respectively, and then the shortage of rain was dramatic with only 24.5 and 0.6 mm in April and May, respectively. Biomass production throughout the season was very similar among genotypes, with no significant differences except at the 3–4 leaf stage. Grain yield was about 2 t ha^{-1} (Table 6).

TABLE 6. DRY MATTER PRODUCTION (kg ha^{-1}) DURING ONTOGENY OF SIX GENOTYPES OF DURUM WHEAT AT TEL HADYA IN 2005–2006

Genotypes	3–4 leaf	Tillering	Stem elong.	Anthesis	Biomass yield	Grain yield
Omruf-2	226.4	1706	9752	11907	9703	2030
Brachoua	212.4	1881	8336	10994	10023	1927
Korifla	238.6	1850	9386	10655	9683	2230
Waha	163.4	1733	7606	11618	9057	1870
Massara-1	147.1	1467	7390	11485	10470	2080
Omrabi-14	145.0	1522	7290	11664	9523	1850
SE (±)	31.7*	173ns	988ns	1119ns	673ns	277ns

ns, not significant; *, significant at $P<0.05$; SE, standard error.

At Breda, vigorous plants were evident at the beginning of the season, even better than that of Tel Hadya (at the 3–4 leaf stage), but thereafter, the crop was badly affected by the drought, and almost produced no grain by the end of the season (total rainfall 135 mm) (Fig. 5). It is interesting to note that at Breda, the yield reached its maximum at stem elongation, stayed almost constant till anthesis and then decreased sharply and dramatically to physiological maturity (Fig. 5). This observation was also made previously for wheat grown in the Syrian Arab Republic under heat stress [24]. There was no significant difference for the dry matter production throughout the season at Breda.

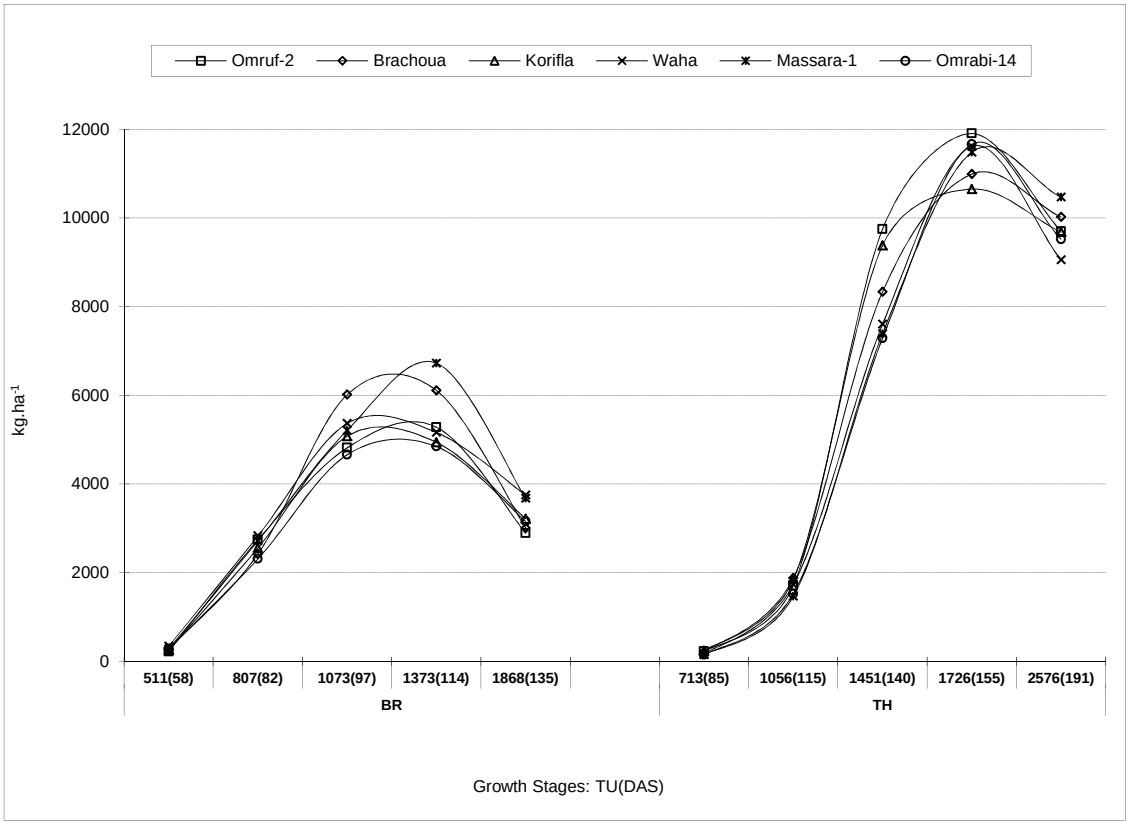

FIG. 5. Dry matter production (kg ha^{-1}) during growth expressed as thermal units, TU, (data in parentheses are days after sowing, DAS) for the six genotypes at two locations (BR, Breda; TH, Tel Hadya) in 2005–2006.

3.1.2. 2006–2007

This season started dry and finished wet, the opposite to that of the previous season (2005–2006). A significant amount of rain had fallen in May reaching 53, 42, 19 and 28.5 mm at TH, BR, MO and YH, respectively. However, the crops suffered from frost damage as well as relatively high air temperature during the grain filling period, causing low yields (Fig. 6). The extreme weather conditions occurred mainly at Muslmieh, where total biomass was less than 4 t ha^{-1}, which was similar to that at Breda despite the wetter conditions at Muslmieh. Also, the crop at Muslmieh reached maturity late compared with Breda (growth periods of 186 and 123 days, respectively). An outstanding yield was recorded at Yahmoul (370 mm of rain, and a growth period of 180 days) with a marked increase in yield from anthesis to maturity (Fig. 6). A similar increase in yield was previously found for a wheat crop grown in The Syrian Arab Republic [25], unlike the other three sites where a marked decrease occurred during this period (Fig. 6). There were no observable differences in yield between the six genotypes.

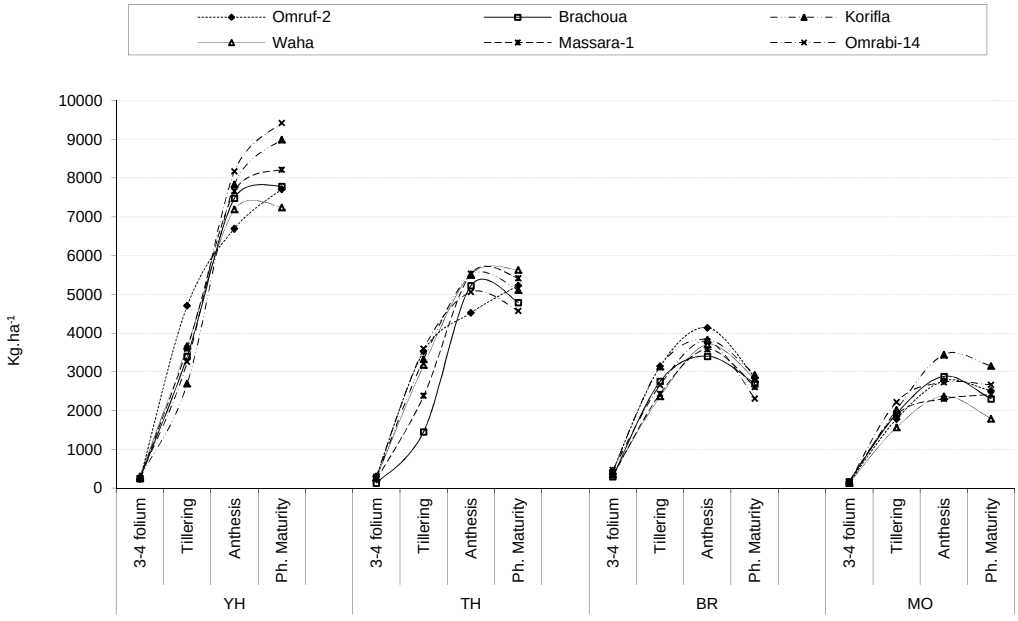

FIG. 6. Dry matter production (kg ha^{-1}) during growth for the six genotypes at four locations (YH, Yahmoul; TH, Tel Hadya; BR, Breda; MO, Muslmieh) in 2006–2007.

3.2. Dry matter accumulation during grain filling

The grain filling periods were 16, 21, 22 and 28 days at Breda, Muslmieh, Tel Hadya and Yahmoul, respectively. Massara-1 and Waha accumulated the highest dry matter production during the grain filling period at Tel Hadya, while Massara-1 continued to be superior at Breda and Yahmoul. Korifla accumulated the highest dry matter at Muslmieh (Table 7).

TABLE 7. LINEAR RELATIONSHIPS BETWEEN DRY MATTER PRODUCTION (Y, kg ha $^{-1}$) AT GRAIN FILLING AND DAYS AFTER SOWING (X) FOR SIX GENOTYPES AT FOUR LOCATIONS IN 2006–2007

Genotype	Tel Hadya	Breda	Muslmieh	Yahmoul
Omruf-2	57.45 x – 5197 (0.98)	24.81 x – 1283 (0.76)	65.71 x – 9081 (0.97)	154.6 x – 19920 (0.96)
Brachoua	50.68 x – 4358 (0.88)	32.67 x – 2381 (0.84)	66.79 x – 9273 (0.99)	140.5 x – 18460 (0.97)
Korifla	57.46 x – 5408 (0.87)	32.9 x – 2228 (0.66)	113.4 x – 16280 (0.94)	142.9 x – 18710 (0.98)
Waha	81.03 x – 7137 (0.99)	29.73 x – 2061 (0.93)	85.54 x – 12130 (0.99)	130.7 x – 16330 (0.99)
Massara-1	88.4 x – 8247 (0.95)	46.83 x – 3632 (0.63)	84.43 x – 12190 (0.97)	163.6 x – 21330 (0.97)
Omrabi-14	74.06 x – 7387 (0.88)	23.67 x – 1308 (0.91)	76.34 x – 10990 (0.88)	140.7 x – 18240 (0.99)

Data in parentheses are R^2 of the linear relationship.

3.3. Radiation use efficiency

Radiation use efficiency (RUE) of Omruf-2 was lower than that of Omrabi-14 at tillering (0.84 and 0.96 g MJ^{-1} m^{-2} d^{-1}, respectively). The RUE for both genotypes decreased at stem elongation (0.42 and 0.22 g MJ^{-1} m^{-2} d^{-1}, respectively) due to the death of green leaves, and remained stable to anthesis (0.35 and 0.23 g MJ^{-1} m^{-2} d^{-1}, respectively). Similar differences in RUE for different plant stages have been reported [26].

3.4. Plant development (Phyllochron)

Phyllochron ranged from 75 to about 130 between genotypes, seasons and sites (Table 8). The dry sites (Breda and Muslmieh) had lower values (between 87 and 100) while the wetter sites (Tel Hadya and Yahmoul) had higher values (between 108 and 123). Waha was the highest, on average, and had the lowest coefficient of variation (12%), while Omruf-2 and Omrabi-14 had the lowest phyllochron values but had very high coefficients of variation (17–19%). Similar results have been reported for wheat [27] and lower results were reported for barley grown in the Syrian Arab Republic [28].

3.5. Water use and water use efficiency

The total water used (evapo-transpiration) was similar between the different genotypes throughout the 2005–2006 growing season at Tel Hadya (Table 9). However, the high Δ genotypes used more water by the end of the season (anthesis and physiological maturity) compared with the low Δ genotypes. The genotypes did not differ significantly in WUE (Table 10).

TABLE 8. PHYLLOCHRON NUMBER FOR SIX DURUM WHEAT GENOTYPES AT TWO SITES IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotypes	2005–2006		2006–2007			
	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul
Omruf-2	100.6	75.4	113.3	101.5	80.2	114.6
Brachoua	126.7	84.7	91.9	105.8	87.4	117.0
Korifla	117.3	101.1	100.5	102.6	96.2	129.9
Waha	101.4	93.6	127.6	102.6	113.2	125.1
Massara-1	120.7	95.7	105.6	99.1	89.5	125.0
Omrabi-14	96.7	74.1	112.3	98.2	86.5	128.4
	Mean	Range	Variance	SD	SEM	CV (%)
Omruf-2	97.6	39.2	271.06	16.46	6.72	16.87
Brachoua	102.3	42.0	292.72	17.11	6.99	16.73
Korifla	107.9	33.7	167.67	12.95	5.29	12.00
Waha	110.6	34.0	188.73	13.74	5.61	12.42
Massara-1	105.9	35.5	200.64	14.17	5.78	13.37
Omrabi-14	99.4	54.3	364.53	19.09	7.80	19.21

SD, standard deviation; SEM, standard error of the mean; CV, coefficient of variation.

TABLE 9. EVAPO-TRANSPIRATION (MM) FOR SIX DURUM WHEAT GENOTYPES DURING ONTOGENY AT TEL HADYA IN 2005–2006

Genotypes	Δ	3–4 leaf	Tillering	Stem elongation	Anthesis	Maturity
Omruf-2	Low	111.4	121.8	189.0	285.2	287.7
Brachoua		109.4	118.6	182.1	270.6	277.3
Korifla		105.1	115.1	183.9	276.1	283.1
Waha	High	109.0	123.7	188.0	289.8	296.0
Massara-1		109.1	116.8	178.6	285.9	289.4
Omrabi-14		108.4	118.6	189.5	279.9	287.0
Fallow		106.1	115.4	139.5	144.3	184.5
SE (±)		10.2ns	12.6ns	13.8*	18.6***	15.3***

ns, not significant; *, ***, significant at $P<0.05$ and $P<0.001$, respectively; SE, standard error.

TABLE 10. WATER USE EFFICIENCY (KG HA^{-1} MM^{-1}) FOR SIX DURUM WHEAT GENOTYPES DURING ONTOGENY AT TEL HADYA IN 2005–2006

Genotypes	3–4 leaf	Tillering	Stem elongation	Anthesis	Biomass yield	Grain yield
Omruf-2	2.1	14.1	51.2	41.8	33.8	7.1
Brachoua	2.0	16.1	46.3	41.3	36.3	7.0
Korifla	2.3	16.1	51.0	38.8	34.1	7.9
Waha	1.6	14.3	40.3	40.1	31.2	7.0
Massara-1	1.4	12.7	42.0	40.0	36.2	7.2
Omrabi-14	1.4	13.0	38.5	41.6	33.2	6.4
SE (±)	0. 4ns	2.4ns	5.7ns	5.2ns	2.9ns	0.9ns

ns, not significant; SE, standard error

Unlike 2005–2006, the genotypes did not differ significantly in total water use at Tel Hadya during the 2006–2007 season (Table 11). However, genotypes differed significantly in WUE at tillering and maturity at Tel Hadya in 2006–2007 (Table 12). Similar results were reported in a field study with wheat in the The Syrian Arab Republicn Arab Republic [29].

TABLE 11. EVAPO-TRANSPIRATION (mm) FOR SIX DURUM WHEAT GENOTYPES DURING ONTOGENY AT TEL HADYA IN 2006–2007

Genotypes	Δ	Tillering	Anthesis	Maturity
Omruf-2	Low	143.6	164.0	202.5
Brachoua		147.5	183.2	227.7
Korifla		151.0	169.4	206.7
Waha	High	148.1	167.4	212.6
Massara-1		152.3	181.8	222.8
Omrabi-14		144.4	165.2	202.2
Fallow		116.4	141.7	206.0
SE (±)		14.7ns	15.2ns	17.2ns

ns, not significant; SE, standard error.

TABLE 12. WATER USE EFFICIENCY (kg ha^{-1} mm^{-1}) FOR SIX DURUM WHEAT GENOTYPES DURING ONTOGENY AT TEL HADYA IN 2006–2007

Genotypes	Δ	Tillering	Anthesis	Biomass yield	Grain yield
Omruf-2	Low	24.8	27.8	26.0	1.3
Brachoua		10.0	28.8	21.0	3.4
Korifla		22.3	32.6	24.7	2.1
Waha	High	21.6	34.6	26.4	3.3
Massara-1		15.8	30.3	24.4	3.4
Omrabi-14		26.1	31.5	23.1	1.6
SE (±)		3.6**	7.7ns	2.5ns	0.5**

ns, not significant; **, significant at $P<0.01$, SE, standard error**.**

3.6. Leaf porosity

Leaf stomatal conductance (mol m^{-2} s^{-1}) differed between genotypes at Tel Hadya in 2005–2006 and at Breda in 2006–2007 (Table 13). There were no apparent differences between the high Δ and low Δ genotypes. Similarly, genotypes differed in leaf stomatal resistance (m^2 s mol^{-1}) at Tel Hadya in both seasons (Table 14). There were no apparent differences between the high Δ and low Δ genotypes.

TABLE 13. LEAF STOMATAL CONDUCTANCE (MOL M^{-2} S^{-1}) FOR SIX DURUM WHEAT GENOTYPES AT THE END OF STEM ELONGATION AT ONE SITE IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotype	Δ	2005–2006	2006–2007				Average
		Tel Hadya	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	Low	0.112	0.11	0.09	0.20	0.20	0.142
Brachoua		0.065	0.19	0.07	0.19	0.15	0.133
Korifla		0.064	0.18	0.05	0.25	0.19	0.147
Waha	High	0.073	0.14	0.05	0.12	0.21	0.119
Massara-1		0.086	0.18	0.04	0.10	0.31	0.143
Omrabi-14		0.079	0.17	0.04	0.16	0.21	0.132
SE (±)		0.003**	0.03ns	0.015*	0.07ns	0.10ns	

ns, not significant; *, **, significant at $P<0.05$ and $P<0.01$, respectively; SE, standard error.

TABLE 14. LEAF STOMATAL RESISTANCE (M^2 S MOL^{-1}) FOR SIX DURUM WHEAT GENOTYPES AT THE END OF STEM ELONGATION AT ONE SITE IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotypes	Δ	2005–2006	2006–2007				Average
		Tel Hadya	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	Low	8.99	9.43	11.85	6.33	7.72	8.86
Brachoua		15.30	5.25	19.80	6.47	8.83	11.13
Korifla		15.32	5.60	20.17	5.27	5.73	10.42
Waha	High	13.35	7.20	20.30	9.14	5.54	11.11
Massara-1		11.72	5.63	28.83	10.19	4.46	12.17
Omrabi-14		12.71	5.95	27.27	6.58	5.30	11.56
SE (±)		0.42***	1.06*	5.75ns	2.50ns	3.70ns	

ns, not significant; *, ***, significant at $P<0.05$ and $P<0.001$, respectively; SE, standard error.

3.7. Canopy temperature

No significant differences were evident between genotypes for canopy temperature at each of the four sites in 2006–2007. A highly significant difference was found between locations for the average of canopy temperature, but the G*E interaction was not significant (Table 15).

TABLE 15. CANOPY TEMPERATURE (ºC) OF SIX DURUM WHEAT GENOTYPES OF FOUR SITES IN 2006– 07

Genotypes	Tel Hadya	Breda	Muslmieh	Yahmoul	Average
Omruf-2	26.75	27.31	28.87	23.77	26.65
Brachoua	26.67	27.25	29.06	23.88	26.72
Korifla	27.04	27.21	27.61	23.73	26.40
Waha	26.20	27.04	29.19	23.97	26.60
Massara-1	26.51	27.26	29.33	23.44	26.64
Omrabi-14	26.77	27.87	29.67	24.28	27.15
Average	26.66	27.32	28.94	23.85	
	Environment	Genotype		G*E	
F test	***	ns		ns	
SE (±)	0.252	0.309		0.617	
$LSD_{0.05}$	0.507	0.621		1.242	
CV (%)	2.8				

ns, not significant; ***, $P<0.001$.

These results agreed with crop biomass production with the highest production at the coldest canopy temperature (Yahmoul), and the lowest production at the hottest canopy temperatures (Muslmieh and Breda). Overall, Omrabi-14 had the highest canopy temperature and Korifla had the lowest.

3.8. Ash and protein content

There were significant differences in the grain ash content between the different genotypes only at Breda for the two seasons 2005–2006 and 2006–2007 (Table 16). Breda is the driest among the four sites. For the other sites, similar trends were observed but with no significant differences between genotypes. For the grain protein content, significant differences between genotypes were observed at Breda for the first season (2005–2006) and at three sites for the 2006–2007 season (Table 17). Similar results were found in wheat in India [30].

TABLE 16. GRAIN ASH CONTENT (%) FOR SIX DURUM WHEAT GENOTYPES AT TWO SITES IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotypes	2005–2006		2006–2007				Average
	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	3.133	3.210	3.208	3.232	3.098	3.101	3.164
Brachoua	3.083	3.107	3.203	3.159	3.064	3.112	3.121
Korifla	3.103	3.203	3.132	3.161	3.123	3.136	3.143
Waha	3.180	3.287	3.213	3.284	3.089	3.096	3.192
Massara-1	3.133	3.320	3.147	3.121	3.072	3.119	3.152
Omrabi-14	3.130	3.343	3.225	3.249	3.098	3.101	3.191
SE (±)	0.050ns	0.029**	0.030ns	0.040*	0.04ns	0.028ns	

ns, not significant; *, **, significant at $P<0.05$, and $P<0.01$, respectively; SE, standard error.

TABLE 17. GRAIN PROTEIN CONTENT (%) FOR SIX DURUM WHEAT GENOTYPES AT TWO SITES IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotypes	2005–2006		2006–2007				Average
	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	14.27	18.10	16.30	18.54	14.06	12.78	15.68
Brachoua	13.40	17.50	15.43	17.16	12.49	13.02	14.83
Korifla	13.70	18.10	16.23	17.47	12.12	13.23	15.14
Waha	15.10	19.10	18.17	18.90	13.07	12.31	16.11
Massara-1	13.97	18.70	15.68	17.83	12.69	12.18	15.18
Omrabi-14	14.20	19.20	17.34	18.85	12.45	12.25	15.72
SE (±)	0.69ns	0.26**	0.20***	0.45*	0.45*	0.92ns	

ns, not significant; *, **, ***, significant at $P<0.05$, $P<0.01$ and $P<0.001$ respectively; SE, standard error.

3.9. CID

3.9.1. Leaf

There were significant differences in leaf Δ between genotypes for the two sites in 2005–2006, but not in 2006–2007 (Table 18). Analysis of the Shukla stability index using Biplot for the 4th leaf Δ showed that Waha was among the most stable genotypes for the different seasons and sites (Fig. 7, Table 19).

3.9.2. Grain

There were significant differences between genotypes in grain Δ at all sites and seasons, except for the wettest site, Yahmoul in 2006–2007 (Table 20). Grain-Δ was lowest at the driest sites (e.g. Breda) and highest at the wettest sites (e.g. Yahmoul). However, analysis of the Shukla stability index using Biplot showed a difference between the genotypes in stability (Fig. 8, Table 21). Waha and Korifla were found to be the best genotypes which showed stable results for grain Δ across sites and seasons (Fig. 8, Table 21).

TABLE 18. FOURTH LEAF Δ (‰) OF SIX DURUM WHEAT GENOTYPES FOR TWO SITES IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotypes	2005–2006		2006–2007				Average
	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	21.95	21.05	21.45	20.71	19.97	22.00	21.19
Brachoua	22.36	21.29	21.12	21.10	20.43	21.83	21.36
Korifla	22.52	21.55	21.71	21.13	20.48	22.19	21.60
Waha	22.51	21.57	22.08	21.08	20.49	22.28	21.67
Massara-1	22.39	21.63	21.96	20.95	20.74	21.28	21.49
Omrabi-14	22.76	21.83	22.20	21.38	20.85	22.57	21.93
SE (±)	0.07***	0.07***	0.27ns	0.14ns	0.30ns	0.55ns	

ns, not significant; ***, significant at $P<0.001$; SE, standard error.

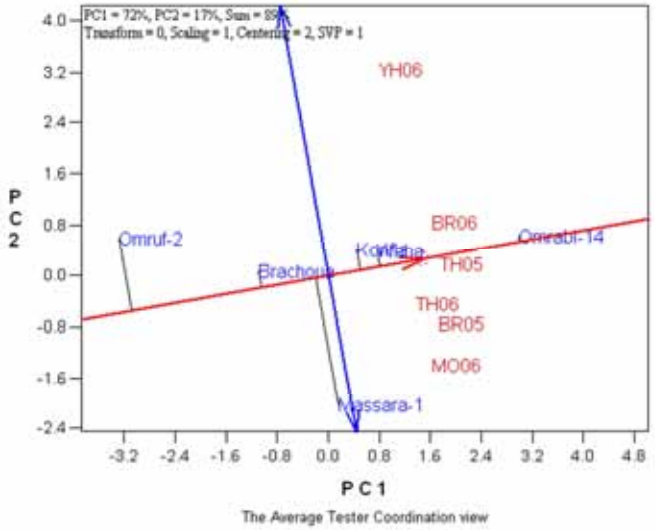

FIG. 7. Analysis of Shukla stability index using Biplot for the 4th leaf Δ of six genotypes over four sites (YH, Yahmoul; TH, Tel Hadya; BR, Breda; MO, Muslmieh) in 2005–2006 and 2006–2007.

TABLE 19. ENTRY MEANS AND SHUKLA'S STABILITY INDEX FOR 4TH LEAF Δ

Parameter	Omruf-2	Brachoua	Korifla	Waha	Massara-1	Omrabi-14
Mean	–0.350	–0.184	0.058	0.130	–0.047	0.393
SBD+	0.163	0.218	0.070	0.121	0.336	0.079

+SBD, stability deviation, calculated as the square root of the GE mean square associated with each entry, and a larger value denotes more GE interaction and less stability

TABLE 20. GRAIN-Δ (‰) OF SIX DURUM WHEAT GENOTYPES AT ONE SITE IN 2004-05, TWO SITES IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotype	ICARDA data	2004–2005	2005–2006		2006–2007				Average
		Tel Hadya	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	14.78	15.36	15.35	13.72	14.08	12.88	14.94	16.00	14.62
Brachoua	14.88	15.33	15.70	14.02	14.78	13.63	15.12	15.61	14.88
Korifla	14.95	15.72	15.68	14.06	14.83	13.69	15.31	15.97	15.04
Waha	15.43	15.80	15.81	14.21	14.88	13.75	14.76	16.41	15.09
Massara-1	15.71	16.13	15.62	14.13	15.21	13.70	14.94	16.18	15.13
Omrabi-14	15.88	16.26	15.71	14.08	15.17	13.71	15.29	16.62	15.26
SE (±)		0.12**	0.07***	0.08**	0.19***	0.16***	0.17*	0.33ns	

ns, not significant; *, **, ***, significant at $P<0.05$, $P<0.01$ and $P<0.001$ respectively; SE, standard error.

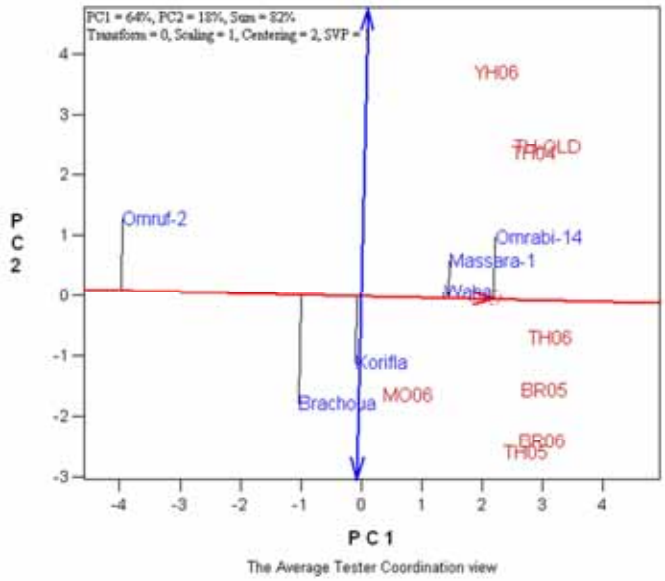

FIG. 8. Analysis of Shukla stability index using Biplot for grain Δ of six genotypes over four sites (YH, Yahmoul; TH, Tel Hadya; BR, Breda; MO, Muslmieh) and three seasons.

TABLE 21. ENTRY MEANS AND SHUKLA'S STABILITY INDEX FOR GRAIN Δ

Parameter	Omruf-2	Brachoua	Korifla	Waha	Massara-1	Omrabi-14
Mean	–0.398	–0.153	–0.011	0.094	0.165	0.303
SBD+	0.216	0.235	0.163	0.165	0.193	0.199

+SBD, stability deviation, calculated as the square root of the GE mean square associated with each entry, and a larger value denotes more GE interaction and less stability.

3.10. Relationships between plant traits

3.10.1. Grain yield versus grain Δ

Positive relationships between grain yield and grain Δ at Tel Hadya (moderate to wet site) were evident for different seasons (Fig. 9) with similar trends for the six genotypes. R^2 differed between the six genotypes and there was a tendency for a higher R^2 and slope of the linear relationship for the high grain Δ genotypes (Fig. 9).

However, results at Breda (driest site) were slightly different. One genotype (Brachoua) showed a negative slope, but the R^2 of 0.0943 was not significant. Otherwise, positive relationships were evident with a tendency for higher slopes of the linear relationship for the high grain Δ genotypes (Fig. 10). When all data were combined, the relationship was positive and significant (Fig. 11).

A positive relationship between yield of wheat and grain CID was reported in the hot environment of Peninsular India [30]. Also, in Syrian Arab Republic, positive and significant relationships were found between yield and grain CID in different dry locations [2, 12].

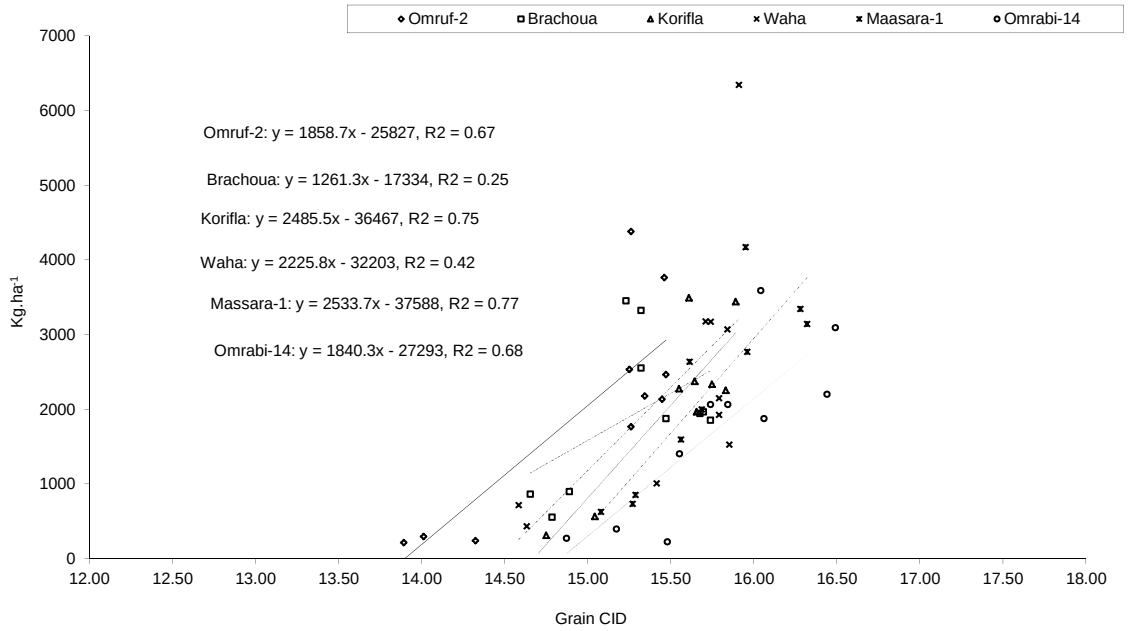

FIG. 9. Relationships between grain yield (kg ha^{-1}) and grain Δ (‰) for six durum wheat genotypes at Tel Hadya for three seasons (2004–2005, 2005–2006 and 2006–2007).

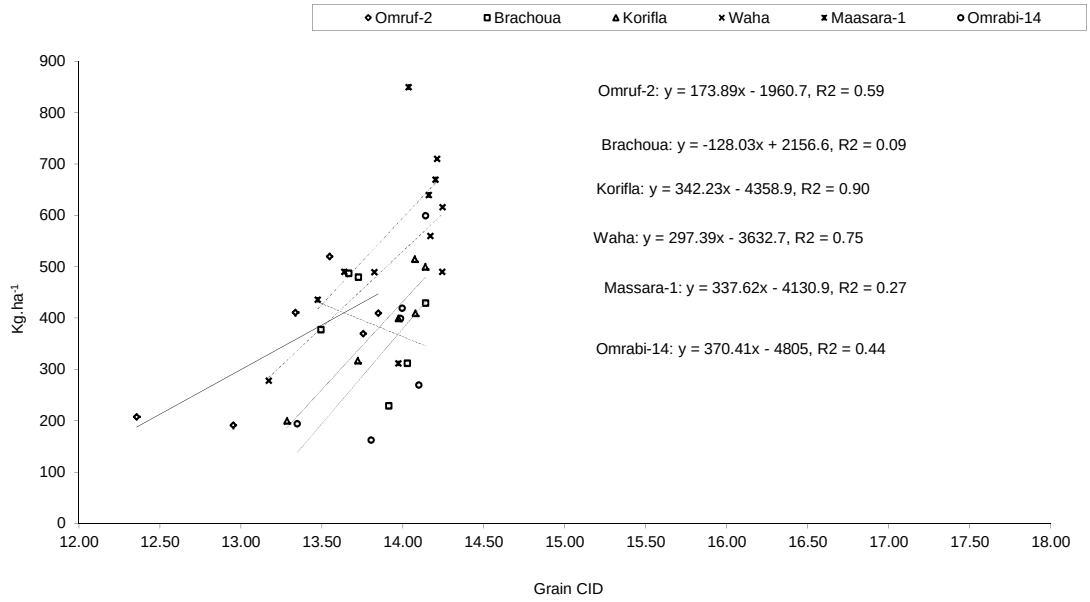

FIG. 10. Relationships between grain yield (kg ha^{-1}) and grain Δ (‰) for six durum wheat genotypes at Breda for two seasons (2005–2006 and 2006–2007).

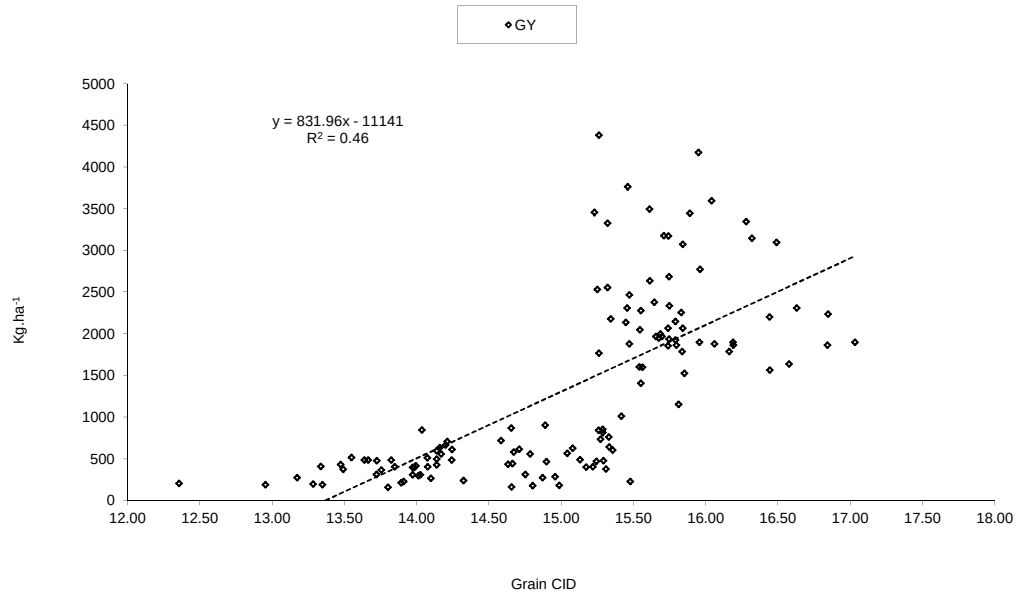

FIG. 11. Relationship between grain yield (GY, kg ha^{-1}) and grain Δ (‰) for all durum wheat genotypes across sites and seasons.

3.10.2. Biomass yield versus grain Δ

A positive and significant linear relationship was found between biomass yield and grain Δ for the combined genotypes, sites and seasons (Fig. 12). Similar results were previously found in the Syrian Arab Republic [2, 12].

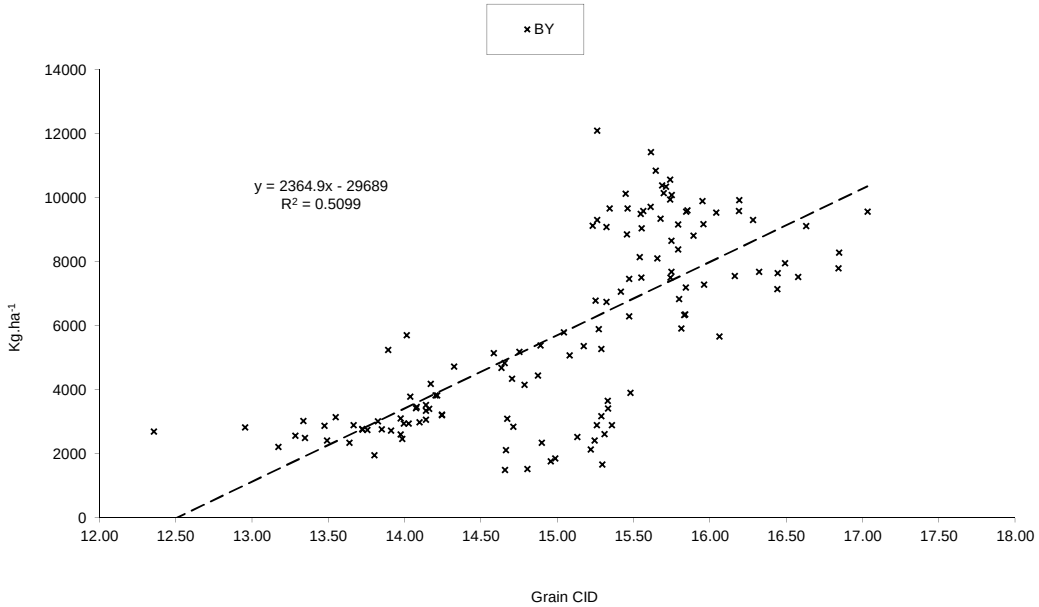

FIG. 12. Relationship between biomass yield (BY, kg ha^{-1}) and grain Δ (‰) for six durum wheat genotypes across sites and seasons.

3.10.3. Grain yield versus grain Δ under different rainfall regimes

At Breda, where annual rainfall was very low (135 and 149.8 mm for the 2005–2006 and 2006–2007 seasons, respectively), the relationships between grain yield and grain Δ were significant and positive (Fig. 10), except for Brachoua, where a negative but non-significant correlation was found. There was a tendency for the high grain Δ genotypes to have higher slopes of the linear regressions (Fig. 10). Similar positive relationships were found at the moderate rainfall sites at Tel Hadya (2004–2005 and 2006–2007, where annual rainfall was 164 and 195.2 mm, respectively), and at Muslmieh in 2006–2007 where annual rainfall was 188.4 mm (Fig. 13). At the wettest sites at Tel Hadya in 2005–2006 and at Yahmoul in 2006–2007, where annual rainfall was 226 and 265.6 mm, respectively, both negative and positive (but non-significant) relationships were found (Fig. 14). Similar negative relationships were found for Australian wheat cultivars [31, 32].

3.10.4. Overall relationships between leaf or grain Δ and grain or biomass yields

Relationships between grain Δ and grain yields for individual sites were positive and significant in the dry sites, but were more diffuse in the wet sites or in high grain yield sites (Fig. 15). Highly significant positive relationships were found between grain or leaf Δ and grain or biomass yields (Fig. 16).

3.11. Stability analyses

3.11.1. Grain yield

On average, genotypes with high grain Δ out-yielded those of low grain Δ (Table 21). Massara-1 and Omrabi-14, with high grain Δ, were high yielding genotypes and Massara-1 was the most yield stable genotype among sites and seasons (Fig. 15).

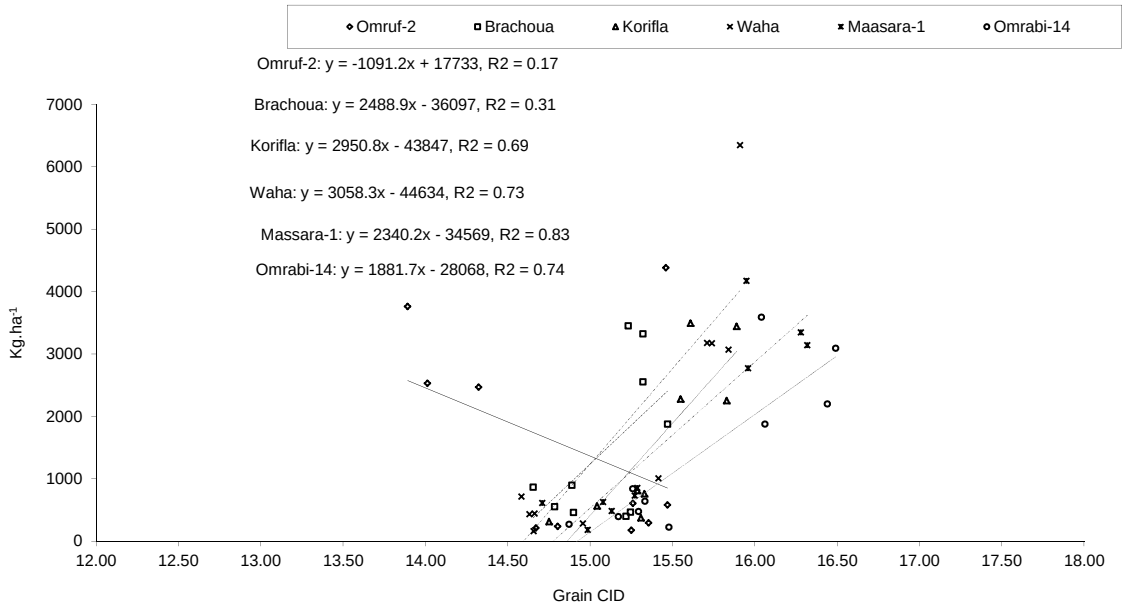

FIG. 13. Relationships between grain yield (kg ha^{-1}) and grain Δ (‰) for six durum wheat genotypes at moderate rainfall sites (Tel Hadya, 2004–2005 and 2006–2007) and Muslmieh (2006–2007).

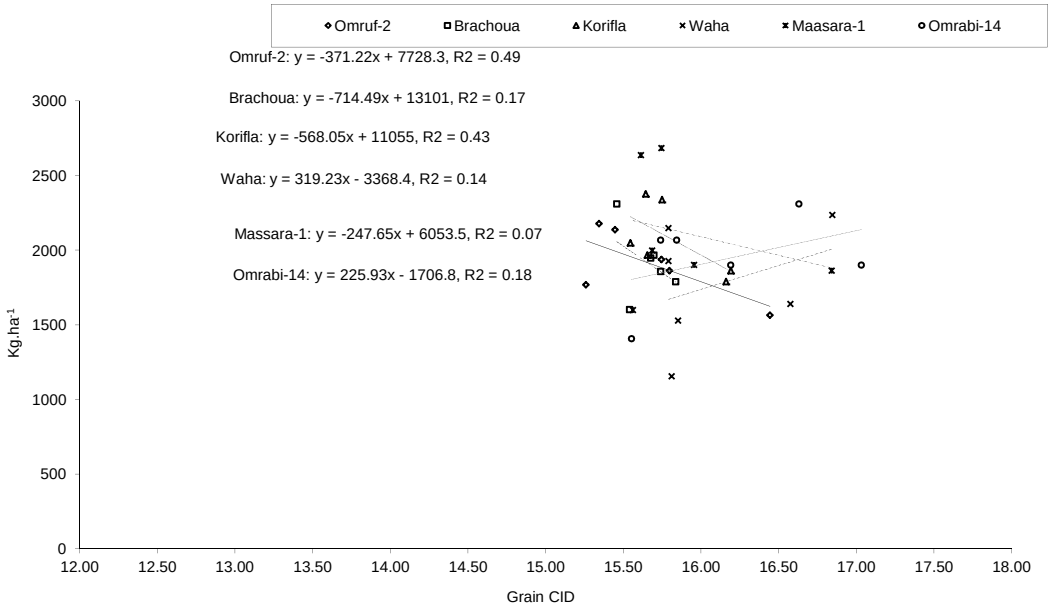

FIG. 14. Relationships between grain yield (kg ha^{-1}) and grain Δ (‰) for six durum wheat genotypes at high rainfall sites (Tel Hadya, 2005–2006 and Yahmoul, 2006–2007).

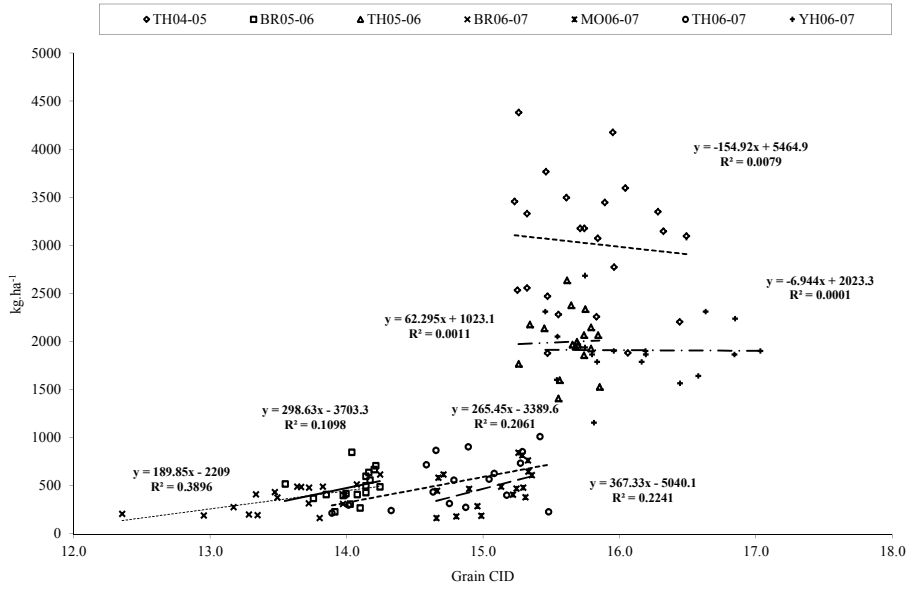

FIG. 15. Relationships between grain yield (kg ha^{-1}) and grain Δ (‰) for individual sites and seasons.

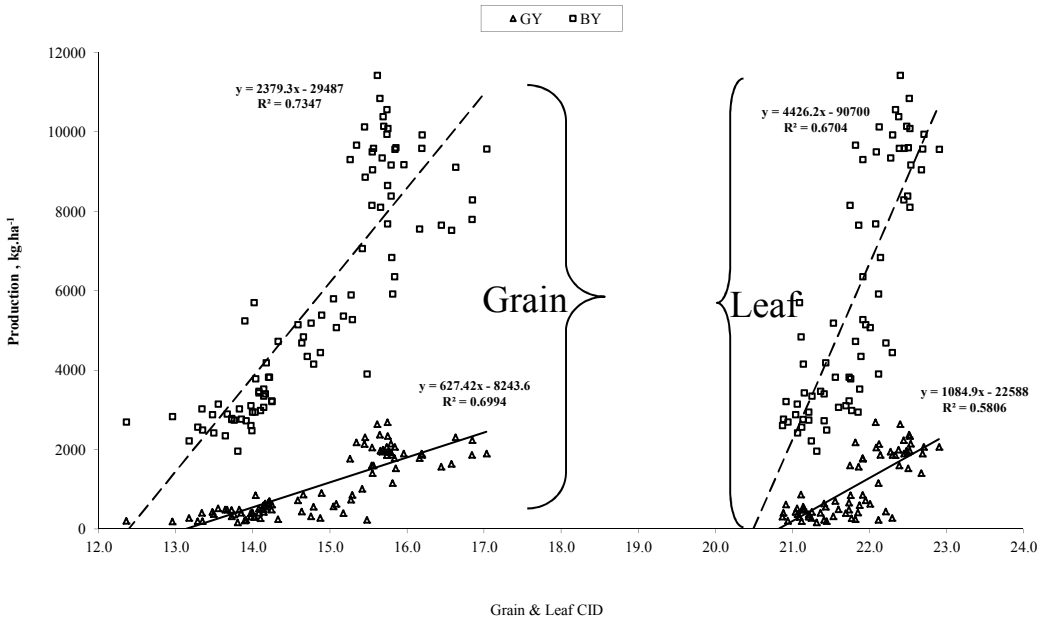

FIG. 16. Relationships between grain (GY) or biomass (BY) yields (kg ha^{-1}) and grain or leaf Δ (‰) across sites and seasons.

TABLE 21. GRAIN YIELD (kg ha^{-1}) OF SIX DURUM WHEAT GENOTYPES AT ONE SITE IN 2004–2005, AT TWO SITES IN 2005–2006 AND AT FOUR SITES IN 2006–2007

Genotypes	2004–2005	2005–2006		2006–2007				Average
	Tel Hadya	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	3290.0	2030.0	433.0	254.3	270.4	458.2	2257.0	1284.7
Brachoua	2807.0	1927.0	323.0	779.3	448.7	449.4	2382.2	1302.4
Korifla	2872.0	2230.0	437.0	340.6	344.3	654.5	2489.2	1338.2
Waha	2591.0	1870.0	587.0	724.3	461.3	300.6	2274.0	1258.3
Massara-1	3362.0	2080.0	720.0	742.8	412.9	432.3	2671.4	1488.8
Omrabi-14	3248.0	1850.0	430.0	304.8	252.7	658.1	3169.3	1416.1
SE (±)	455.4ns	277.3ns	88.2*	138.3*	104.4ns	155.5ns	339.1ns	

ns, not significant; *, significant at $P<0.05$; SE, standard error.

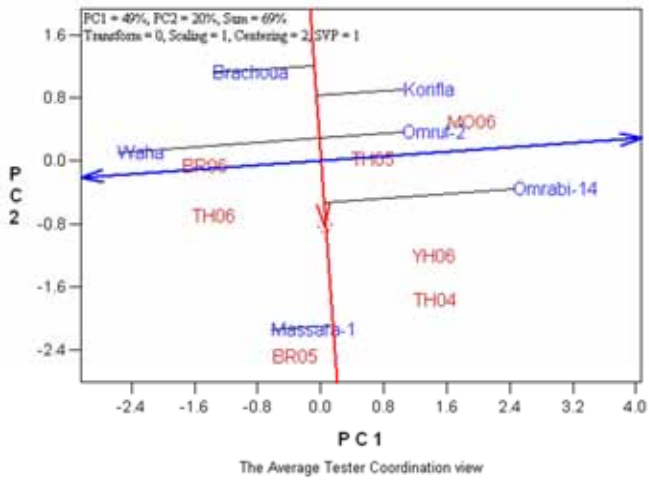

FIG. 15. Analysis of Shukla stability index using Biplot for the grain yield of six genotypes across four sites (YH, Yahmou; TH, Tel Hadya; BR, Breda; MO, Muslmieh) and three seasons (2004–2005, 2005–2006 and 2006–2007).

TABLE 22. ENTRY MEANS AND SHUKLA'S STABILITY INDEX FOR GRAIN YIELD

Parameter	Omruf-2	Brachoua	Korifla	Waha	Massara-1	Omrabi-14
SBD+	172.32	153.81	143.16	213.11	121.86	273.80

+SBD, stability deviation, calculated as the square root of the GE mean square associated with each entry, and a larger value denotes more GE interaction and less stability.

3.11.2. Biomass yield

No significant differences in biomass yield were found between the genotypes (Table 23), with Massara-1 and Brachoua showing similar trends across sites and seasons (Fig. 16, Table 24). These two genotypes showed a very stable and almost the highest yields compared with the others genotypes (Table 24).

TABLE 23. BIOMASS YIELD (kg ha^{-1}) OF SIX DURUM WHEAT GENOTYPES AT ONE SITE IN 2004–2005, TWO SITES IN 2005–2006 AND FOUR SITES IN 2006–2007

Genotypes	2004–2005	2005–2006		2006–2007				Average
	Tel Hadya	Tel Hadya	Breda	Tel Hadya	Breda	Muslmieh	Yahmoul	
Omruf-2	9011.0	9703.0	2890.0	5230.0	2855.2	2513.5	7719.9	5703.2
Brachoua	7819.0	10023.0	3010.0	4796.7	2694.7	2306.9	7794.6	5492.1
Korifla	8100.0	9683.0	3217.0	5113.3	2930.4	3156.0	8998.2	5885.4
Waha	7076.0	9057.0	3750.0	5636.7	2821.3	1798.3	7250.1	5341.3
Massara-1	8550.0	10470.0	3677.0	5420.0	2616.2	2415.8	8224.8	5910.5
Omrabi-14	8861.0	9523.0	3157.0	4576.7	2313.0	2666.6	9430.6	5789.7
SE (±)	1048.1ns	673.3ns	269.8*	621.1ns	285.3ns	492.8ns	837.4ns	

ns, not significant; *, significant at $P<0.05$; SE, standard error.

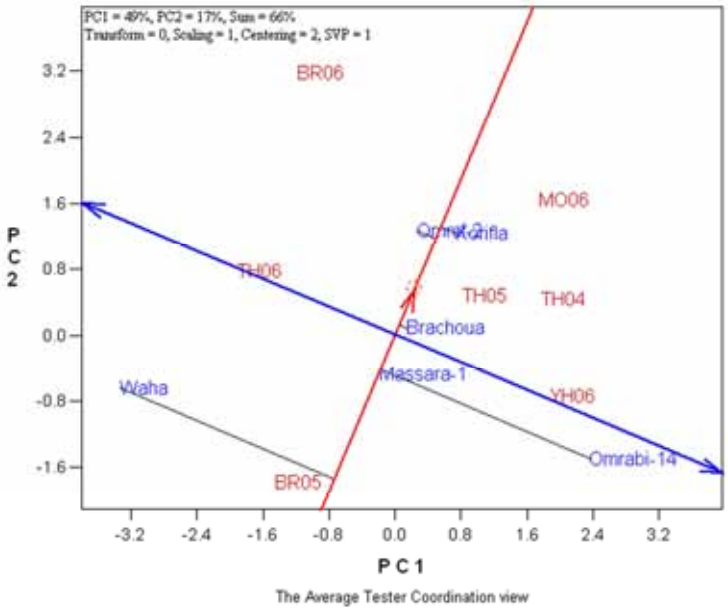

FIG. 16. Analysis of Shukla stability index by using Biplot for the biomass yield of six genotypes across sites and seasons

TABLE 24. ENTRY MEANS AND SHUKLA'S STABILITY INDEX FOR BIOMASS YIELD

Parameter	Omruf-2	Brachoua	Korifla	Waha	Massara-1	Omrabi-14
Mean	16.17	–194.93	198.36	–345.72	223.48	102.64
SBD+	388.38	237.68	347.38	643.81	274.76	573.50

+SBD, stability deviation, calculated as the square root of the GE mean square associated with each entry, and a larger value denotes more GE interaction and less stability.

3.11.3. Relationships among plant traits and genotypes

The relationships among plant traits showed that ash, protein and phyllochron were significantly correlated with each other for the different sites and seasons (Fig. 17). Grain- and leaf Δ and phyllochron were also closely related to each other. Genotypes of low grain Δ (Omruf-2, Brachoua and Korifla) were closely related to each other, but high grain Δ genotypes were not (Massara-1, Omrabi-14 and Waha, Fig. 17).

The superiority of genotypes for the individual plant traits is illustrated in Fig. 18. Omrabi-14 has the highest leaf and grain Δ across seasons and sites. Massara-1 and Korifla had the greatest biomass and grain yield. However, Waha had the highest grain protein and ash content (Fig. 18).

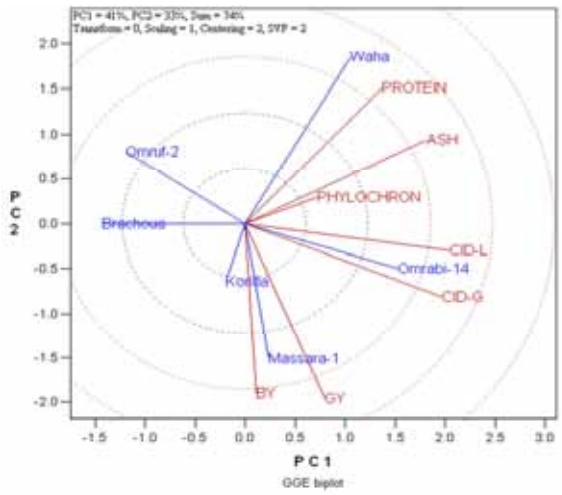

FIG. 17. GGE biplot showing the performance of each cultivar in each environment for individual plant traits (protein, ash, phyllochron, leaf Δ, grain Δ, GY and BY).

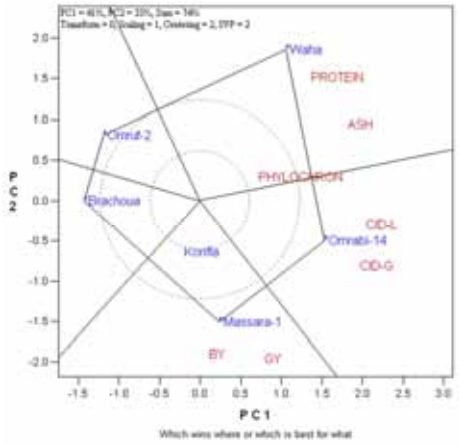

FIG. 18. GGE biplot showing the best performing cultivar for each environment for individual plant traits (protein, ash, phyllochron, leaf Δ, grain Δ, GY and BY).

4. CONCLUSIONS

Grain-Δ values varied between 12.88 and 16.62‰ and were higher for the 4th fully expanded leaf and ranged between 19.97 and 22.57‰. The dry sites or seasons produced low yields and plants with low Δ values compared with the wet sites or seasons. Higher variation was apparent in the grain Δ values compared with variation of the 4th fully expanded leaf Δ, with a similar ranking order between genotypes for grain and leaf Δ. No significant G*E interactions were evident for two genotypes, Waha ($Cham_3$) and Korifla ($Cham_1$) for almost all of the measured traits. The genotypes with high Δ produced high grain yield with low 1000 grain weight i.e. small grain size. Higher and positive correlations were found between grain yield and grain Δ in dry locations compared to moderately wet locations. Significant correlations were evident between grain Δ and grain yield, protein and stomatal resistance. The 4th fully expanded leaf Δ was closely associated with biomass yield, spikelet number $spike^{-1}$, peduncle length, 1000 grain weight and grain ash content.

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RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION ($\Delta^{13}C$) AND WATER USE EFFICIENCY OF DURUM WHEAT IN THE SYRIAN ARAB REPUBLIC. 2. GLASSHOUSE EVALUATION

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Abstract

A greenhouse pot experiment was conducted to compare transpiration rates of six durum wheat genotypes grown in two soil types, a clay (Tel Hadya) and a sandy clay loam (Breda). Six durum wheat genotypes varying in grain carbon isotope discrimination (Δ), an index to transpiration efficiency, were used. Pots were subjected to controlled and gradual dehydration, with a wet treatment as a control. The transpiration ratio (TR) was calculated as the ratio between daily water loss for each of the pots undergoing gradual dehydration, and the average daily water loss in the wet pots. Then the data were further normalized. The daily fraction of transpirable soil water (FTSW) for each pot was calculated by dividing the difference between daily pot weight and final weight by the overall transpirable soil water (difference between initial and final pot weight). The data were analyzed by plotting normalized transpiration ratio (NTR) against the FTSW using logistic, linear plateau and exponential models. Genotypes differed in transpiration rates during gradual dehydration and between the two soil types for pooled data. A significant relationship was found between dry matter production and threshold values (the point when the transpiration rate starts to be less in the gradual dehydration treatment than in the control treatment). The cultivar Brachoua (which had low grain Δ) recorded the highest dry matter production and the highest threshold value. Significant differences in threshold values were evident between the two soil types. The lowest threshold value was for the cultivar Waha (which had high grain Δ, and consequently had a potentially high transpiration efficiency), indicating a superior ability to extract water at high soil water potentials (when soil is dry). On the other hand, the genotype Brachoua was very sensitive to low soil moisture, and transpiration rates decreased at the beginning of the gradual dehydration.

1. INTRODUCTION

Drought is a major threat to food security worldwide, and tolerance to drought stress in crops is a principal focus of plant breeding efforts, especially in arid Mediterranean areas. The impact of drought on crop productivity is associated with physiological abnormalities, which include changes in respiration, photosynthesis, protein synthesis, mineral nutrition, hormone balance, together with increased exposure to a variety of phototoxic compounds [1]. Drought reduces nutrient and water uptake by roots because of restricted transpiration rates [2].

The climate in northern Syrian Arab Republic is classified as a warm continental Mediterranean climate, with a moisture regime characterized as semi-arid to arid [3]. Water shortage is the major constraint to agricultural production in this area [4], and the negative impact of global climate change on rain fed farming systems will be exacerbated by ubiquitous land degradation and increasing water scarcity [5].

Wheat is a major cereal crop globally. The main challenge is to increase the production of this crop vertically without causing an imbalance in overall agricultural output, since the horizontal expansion is very limited. Therefore, increasing production per unit of water is essential, especially with water becoming more limited to meet human as well as agricultural demands. Wheat in the Syrian Arab Republic is normally sown in November,

headed in April, and harvested in June. Rainfall decreases during March and April, and sporadic rainfall and high evaporative demand are observed after May [4, 6].

Increasing water use efficiency (WUE) of crops (i.e yield per unit of water consumed) has been a major long term goal of agronomists and plant breeders. Several morpho-physiological traits were identified and used by the breeders to produce greater WUE at the plant level i.e. greater transpiration efficiency (TE). However, agronomists were more concerned with total water usage i.e. evapo-transpiration. However, it is difficult experimentally to separate plant transpiration from evaporation from the soil.

Morphological and physiological traits will provide qualitative data related to TE, e.g. grain and biomass yield, leaf area, canopy temperature, stomatal conductivity and chlorophyll content [7]. However, another approach involves the use of carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ), since plants with high CID have been shown to have high transpiration efficiency [8]. This technique is based on discrimination against the heavy isotope, ^{13}C, in favour of the lighter ^{12}C isotope during photosynthesis. Discrimination occurs during stomatal diffusion of CO_2 and during the enzymatic processes involved in C fixation in the plant.

Positive relationships between Δ and yield were observed in many studies in Mediterranean-type environments [9, 10, 11, 12, 13, 14, 15], and this relationship was also found to be positive in durum wheat varieties at two locations and over several seasons in northern Syrian Arab Republic [15, 16]. The variation in Δ among varieties within a site reflects their different CO_2 assimilation and transpiration efficiencies. In this case, the breeders can choose the variety that has high transpiration efficiency without the need for many years of testing [13].

Soil water deficit is frequently the environmental factor that imposes the greatest constraint on plant growth. Therefore, a great deal of research has been targeted to understand the plant's response to the imposition of water stress. Experimental approaches vary widely, but a common approach is to simulate drought by withholding water from the medium in which the plants are grown [17, 18]. The objective of the present investigation was to compare transpiration rates of six durum wheat cultivars during the gradual dehydration of two Syrian soil types.

2. MATERIALS AND METHODS

2.1. Plant material

The pot experiments were carried out in a greenhouse at the Faculty of Agriculture, University of Aleppo, using six durum wheat cultivars (Omruf-2, Brachoua, Korifla (Cham3), Waha (Cham1), Massara-1 and Omrabi-14), which were selected and provided by the Durum Wheat Program, ICARDA. These cultivars had a similar phenology, but had contrasting Δ values (Table 1).

2.2. Soils

The two soils (0-20 cm) used in the pot experiment were sampled at Tel Hadya (36.01 ^{o}N, 36.56 ^{o}E) and Breda (35.56 ^{o}N, 37.10 ^{o}E). The soil was collected in the summer, air dried on a greenhouse bench and large soil clods were broken with a mallet. The air-dry soil was screened through a 6 mm sieve. Tel Hadya soil (1210 g) and Breda soil (1320 g) were weighed into pots (1100 cm^3) with 5 holes in the bottom to allow free water drainage, to give a bulk density of 1.1 (Tel Hadya) and 1.2 g cm^{-3} (Breda). 60 pots were prepared for each soil, with each cultivar replicated 10 times.

TABLE 1. $\Delta^{13}C$ VALUES FOR SIX DURUM WHEAT CULTIVARS

Cultivar	Nachit †	Wahbi et al. [19] ‡
Omruf-2	14.78	15.36c
Brachoua	14.88	15.33c
Korifla (Cham3)	14.95	15.72b
Waha (Cham1)	15.43	15.80b
Massara-1	15.71	16.13a
Omrabi-14	15.88	16.26a
		$LSD_{0.05}$ = 0.21

† Personal communication.
‡Data followed by the same letter are not significantly different (P<0.01).

The Tel Hadya soil was classified as a very fine, montmorillonitic, thermic, Chromic Calcixert merging into a Calcixerollic Xerochrept [20]. The surface soil (0–20 cm) was calcareous with a fine to medium granular structure and a pH of 8.1. The texture was clayey (Table 2). Gravimetric water content at field capacity (0.2 bar) was 47% and permanent wilting point (15 bar) was 25% (Fig. 1) [21]. Available moisture was therefore moderately low to low at 22% gravimetric water content.

The Breda soil was classified as a clayey, montmorillonitic, thermic, Calcixerollic Xerochrept [20, 21]. The surface soil was calcareous with a sandy clay loam texture (Table 2) and a moderate fine-to-medium granular structure, slightly hard when dry, but very friable, slightly sticky and slightly plastic when moist [21]. Surface (0–20 cm) soil pH was 7.8. Gravimetric water content at field capacity (0.2 bar) was 39% and permanent wilting point (15 bar) was 23% (Fig. 1) [21], and available moisture was therefore comparatively low at 16% gravimetric water content.

TABLE 2. PARTICLE SIZE DISTRIBUTION (G KG^{-1}) FOR 0–20 CM DEPTH AT TWO SITES

Soil	Texture class	Sand	Silt	Clay
Tel Hadya	Clay	230	320	450
Breda	Sandy clay loam	470	250	280

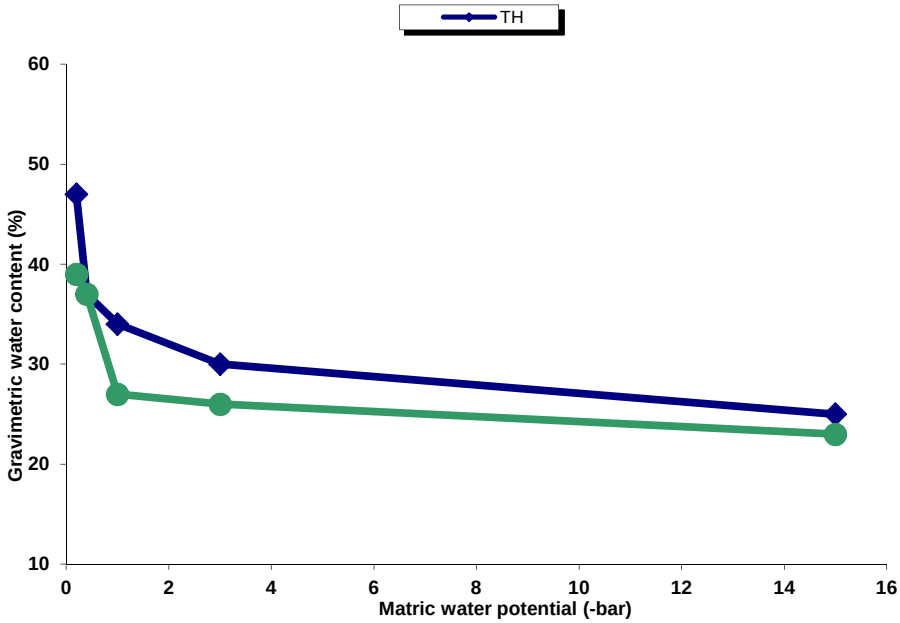

FIG. 1. Moisture characteristic curves for Tel Hadya (TH) and Breda (BR) soils for the 0–20 cm depth [21].

2.3. Experimental

The sowing date was 25th September, 2006. Seeds were placed at 1.5 cm depth, with 10 seeds planted in each pot. Water was maintained at about field capacity during early plant growth. Germination occurred 3–5 d after sowing (DAS). N fertilizer (together with dissolved micronutrients) was applied on 7th October by using 105 g urea (46% N) dissolved in 20 l of water. A second application (23 g urea in 20 l water) was made on 8th November. Plants were thinned on 10th October (third leaf stage, Zadock scale = 13), and a second thinning was carried out on 26th October, leaving one plant per pot. Internal glasshouse temperature was maintained by air-conditioning, and varied from about 30 ^{o}C at 1200 h to 22 ^{o}C at 1730 h.

The dehydration treatment was initiated 54 DAS on 17th November, 2006. Pots were watered excessively and left to drain freely for 24 h. Individual pots were then covered with a polyethylene bag to prevent loss of water by evaporation. Pots were weighed to the nearest 1.0 g on 18th November. On the following day, pots were weighed first at 1050 h to determine the drained upper weight. Then, three pots were selected out of 10 replicates and kept well-watered by weighing daily (at 1730 h) and adding water to maintain the fraction of extractable water at approximately 0.75. However, the drying pots (seven replicates per treatment) were allowed to gradually dehydrate, by weighing each pot daily (at 1730 h) and adding water if needed so that there was only a maximum of 5 g net loss of water each day. To accelerate the drying, the amount was increased from 5 g net loss to 15 and to 20 g day^{-1} on 18th December and 21st December 2006, respectively. The dehydration cycle was 40 days. At the termination of the dehydration cycle (27th December), the plants were harvested by cutting at the soil surface and were dried at 70 ^{o}C to determine plant dry weight.

2.4. Data processing

The transpiration ratio (TR) was calculated as a ratio between daily water loss in each of the drought pots, and the average daily water loss in the wet pots. Data were further normalized by estimating the average weight of pots before the TR value declined to less than 1.0, and then dividing the initial pot weight by the average.

The daily fraction of transpirable soil water (FTSW) for each pot was calculated by dividing the difference between daily and final pot weight by the overall transpirable soil water (difference between initial and final pot weights):

$$\text{daily FTSW} = \frac{\textit{daily pot weight} - \textit{final pot weight}}{\textit{initial pot weight} - \textit{final pot weight}}$$

The data were analyzed by plotting the normalized transpiration ratio (NTR) against the FTSW using Curve Expert V1.3 and Prisme V3.0 programmes. The following logistic model was used:

$$Y = \frac{1}{1 + a\, e^{-bX}}$$

where Y is the Normalized Transpiration Ratio (NTR), X is (FTSW) and a, b were equation constants.

Also, two shapes of curves were found; one with a linear-plateau RT = 1.0, and the other with a linear-plateau RT <1.0 by using plateau regression procedures:

$RT = A + (B \times FTSW)$ (when $FTSW < Cp$)
$RT = 1.0$ (when $FTSW > Cp$)

where A and B are regression coefficients and Cp is the critical value of FTSW demarcating the two stages of the model. In this case, the subscript denotes that Cp is for the extractable soil water based on physiological defined end-points [22]. The value of Cp is defined by the regression coefficients in the following equation:

$Cp = (1 - A)/B$

In addition, a simple exponential model was fitted to the data [23]:

$RT = 1 - \exp(-k \times FTSW)$

The value of k reflected the rate of decrease in RT as FTSW decreased. The regression curves were constructed using GraphPad Prism v. 3.0 (GraphPad Software Inc., San Diego, USA) and SPSS v. 15.0.

Dry matter and threshold data were subjected to analysis of variance (ANOVA). The Duncan test was used to establish differences between cultivars, and between soils. Statistical analyses were performed using GenStat Release 7.2.

3. RESULTS AND DISCUSSION

3.1. Coefficients of equations

Three equations (logistic, exponential and plateau linear regression) were analyzed and the coefficients of each equation were compared.

The values of R^2 were 0.63 and 0.83 at Tel Hadya and Breda soils, respectively, using the logistic model (Table 3) [24], whereas the values were 0.65 and 0.83 for Tel Hayda and Breda soils, respectively, using the exponential model (Table 4). Similarly, a better fitting was obtained using the exponential compared with the first model [23]. By using the plateau linear regression mode, the values of R^2 were 0.84 for Tel Hadya and 0.76 for Breda soils (Table 5). In general, the values of R^2 for the cultivars grown in the Breda soil were higher than cultivars in the Tel Hadya soil, especially Massara-1.

TABLE 3. VALUE OF PARAMETERS IN THE LOGISTIC EQUATION†

Soil	Cultivar	Tel Hadya				Breda			
		a	b	R^2	N°.	a	b	R^2	N°.
Breda						4.70	12.54	0.83	2032
Tel Hadya		4.46	12.89	0.63	1839				
	Omruf-2	3.77	11.77	0.67	325	4.07	15.34	0.85	321
	Brachoua	6.48	9.29	0.72	388	3.56	10.26	0.87	321
	Korifla	3.94	16.15	0.77	300	8.35	13.74	0.80	362
	Waha	6.96	24.07	0.58	270	2.52	11.24	0.67	334
	Massara-1	4.93	21.47	0.53	225	4.90	12.93	0.9	341
	Omrabi-14	3.15	10.65	0.74	331	7.58	13.62	0.93	353

† NTR = 1/1 + a exp (–b*FTSW).

TABLE 4. VALUE OF PARAMETERS IN THE EXPONENTIAL EQUATION†

Soil	Cultivar	Tel Hadya				Breda			
		k	k, 95% confid.	R^2	N°.	k	k, 95% confid.	R^2	N°.
Breda						6.754	6.573-6.936	0.83	2032
Tel Hadya		6.613	6.384-6.842	0.65	1839				
	Omruf-2	7.688	6.967-8.408	0.66	325	8.528	7.914-9.142	0.85	321
	Brachoua	4.112	3.893-4.330	0.72	388	6.628	6.163-7.093	0.86	321
	Korifla	8.546	7.924-9.168	0.78	300	5.779	5.440-6.119	0.76	362
	Waha	10.09	8.956-11.22	0.57	270	8.796	8.067-9.526	0.66	334
	Massara-1	7.349	6.672-8.026	0.63	225	6.781	6.410-7.152	0.89	341
	Omrabi-14	6.606	6.180-7.033	0.76	331	5.839	5.557-6.122	0.92	353

† TR = 1 – exp (–k*FTSW).

TABLE 5. VALUE OF PARAMETERS IN THE REGRESSION EQUATION†

Soil	Cultivar	Slope (B)	Y–intercept (A)	R^2	N°.
Tel Hadya		3.103 ± 0.1463	0.1996 ± 0.0193	0.84	88
	Omruf-2	2.372 ± 0.2352	0.2459 ± 0.0440	0.58	76
	Brachoua	1.949 ± 0.1109	0.0932 ± 0.0324	0.67	153
	Korifla	2.832 ± 0.1911	0.2357 ± 0.0303	0.71	93
	Waha	3.804 ± 0.4594	0.1791 ± 0.0620	0.61	45
	Massara-1	3.605 ± 0.2633	0.1684 ± 0.0324	0.73	72
	Omrabi-14	2.298 ± 0.1720	0.2379 ± 0.0317	0.65	98
Breda		2.504 ± 0.0553	0.1839 ± 0.0100	0.76	630
	Omruf-2	2.078 ± 0.0893	0.2254 ± 0.0168	0.84	102
	Brachoua	2.792 ± 0.1513	0.0698 ± 0.0312	0.73	126
	Korifla	2.644 ± 0.2530	0.2847 ± 0.0410	0.59	78
	Waha	2.623 ± 0.1055	0.1622 ± 0.0171	0.86	104
	Massara-1	2.623 ± 0.0659	0.0950 ± 0.0126	0.93	115
	Omrabi-14	2.211 ± 0.0953	0.2535 ± 0.0175	0.52	508

† RT = A + (B*FTSW).

The logistic model was used to generate threshold values which were compared with Cp values from the exponential model (Table 6). The same trend was observed between critical values from the two models. Therefore, the thresholds from the logistic model were selected for detailed discussion.

TABLE 6. COMPARISON OF THRESHOLD VALUES

Cultivar	Threshold values†		Generated threshold values‡	
	Tel Hadya	Breda	Tel Hadya	Breda
Omruf-2	0.32	0.37	0.31	0.28
Brachoua	0.46	0.33	0.45	0.39
Korifla	0.27	0.27	0.28	0.33
Waha	0.21	0.32	0.24	0.26
Massara-1	0.23	0.34	0.23	0.33
Omrabi-14	0.33	0.34	0.33	0.37
Average	0.26	0.33	0.31	0.33

†From exponential model.
‡From logistic model.

3.2. Dry matter and CID

Significant differences in dry matter (DM) were found between cultivars (Table 7). Omruf-2 and Brachoua had significantly higher DM compared with the other cultivars, while Omrabi-14 and Massara-1 had significant higher DM compared with Waha (Table 7). Cultivars in the Breda soil had significantly higher DM compared cultivars in the Tel Hadya soil (Table 8). Dry matter for low Δ cultivars was significantly higher than DM for high Δ cultivars (Table 8).

Values for leaf Δ were considerably higher than grain Δ for each cultivar (Table 7). Significant differences in leaf Δ were evident between cultivars in the order Omrabi-14 > Massara-1 = Waha = Korifla > Brachoua > Omruf-2 (Table 7). However this order was not maintained with grain Δ, with Waha being significantly higher and Omruf-2 being significantly lower than the other four cultivars, which did not differ significantly among themselves (Table 7). The grouping of three cultivars as low Δ and three as high Δ was confirmed by significant differences between their mean values for both leaf and grain (Table 8). Both leaf and grain Δ were significantly higher at Tel Hadya than Breda, the opposite of the results obtained for DM (Table 8).

TABLE 7. MEAN DRY MATTER (G PLANT^{-1}), LEAF AND GRAIN Δ (‰) AND THRESHOLD VALUES OF SIX DURUM WHEAT CULTIVARS GROWN IN TEL HADYA AND BREDA SOILS

Cultivars	Dry matter	Leaf-Δ	Grain-Δ	Threshold †
Omruf-2	0.85a	21.50d	14.53c	0.296bc
Brachoua	0.87a	21.82c	14.86b	0.420a
Korifla	0.54bc	22.03b	14.87b	0.307bc
Waha	0.41c	22.04b	15.01a	0.250c
Massara-1	0.65b	22.01b	14.87b	0.280c
Omrabi-14	0.64b	22.29a	14.89b	0.349b
F test	0.049	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.26*	0.114***	0.11***	0.18***
SE (±)	0.13	0.055	0.05	0.09

† Generated by the logistic equation
Means followed by the same letter are not significantly different (Duncan test, $P<0.05$);
*, ***, significant at $P<0.05$ and $P< 0.001$, respectively; SE, standard error.

TABLE 8. MEAN DRY MATTER (G PLANT^{-1}), LEAF AND GRAIN Δ (‰) AND THRESHOLD VALUES OF SIX DURUM WHEAT CULTIVARS GROWN IN TEL HADYA AND BREDA SOILS

Parameter	Dry matter	Leaf-Δ	Grain-Δ	Threshold †
Low-Δ	0.75a	21.8b	14.75b	0.34a
High-Δ	0.56b	22.1a	14.93a	0.29b
F test	0.002	<0.001	0.004	0.014
$LSD_{0.05}$	0.12	0.10	0.11	0.04
Tel Hadya	0.55b	22.4a	15.65a	0.33a
Breda	0.77a	21.5b	14.04b	0.31a
F test	<0.001	<0.001	<0.001	0.28
$LSD_{0.05}$	0.12	0.10	0.11	0.04

† Generated by the logistic equation.
Means followed by the same letter are not significantly different (Duncan test, $P<0.05$).

3.3. Water extraction

Soil water uptake by six durum cultivars grown in Tel Hadya and Breda soils was expressed by relative transpiration rate with the fraction of transpirable soil water based on physiological values (Fig. 2 and Fig. 3). The plants maintained transpiration ratios of 1.0 until the critical threshold of FTSW reached 0.31, 0.45, 0.28, 0.24, 0.23 and 0.33 for Omruf-2, Brachoua, Korifla, Waha, Massara-1 and Omrabi-14 cultivars, respectively, in the Tel Hadya soil (by generated logistic equation), whereas critical thresholds of FTSW in the Breda soil reached 0.28, 0.39, 0.33, 0.26, 0.33 and 0.37 for the respective cultivars. The values reported were within the same range to that reported for soybean (FTSW of 0.35), and maize (FTSW of 0.31) [25], whereas a wider range of 0.20 to 0.60 was reported for two cultivars of maize [26].

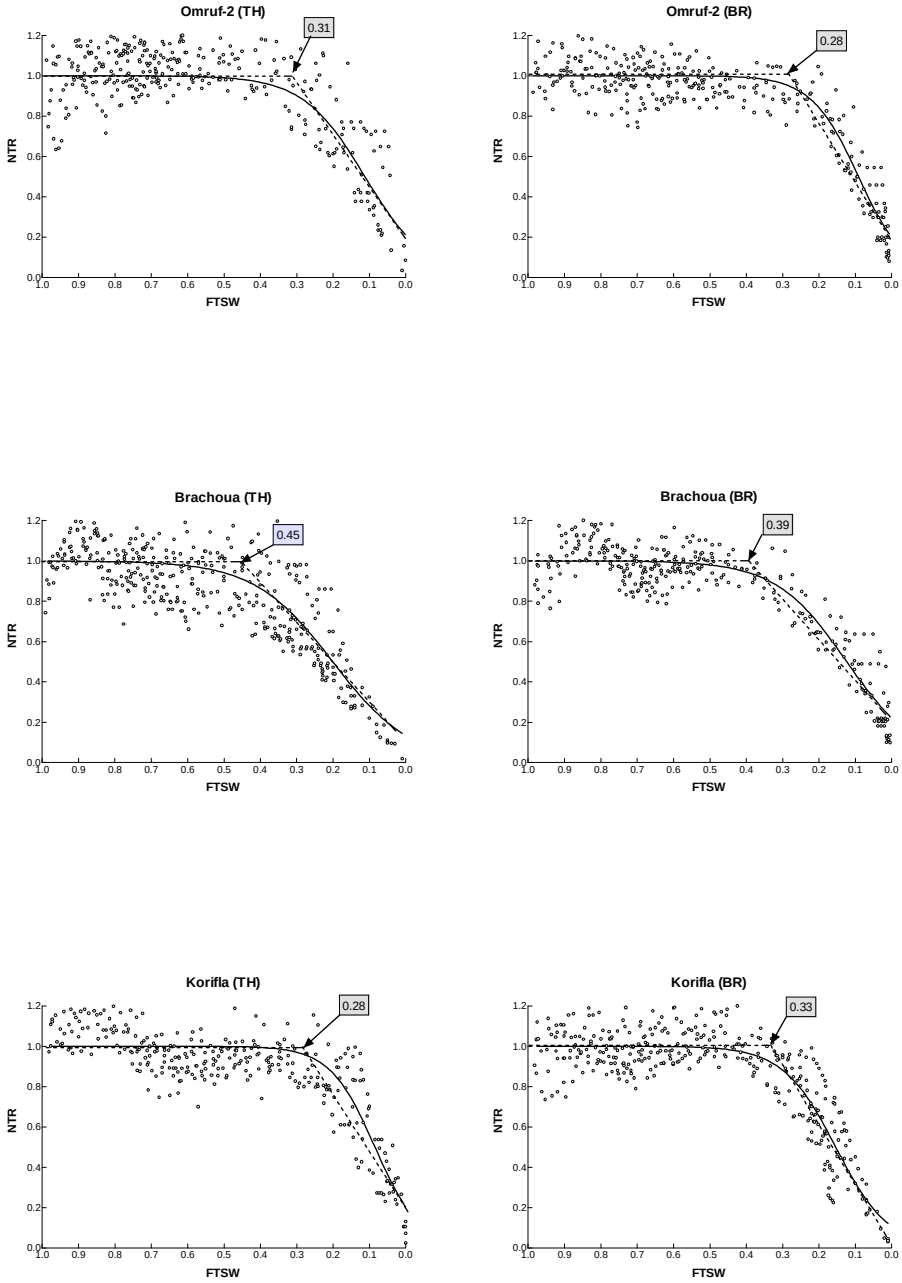

FIG. 2. Relationships between normalized transpiration ratio (NTR) and fraction transpirable soil water (FTSW) for three low Δ durum wheat cultivars grown in Tel Hadya (TH) and Breda (BR) soils.

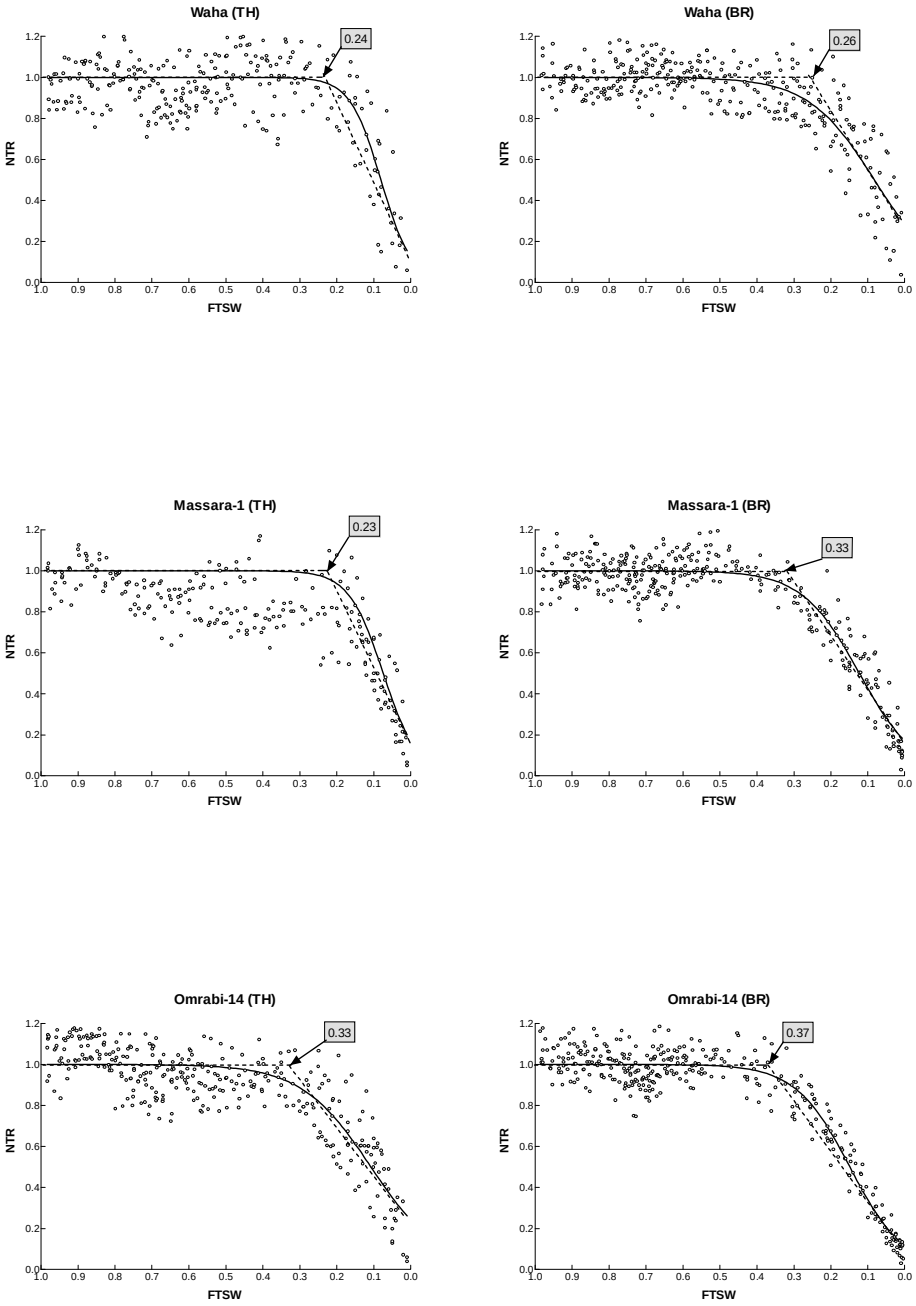

FIG. 3. Relationships between normalized transpiration ratio (NTR) and fraction transpirable soil water (FTSW) for three high Δ durum wheat cultivars grown in Tel Hadya (TH) and Breda (BR) soils.

Significant differences were found between cultivars in the individual threshold values averaged across sites (Table 7). Genotypic differences in threshold values of FTSW among 17 genotypes of peanut ranged from 0.33 and 0.71 [27], whereas threshold values even between different species of maize, soybean and Arabidopsis did not differ [23].

The threshold for Brachoua was significantly higher than the other cultivars. This was followed by Omrabi-14 (0.35), Omruf-2 and Korifla (0.30 and 0.31) and Massara-1 and Waha (0.28, 0.25), respectively (Table 7). Omrabi-14 had a significantly higher threshold compared with Waha and Massara-1. The results suggest that Brachoua could be more sensitive to water deprivation. Significant differences were found between the threshold means of low and high Δ cultivars (Table 8). However, there was no significant difference between the cultivar

threshold means in the two soil types, although differences were noted for individual cultivars (Table 7).

The relationships between normalized transpiration ratio (NTR) and fraction transpirable soil water (FTSW) for six durum wheat cultivars showed a much wider scatter of the pooled data in the Tel Hadya soil compared with the Breda soil (Fig. 4).

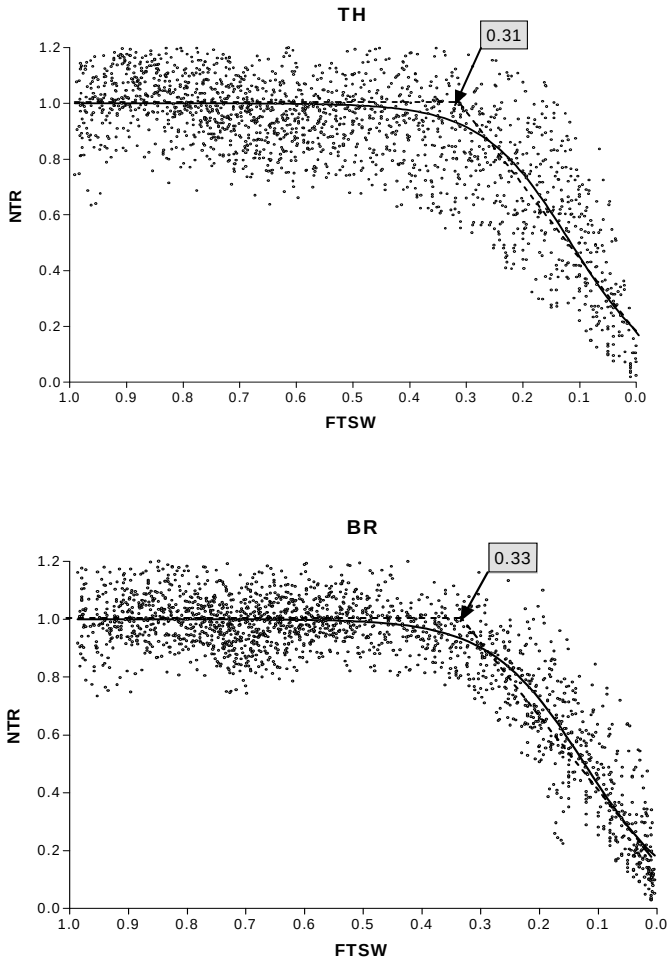

FIG. 4. Relationships between normalized transpiration ratio (NTR) and fraction transpirable soil water (FTSW) for pooled data of six durum wheat cultivars grown in Tel Hadya (TH) and Breda (BR) soils.

Exponential relationships between NTR and FTSW for six individual durum wheat cultivars grown in Tel Hadya and Breda soils showed a closer trend among the cultivars in the Breda soil compared with the Tel Hadya soil, where the cultivars were more divergent (Fig. 5).

There were no significant differences between cultivars in the Breda soil in the time course of the daily fraction of transpirable soil water (FTSW) after imposition of the dehydration stress, whereas the cultivars showed a different behavior in the Tel Hadya soil (Fig. 6). Omruf-2 and Brachoua diverged noticeably compared with the other four cultivars beginning at day 5. On day 12, differences between Omrabi-14 and other cultivars became obvious. Massara-1 had the lowest trend for FTSW and there was a significant difference between cultivars after 30 days (Fig. 6).

The same behaviour was shown in cumulative transpiration. Omruf-2 in the Tel Hadya soil had the highest trend with a significant difference compared with other cultivars after 12 days (Fig. 7). Waha had the lowest cumulative transpiration with a significant difference compared with other cultivars after 15 days, except Korifla and Massara-1. Waha had the lowest cumulative transpiration in the Breda soil with a significant difference compared with Omruf-2 and Omrabi-14 after 12 days (Fig. 7). On average, the cumulative transpiration at Breda had a significant difference compared with Tel Hadya after 9 days (Fig. 7).

The means of cumulative transpiration for low Δ durum wheat cultivars (Omruf-2, Brachoua and Korifla) in the Tel Hadya soil showed a significant difference compared with the high Δ durum wheat cultivars (Waha, Massara-1 and Omrabi-14) after five days of dehydration stress (Fig. 8), and this differences was shown in FTSW values after 14 days of dehydration of stress. These differences were not observed in the Breda soil.

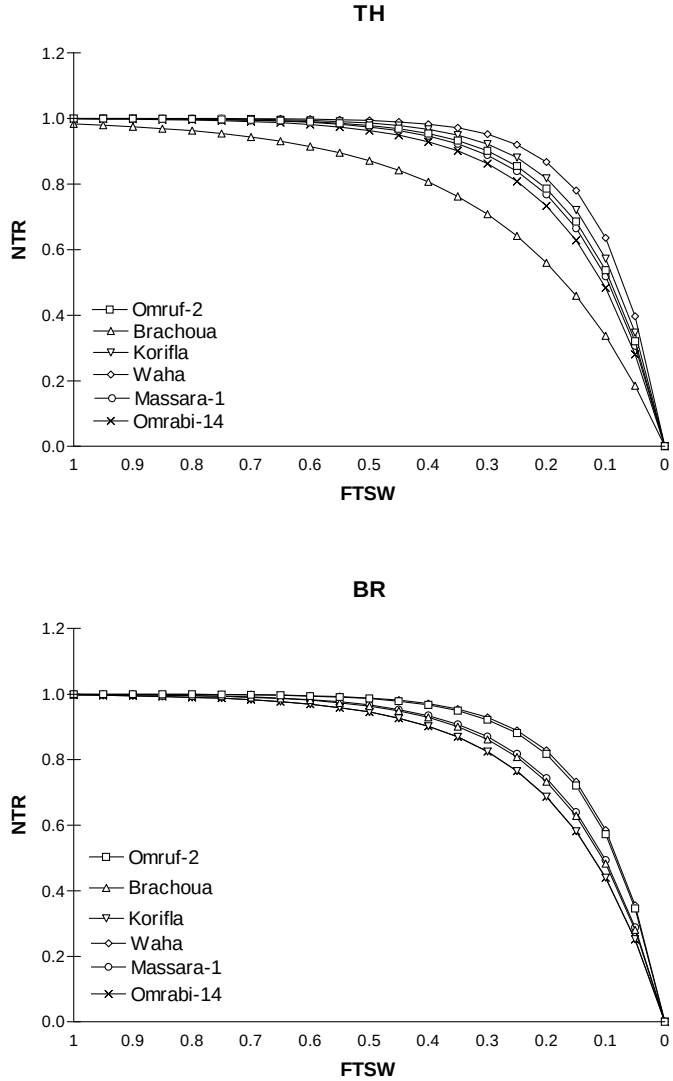

FIG. 5. Exponential relationships between NTR and FTSW for six individual durum wheat cultivars grown in Tel Hadya (TH) and Breda (BR) soils.

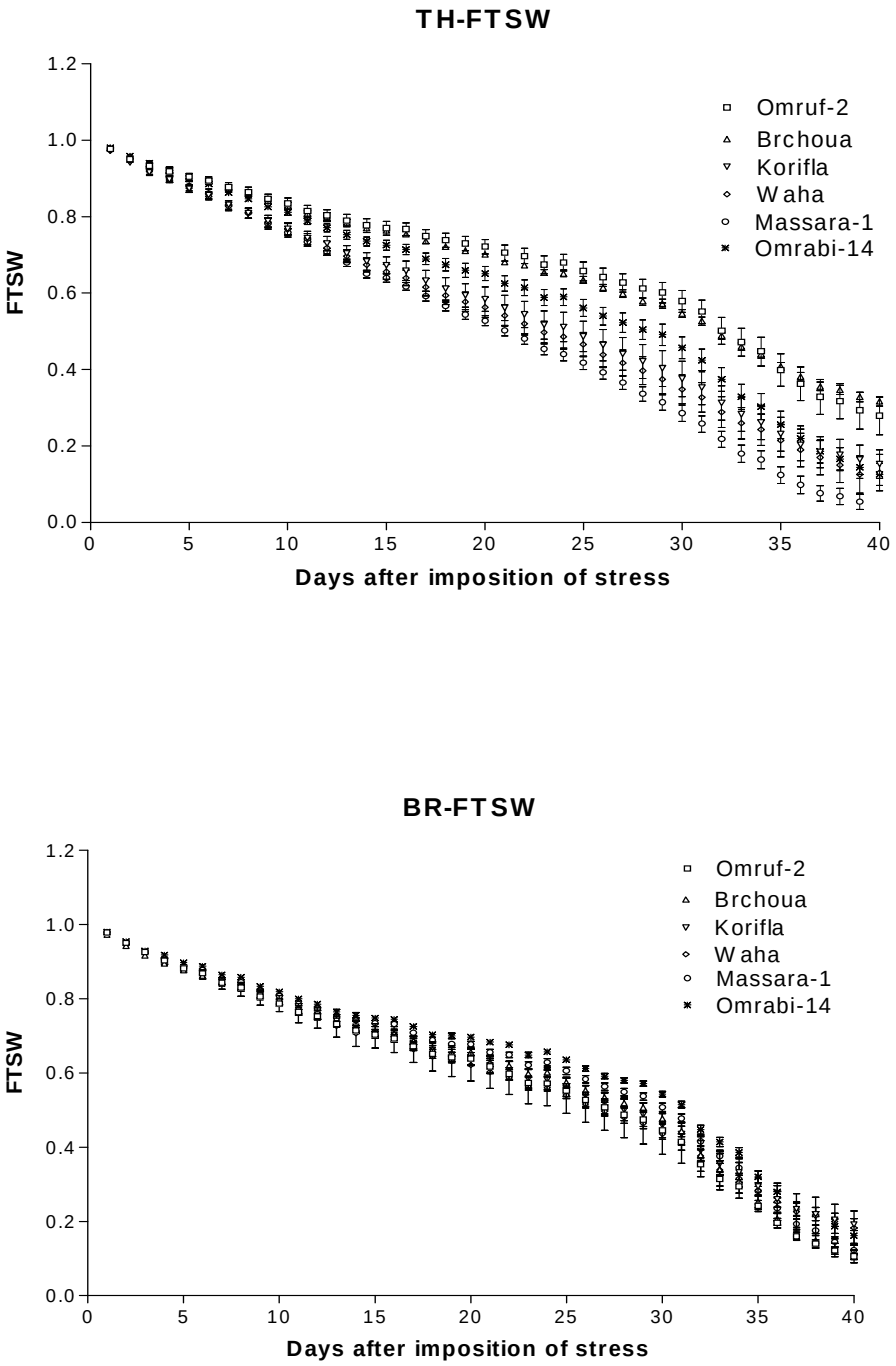

FIG. 6. Time course of FTSW in six individual durum wheat cultivars grown in Tel Hadya (TH) and Breda (BR) soils.

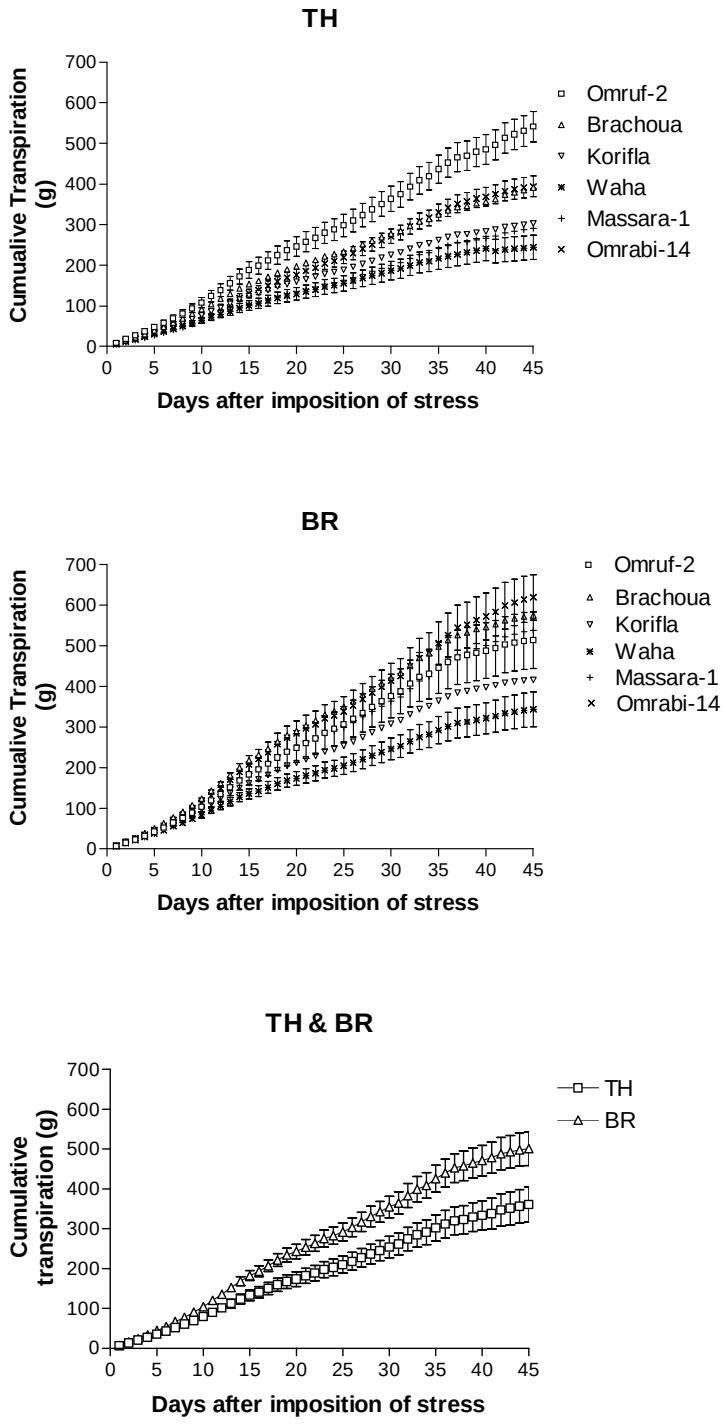

FIG. 7. Cumulative transpiration in six individual durum wheat genotypes and pooled genotypes in Tel Hadya (TH) and Breda (BR) soils.

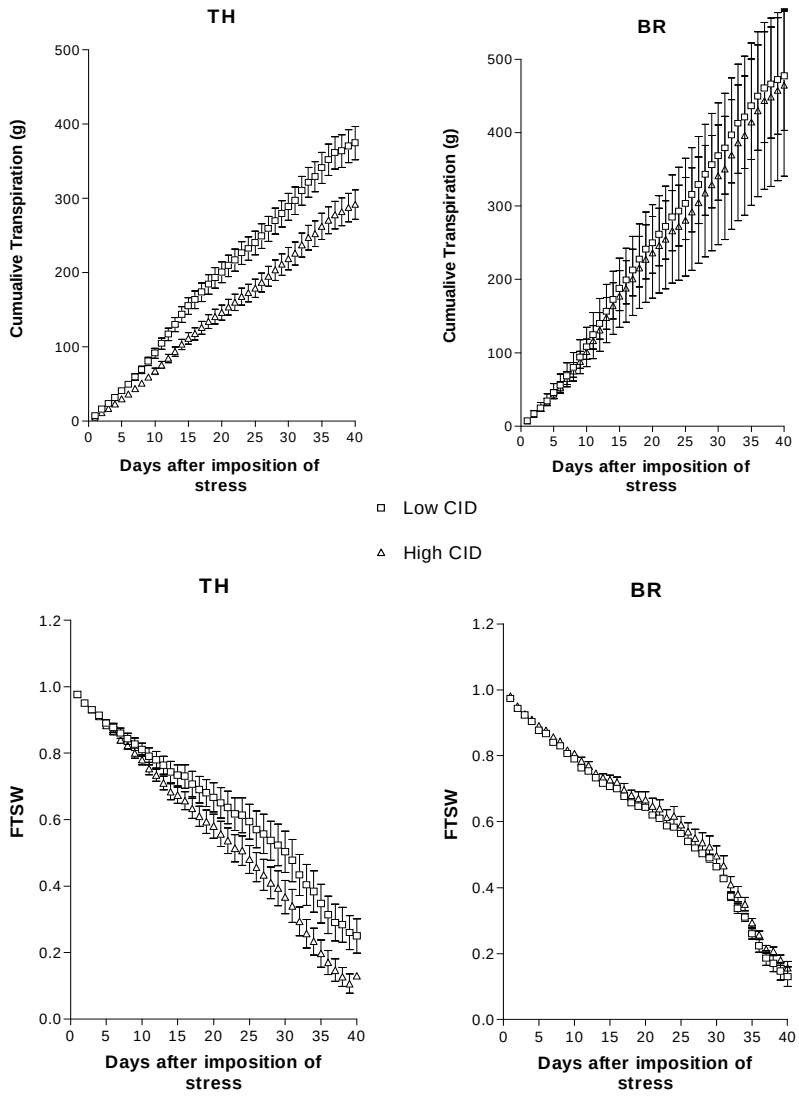

FIG. 8. Time course of FTSW and cumulative transpiration in low and high Δ durum wheat genotypes in Tel Hadya (TH) and Breda (BR) soils.

3.4. Relationships between dry matter, CID and threshold

A significant and positive correlation was found between dry matter and threshold (Fig. 9), indicating that a cultivar which had a low threshold could produce low dry matter because its transpiration rate could be slower than the other cultivars.

There were no significant linear relationships between grain or leaf Δ and threshold values.

4. CONCLUSIONS

Transpiration rates during gradual soil dehydration differed between cultivars in individual soils and between the two soil types for pooled cultivar data. A significant relationship was found between dry matter production and threshold values. Waha (Cham3) genotype was capable of extracting a higher amount of soil water even with very low soil moisture (threshold of 0.25) compared with other cultivars. On the other hand, the cultivar Brachoua was very sensitive to shortage of soil moisture (threshold of 0.42), and transpiration rates decreased at the beginning of the gradual dehydration.

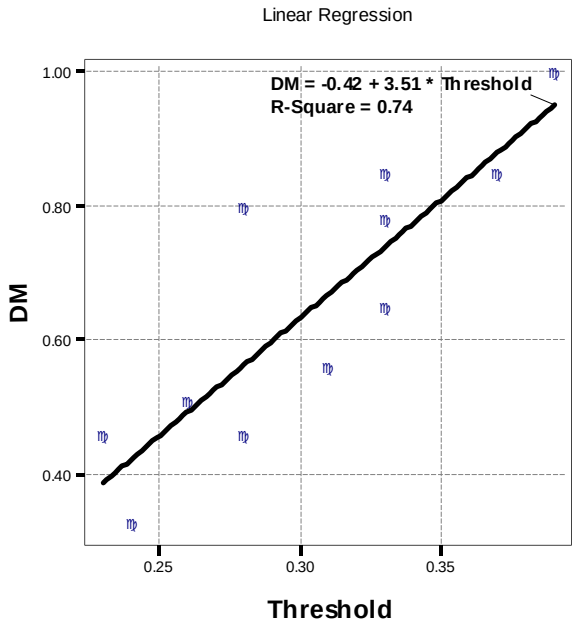

FIG. 9. Relationship between dry matter and threshold for six durum wheat cultivars grown in two soils.

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GENETIC IMPROVEMENT OF AGRONOMIC WATER USE EFFICIENCY OF WHEAT IN NORTHWEST CHINA

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Abstract

The five North-western provinces of China (Shaanxi, Gansu, Ningxia, Qinhai and Xinjiang) are located in arid or semiarid areas, where evaporation is 4- to 10-fold higher than precipitation. The serious deficiency of water results in poor yields and low profits from agricultural production. Grain production in the limited irrigated areas is seriously threatened by the continuous water decline in the Yellow River. Wheat is the major crop cultivated in the region. Therefore, breeding wheat varieties resistant to drought with increased water use efficiency (WUE) is one of main strategies for increasing food security and stabilizing farmers' incomes. The purpose of the research was to study the relationships between carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) and yield, agronomic WUE and other related traits in wheat, and to select high WUE wheat varieties adapted to different ecological environments. We evaluated the genetic variation of Δ, studied the relationships between Δ and yield, developed populations from progenitors which contrasted in leaf and kernel-Δ, and studied the genetics of Δ in wheat from 2004 to 2006. In 2004, 32 bread wheat cultivars were planted in four locations, including two rain fed sites (Guyuan and Penyang) and two limited irrigated sites (Yinchuan and Huinong) to study the genetic variation in Δ. In 2005, 20 bread wheat genotypes were studied for the relationship between Δ and yield, and the varieties with contrasting Δ values were selected to make crosses in three locations. In 2006, 2007 and 2008, F_3, F_4, F_6 progenies and stabilized genotypes were planted in Yinchuan (limited irrigation) and in Guyuan (rain fed). The genetics of Δ were studied in 2006, 2007 and 2008. Correlations between Δ and yield were studied in progenies and stabilized genotypes in 2008. Significant variation in Δ was found across genotypes, locations and years. The Δ of local varieties was significantly lower than that of improved varieties and advanced lines. Lower Δ was found under rain fed conditions than under limited irrigation. The relationships between grain yield and the Δ of mature grain (ΔG_m) and the flag leaf at anthesis (ΔL_a) varied greatly across locations and years. In Guyuan, the correlation between grain yield and Δ in two years was not significant. In Penyang, grain yield was highly significantly correlated with both ΔL_a and ΔG_m in 2004. In Yinchuan, grain yield was significantly and positively correlated with grain Δ only in 2005. In Huinong, grain yield was correlated with ΔG_m and ΔL_a in 2005, but not in 2004. Higher ash content in the leaf and whole plant was found under limited irrigation than under rain fed conditions in each of the two studied years, but the higher ash content in grain was found in rain fed compared with limited irrigated conditions. Leaf ash content was significantly correlated with ΔG_m and ΔL_a under both rain fed and limited irrigation in 2005. Higher specific leaf dry mass (LDM) was found under rain fed compared with limited irrigation, and LDM at heading was significantly and positively correlated with ΔL_a under rain fed conditions in one year of the study. Five crossing types (high Δ × high Δ, high Δ × low Δ, low Δ × low Δ, high Δ × Florence, low Δ × Florence) were carried out in 2005. Two Australian check varieties were used to make crosses in 2006 (Drysdale × high Δ, Drysdale × low Δ, Quarry × high Δ, and Quarry × low Δ). High broad sense heritability (h_B^2) of Δ was found under both rain fed and irrigated conditions in 2006 and in Yinchuan in 2008. High-Δ progenies were generated more frequently from the high Δ parent combination. Several genetic resources with low and high WUE were identified. 200 F_6 lines originated from three combinations were obtained and some elite lines were selected in 2008. The relationship between grain yield and Δ was unstable across environments and water regimes. It depended highly on the quantity and distribution of rainfall, the occurrence of irrigation and consequently the water status of the crop across the growth cycle. The use of Δ as a yield predictor should be recommended under moderate pre-anthesis water stress and limited irrigation conditions, but not in environments characterized by very severe pre-anthesis drought stress. On the basis of the results obtained in 2007 to 2008, high Δ × high Δ seems to be the optimal crossing

combination, and the selection of progenies with high Δ values would lead to a yield gain under limited irrigation, while high Δ × low Δ would be a better crossing combination in breeding programmes in rain fed environments.

1. INTRODUCTION

Ningxia Hui autonomous region is located on the loess Plateau of Northwest China, between 104.17° – 107.38° E and 35.14° – 39.23° N with a total area of 60 400 km². Altitude ranges from 1000 – 2000 m above sea level and decreases from south to north. The climate is temperate with long, cold winters and short, hot summers. Average annual rainfall varies from 190 – 700 mm, gradually decreasing from south to north. Rainfall from June to September accounts for 50 to 73% of the total, but the year-to-year distribution and intensity are irregular [1].

In the northern part of the Province, wheat is irrigated with Yellow River water, through well-established irrigation systems [2]. In the hilly southern part of the Province, characterized by low rainfall, poor soil fertility, and heavy soil erosion, wheat is cultivated under rain fed conditions, and is exposed to severe pre-anthesis water stress [3]. Because of drought occurring periodically in the loess Plateau [4], the recent decrease in Yellow river water flow [5] and the expected effects of climate change [6], enhancing water use efficiency has become a major challenge for wheat cultivation in this region. In addition, an important part of the irrigated area is affected by soil salinity, and salinity tolerance is consequently another major objective for wheat breeders [7].

Carbon isotope discrimination (CID, $\Delta^{13}C$, Δ) has been proposed by several authors as an indirect selection criterion for wheat grain yield under drought [8, 9, 10] and salinity [11]. In C_3 plants, Δ was found to be positively correlated with C_i/C_a (i.e. the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration) [12, 13]. The variation in Δ results from variation in both stomatal conductance and photosynthetic capacity [14]. Under stressed conditions, the variation in Δ is mainly driven by stomatal conductance than by photosynthetic capacity [3, 15]. Under terminal (post-anthesis) drought stress, the relationship between Δ and grain yield is significantly positive and stable across environments [8, 10], and high Δ genotypes maintaining higher stomatal conductance perform better than low Δ genotypes [8, 10, 16]. Under other drought stress scenarios, the relationship between grain Δ and yield varies highly with the amount and distribution of rainfall, and with the quantity of water stored in the soil at sowing. A wide variation in this relationship was found across years under early (pre-anthesis) water stress [3, 17] and residual soil moisture conditions [18]. Δ was reported to decrease with soil salinity in several C_3 plants [19]. A positive correlation between Δ and the production of grain and total biomass was noted in wheat under controlled conditions [11]. Grain yield and grain or leaf Δ were not significantly correlated under well-watered conditions [10]. However, when irrigation is limited, and when a subtle water stress occurs during grain filling, yield of irrigated cereals was found to positively correlate with Δ in the peduncle [15] and grain [20, 21].

In order to further validate the potential value of Δ in breeding programmes, it is necessary to better understand the relationships between Δ, morpho-physiological traits and conventional agronomic traits, particularly those related to water status and/or used as indirect selection criteria. There are some reports of positive correlations between Δ and harvest index (HI) in wheat [8, 22]. Although positive or no correlation between biomass and Δ have been reported [14, 23, 24, 25], a few negative relationships between the two traits have been found [9, 24, 26]. Specific leaf dry weight (SLDW) has been proposed as a surrogate of Δ in

sunflower [27] and groundnut [28]. Negative correlations between SLDW and Δ were reported in wheat [25] and barley [29] under favourable conditions. A weak or no relationship between Δ and SDLW was reported in durum wheat [8] and in cowpea [30] under drought conditions. Positive correlations between Δ and water status parameters were reported in durum wheat under Mediterranean conditions [8]. Significant negative correlation between leaf Δ and canopy temperature at anthesis were reported [10, 21].

Correlations were reported between Δ and ash content of leaf and grain of different C_3 species. Positive correlations between leaf Δ and leaf m_a, and negative correlations between grain Δ and grain m_a were found in cereals [3, 8, 31, 32]. Leaf-m_a was also found to be positively correlated with grain yield in bread and durum wheat [33]. Most minerals are transported passively in the xylem and accumulate in transpiring plant tissues. Greater transpiration which is generally associated with higher Δ consequently increases the amount of passively-transported minerals in the leaves. However, the accumulation of individual minerals in plant organs is complex because these elements transported actively can be redistributed to the most active parts of the plant. Potassium (K) was found to be the element contributing more to soluble minerals in xylem sap and transpiration ratio [31]. It was found to play an important role in plant water economy via regulation of stomatal function [34, 35]. The transport of assimilates from source organs to sink organs via the phloem is also coupled with the movement of K [36, 37]. Magnesium (Mg) plays an important role in reducing active oxygen and malondialdehyde (MDA), which can delay senescence [38, 39]. Calcium (Ca) in the plant is transported via the xylem rather than via the phloem. The movement of Ca in plant xylem is mainly driven by transpiration flow [40]. The Ca concentration is consequently high in the organs that have intensified transpiration and have a long growing period [41]. Most studies dealing with the relationships between Δ and minerals in cereals have focused on silicon (Si) whose absorption was considered to be passive and closely related to water flow [42]. Significant relationships between Δ and the concentrations of four individual minerals (K, Mg, P and Si) in three organs of durum wheat grown under Mediterranean field conditions were reported [8].

However, most reported relationships between Δ and yield and morpho-physiological traits were obtained under irrigated or post-anthesis drought stress scenarios and there are few studies carried out under pre-anthesis drought stress or saline conditions. It is of importance to study the associations between Δ, plant water status and leaf gas exchange parameters in wheat grown in rain fed (pre-anthesis drought stress), irrigated (including saline and non saline) environments in China. Such study would also identify a collection of wheat materials and makes it possible to develop populations from progenitors with contrasted Δ value and to evaluate different strategies for using CID as a selection tool for identifying higher yielding genotypes in water limited rain fed cropping and irrigated cropping systems by assessing the integrated performance of progenies derived from different crossing combinations.

The aims of this study were (a) to describe the genetic variation for CID and analyze the relationship between leaf and kernel-Δ and grain yield (b) to determine the relationships between CID and several morpho-physiological traits under Ningxia conditions (c) to identify genetic resources with low or higher water use efficiency (d) to evaluate an Australian population obtained from low Δ progenitors (e) to develop populations from progenitors with contrasting leaf and kernel-Δ, and sets of elite isomorphic lines varying in CID, to be used in breeding programmes and genetics and/or molecular studies (f) to evaluate different strategies for using CID as a selection tool for identifying higher yielding genotypes in water limited rain fed and irrigated cropping systems (g) to study the genetics of CID in wheat.

2. MATERIALS AND METHODS

2.1. Materials

32 genotypes were planted in 2004 and 20 genotypes were planted in 2005, 2006, 2007 and 2008, which were collected from the crop breeding organizations in northwestern China and Ningxia Province, including landraces, elite improved varieties and advanced lines, as well as two check varieties. The origin and genetic background of the 32 genotypes are shown in Table 1.

TABLE 1. ORIGIN AND GENETIC BACKGROUND OF GENOTYPES

Material †	Origin‡	Genetic background
Hongtuzi*	Pingluo and Yinchuan	Local variety
Xiaoheshangtou*	Zhongwei and Zhongning	
Maohuomai**	Wuzhong and Yongning	
Shanmai*	Southern mountainous region of Ningxia	
Hongmangmai**		
Ningchun 30**	Crop Research Institute of NAAFS	Conventional breeding variety
82361-3*		
Xin93-32**	Xinjiang	
Shan SW1206**	Shaanxi	
Jinyan 746-9**	Shanxi	
2003A4016**	Crop Research Institute of NAAFS	*T. aestivum x Aegilops* crossbred
2003A4022**		
2003A4045**		
2003A4057*		
2003A4269**		
Yong 2638*		Conventional variety of crossbred
Yong 3119**		
Ningchun 4**		
Ningchun 35*	NAAFS	
5909*		
Ningchun 32**		
93-8*		
03S47**		*T. aestivum x Aegilops* crossbred
03S111**		
03Y8**		*T. aestivum x T. Durum* Dsef
01H39*		Conventional crossbred incubated by
01H219**		wheat anther
98H30**		
2003A4044*	CIMMYT, Mexico	SHA3/SERI//PSN/BOW
F3-7**	France	Descendant of hard particle wheat
Ningchun 36*	Guyuan Academy of Agricultural Science,	Conventional variety of crossbred
Ningchun 27**	Ningxia	

†* denotes planted for one year; ** denotes five years.
‡ NAAFS, Ningxia Academy of Agriculture and Forestry Sciences; Origin is China unless otherwise indicated.

In 2006 and 2007, 150 lines selected from 3 combinations, i.e. Yong 3119 × 03S111 (high Δ × high Δ), Ningchun 4 × Ningchun 27 (high Δ × low Δ), and Ningchun 32 × Ningchun 27 (low Δ × low Δ) were evaluated in Yinchuan and Guyuan.

In 2008, 200 F_6 lines were planted in Yinchuan and Guyuan. 99 taller F_6 lines of Ningchun 32 × Ningchun 27 (low Δ × low Δ) and Ningchun 4 × Ningchun 27 (high Δ × low Δ) combinations were sown at the Guyuan site, while 101 shorter lines of Yong3119 ×

03S111 (high Δ × high Δ) and Ningchun 4 × Ningchun 27 (high Δ × low Δ) combinations were sown at the Yinchuan site.

2.2. Experimental sites

2.2.1. Climate and soil conditions

The characteristics of the four experimental sites are shown in Table 2.

TABLE 2. PEDO-CLIMATIC CHARACTERISTICS OF THE EXPERIMENTAL SITES

Location	Water regime	Altitude (m)	Annual rainfall (mm)	Evaporation (mm)	Organic matter ($g\ kg^{-1}$)	Salt content ($g\ kg^{-1}$)	Soil pH
Guyuan †	Rain fed	1730	300-400	1700-1900	15.0	0.67	7.8
Pengyang‡		1500	200-300	1440-1670	11.9	0.67	7.8
Yinchuan #	Limited	1111	150-200	1700-1900	16.9	0.55	8.0
Huinong *	irrigation	1089	180-200	2200-2300	14.5	1.90	8.6

†Foot of Dongyue Hill near Guyuan County of southern-mountain-area in Ningxia.
‡Pengyang County of southern Ningxia.
#Crop Research Institute of Wangtaipu, Yinchuan-plain in central Ningxia.
*Huinong agricultural demonstration area in northern Ningxia.

2.2.2. Rainfall and temperature during the wheat-cropping seasons

The soil water content in Guyuan was highest in 2004 and lowest in 2008, which was consistent with growing season rainfall in each year (Table 3). The highest soil water content was found at Huinong followed by Yinchuan, Pengyang and Guyuan. Total water input (Wi) was calculated as the sum of rainfall and irrigation during the wheat growth cycle, and was highest at the irrigated Yinchuan site, followed by Huinong, Guyuan and Pengyang.

The rainfall in Guyuan was higher than Yinchuan in 2008 (Table 4). The rainfall was higher in April in Yinchuan than the years before, which was beneficial to the wheat's emergence and crop establishment. In Guyuan, the crop growth mainly depended on rainfall. However, rain mainly occurred after June which missed the critical period, partly accounting for the lower yield in Guyuan than Yinchuan.

TABLE 3. AGRONOMIC AND ENVIRONMENTAL DATA OVER FIVE SEASONS

Location	Year	Sowing date	Soil H_2O ($g\ kg^{-1}$)	Ave. temp. (^oC)	Rainfall (mm)	Irrigation (mm)	W_i (mm)
Guyuan	2004	March 20	196	9.41	219	0	219
	2005	March 20	142	9.08	212		212
	2006	April 1	126	10.13	184		184
	2007	March 28	144	8.68	171		171
	2008	March 26	109	7.25	116		116
Pengyan	2004	March 21	193	10.01	162		162
Yinchuan	2004	Feb. 27	212	12.34	59	180 (2)	239
	2005	March 1	244	12.13	24	180 (2)	204
	2006	March 30	223	12.53	68	180 (1)	248
	2007	March 2	209	11.97	153	180 (2)	333
	2008	Feb. 28	200	11.10	87	120 (1)	207
Huinong	2004	Feb. 28	260	11.80	88	180 (2)	268
	2005	March 5	252	11.81	30	180 (2)	210

†Months 1-7
‡Data in parentheses are the number of irrigations

TABLE 4. MONTHLY CLIMATE PARAMETERS IN 2008 AT TWO SITES

Location	Month	Ave. daily air temp. (°C)	Rainfall (mm)	Relative humidity (%)	Ave. daily wind speed (m s^{-1})
Yinchuan	January	–9.4	15.5	68.1	1.40
	February	–6.9	1.2	66.5	1.54
	March	8.4	0	38.0	2.24
	April	14.6	18.9	42.3	1.81
	May	20.2	0.3	36.1	1.94
	June	24.7	2.2	45.4	2.05
	July	26.0	65.6	59.9	2.03
Guyuan	January	–10.9	10.7	62.5	1.89
	February	–7.2	2.1	60.0	2.14
	March	5.1	14.3	47.7	2.27
	April	9.5	20.0	49.6	2.76
	May	16.0	6.4	40.6	2.79
	June	18.1	41.9	53.3	2.89
	July	20.1	33.8	64.6	2.92

The water balance parameters calculated by the softwares ETO and Budget [43] are shown in Table 5. The daily evaporation was strongest in March and reduced thereafter at the two experimental sites. Higher transpiration and evapo-transpiration were observed at Yinchuan than at Guyuan. Yinchuan had the lowest daily evaporation in June, while at Guyuan the lowest evaporation occurred in July. Daily transpiration increased after March and the peak was reached in June at Yinchuan and in May at Guyuan. The change in evapo-transpiration from March to July was similar to that for transpiration.

TABLE 5. GROWING SEASON WATER BALANCE PARAMETERS† IN 2008 AT YINCHUAN AND GUYUAN

Location	Month	R	D	E_{pot}	E_{act}	T_{pot}	T_{act}	T_{act}/T_{pot}	ET_{pot}	ET_{act}	ET_{act}/ET_{pot}
Yinchuan	March	0.000	0.000	4.117	3.883	0.183	0.183	100.000	4.300	4.050	94.500
	April	0.000	1.753	2.160	0.637	2.197	1.737	83.067	4.357	2.367	52.167
	May	0.000	0.158	0.345	0.490	6.058	3.410	40.571	6.429	3.816	42.355
	June	0.000	0.000	0.330	0.130	6.973	0.030	0.400	7.320	0.147	1.967
	July	0.000	0.000	0.357	0.357	6.957	0.786	11.000	7.286	1.114	15.286
Guyuan	March	0.000	0.042	3.133	1.858	0.333	0.333	100.000	3.467	2.200	64.667
	April	0.000	0.000	1.343	0.610	2.623	1.207	57.333	3.953	1.810	46.033
	May	0.000	0.000	0.303	0.219	5.816	0.510	9.452	6.119	0.739	12.677
	June	0.000	0.000	0.300	0.300	5.587	0.953	17.067	5.847	1.230	20.967
	July	0.000	0.000	0.247	0.247	4.218	0.865	20.765	4.465	1.124	25.176

†Calculated on a daily basis; R = runoff; D = drainage; E_{pot} = potential evaporation; E_{act} = actual evaporation; T_{pot} = potential transpiration; T_{act} = actual transpiration; ET_{pot} = potential evapotranspiration; ET_{act} = actual evapotranspiration.

2.2.3. Crop management

In the rain fed experimental sites, Guyuan and Pengyang, sowing was in late March or the beginning of April (Table 3). A randomized complete block design for 20 accessions was applied, with 3 replicates genotype^{-1}. Seeds were sown in five 2-m rows spaced 20 cm apart (180 seeds in each row). No irrigation was applied during the whole growth period.

In the limited irrigation experimental sites, sowing was in late February and early March in Yinchuan and Huinong, respectively. A randomized complete block design for 20 accessions was used, with 3 replicates genotype^{-1}. Seeds were sown in five 2-m rows spaced 20 cm apart (200 seeds in each row). 180 mm water was applied by flood irrigation, at tillering (100 mm) and elongation (80 mm). Each treatment was isolated by plastic film which was embedded 100 cm deep to prevent water penetration between different treatments. Water weirs were used to measure the amount of irrigation applied.

2.3. Pot experiment 2007

The experiment was conducted in the Crop Institute of Ningxia Academy of Agricultural and Forestry Sciences (NAAFS), Yinchuan (38°17′N, 106°15′E), Ningxia, China, during the 2007 wheat growing season. All pots were placed outdoors, and rain impact was prevented by a moveable shelter. Plants were cultivated under three different water regimes corresponding to moderate, intermediate and severe drought stress obtained by maintaining soil humidity at 75, 55 and 45% of the humidity at field capacity (FC), respectively.

Pots were made of PVC pipes (16cm × 105cm), whose empty weight was 2 kg. Prior to pot filling, the soil was air dried and then passed through a 5-mm sieve to get rid of large stones and plant root debris. The soil was then thoroughly mixed. Totally, each pot was filled with 22 kg prepared soil. The basal soil characters were organic matter 16.9 mg g^{-1}, field capacity (FC) 26.3%, total nitrogen content 1.2 mg g^{-1}, available nitrogen content 58.2 mg kg^{-1}, available phosphorus content 25.6 mg kg^{-1}, available potassium content 68.2 mg kg^{-1} and total salt content 0.55 mg g^{-1}. The experimental design was a randomized complete block with six replications. Twenty-five seeds were sown in each pot on April 1st. Plant number per pot was thinned to 11 after crop establishment. The PVC pipes were buried in soil after 20 May. The soil surface of each pot was covered with wheat straw for limiting water evaporation. The pots were weighed at 7-day interval and water was applied to pots for compensating the weight loss. The sum of water applied was recorded.

2.4. Methods

2.4.1. Carbon isotope discrimination

20 flag leaves and 10 g of grain were collected at anthesis and maturity, respectively, oven dried to constant weight at 70 ^{o}C for 24 h, and ground to a fine powder. The $\delta^{13}C$ in each sample was determined by isotope ratio mass spectrometry, either in the Laboratory of Stable Isotopes in the Plant Research Institute of the Chinese Academy of Sciences, or the Soil Science Unit of IAEA, Seibersdorf, Austria.

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{reference}) - 1] \times 1000 \quad (1)$$

where $R = {}^{13}C/{}^{12}C$ ratio. CID was calculated using the following formula [44]:

$$\Delta\ (‰) = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000 \quad (2)$$

where δ_p is the $\delta^{13}C$ of the plant sample and δ_a is the $\delta^{13}C$ of atmospheric CO_2 (–8‰).

2.4.2. Specific leaf dry matter (LDM, expressed in mg cm^{-2})

At anthesis, 3 flag leaves genotype^{-1} were excised and immediately brought to the laboratory. The leaf area (LA, in cm^2) was determined by round disks which were punched from cleaned wheat leaves by a puncher of known radius. The disks' dry weights (DW) were obtained by weighing after oven-drying at 70 ^oC for 24 h. The specific leaf dry matter (LDM) was then calculated as LDM = DW/LA.

2.4.3. Ash content (m_a, expressed in mg g^{-1} DM)

Approximately 1.0 g of dry matter was incinerated at 880 ^oC for 7–8 h. Ash content (%) was expressed on a dry mass basis. Ash samples were also used for the determination of individual mineral. Magnesium (Mg), potassium (K) and calcium (Ca) contents were determined in flag leaf at anthesis and maturity, grain at maturity by Flame Atomic Absorption Spectrometry (Hitachi 180-80, Japan). The content of the three minerals elements was expressed in mg g^{-1} dry weight.

2.4.4. Soil water content

Soil water content was measured at different growth stages by time domain reflectometry (TDR) at depth intervals over 0–200 cm (Yinchuan and Guyuan) and 0–130 cm at Huinong due to a high water table.

2.4.5. Relative water content (RWC, %)

Three flag leaves were detached randomly at heading and the beginning of grain filling and placed in a sealed container, and the fresh weight (FW) was determined. The full turgid weight (TW) was obtained after the re-hydration of the leaves by placing them in a test tube containing distilled water for 12 h at ambient temperature. The RWC was calculated from the following equation:

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100.$$

2.4.6. Photosynthetic rate

Photosynthesis gas exchange parameters were measured using a CIRAS-1 portable Photosynthesis System (PP Systems, UK), which was synchronized to obtain a series of physiological parameters. Transpiration efficiency (TE) was calculated as A_n/T, where A_n = net photosythetic rate and T = transpiration rate.

2.4.7. Chlorophyll content

Total leaf chlorophyll content expressed on an area basis was estimated in intact leaves at heading using a SPAD-502 portable chlorophyll meter (Minolta Camera Co., Osaka, Japan).

2.4.8. Broad sense heritability

Broad sense heritability ($h^2\Delta_p$) for Δ of plant parts (flag leaf at heading and grain at maturity) sampled at two locations on a single plot basis in 2006 were estimated as [45]

$$h^2\Delta_p = \frac{\sigma^2{}_g}{\sigma^2{}_g + \sigma^2{}_{ge}/e + \sigma^2{}_r/(e \cdot r)}$$

where σ^2_g, σ^2_{ge} and σ^2_r are the genotype, genotype × environment and residual variances, *e* is the number of environments and *r* is the number of replicates.

3. RESULTS

3.1. Genetic variation in CID and relationships between leaf and kernel Δ and yield

Δ values across genotypes, sites and seasons are given in Table 6.

TABLE 6. MEAN, MAXIMUM AND MINIMUM VALUES OF Δ AND BROAD SENSE HERITABILITY FOR ALL GENOTYPES OVER SITES AND SEASONS

Year	Location	Leaf-Δ (‰)				Grain-Δ (‰)			
		Mean†	Max.	Min.	Range	Mean†	Max.	Min.	Range
2004	Yinchuan	20.60a	21.30	19.83	1.47	19.61a	20.37	18.46	1.90
	Huinong	19.80d	20.40	18.42	1.98	17.11e	17.76	16.62	1.14
	Guyuan	19.22e	19.59	18.64	0.95	17.15e	17.77	16.18	1.59
	Pengyang	20.06bcd	20.62	19.39	1.23	17.65d	18.21	16.95	1.26
	h_B^2 (%)	95.05				95.36			
2005	Yinchuan	20.21b	21.08	19.43	1.65	18.44c	19.39	17.35	2.04
	Huinong	19.16e	20.03	18.02	2.01	16.67f	17.34	16.08	1.26
	Guyaun	19.40e	19.91	18.51	1.40	15.65g	16.38	15.16	1.22
	h_B^2 (%)	93.78				92.12			
2006	Yinchuan	20.11bc	20.89	19.35	1.55	18.64bc	19.26	17.70	1.56
	Guyuan	18.35f	19.17	16.98	2.14	15.51g	16.47	14.74	1.73
	h_B^2 (%)	74.49				88.43			
2007	Yinchuan	19.83cd	20.56	18.79	1.78	18.71b	19.04	18.20	0.84
	Guyuan	17.56g	18.25	16.83	1.42	15.52g	16.14	15.00	1.14
	h_B^2 (%)	71.98				70.53			
2008	Yinchuan	20.13b	21.44	18.95	2.49	17.52d	18.48	15.97	2.50
	Guyuan	-	-	-	-	17.00e	15.59	16.06	1.40
	h_B^2 (%)					87.28			

†Means within a column followed by a different letter are significantly different at $P<0.05$ (Duncan test).

Large genetic variation for CID was found among the genotypes tested. The biggest range for ΔL_a was 2.49‰, and the biggest range for ΔG_m was 2.50‰. The lowest leaf and grain Δ values and yield were noted in Guyuan, and the highest in Yinchuan (Table 6).

The grain Δ of Yinchuan correlated significantly and positively with that of Huinong in 2004 and with that of Guyuan in 2004 and 2006. A significant and positive correlation between ΔL_a of Yinchuan and Huinong was noted in 2004, but not in other years. ΔL_a and ΔG_m in Huinong correlated with that in the other three sites in 2004 and 2005. ΔL_a and ΔG_m in Pengyang were found to be positively correlated with that in Huinong and Guyuan in 2004 (Table 7).

At Yinchuan, Δ in 2004 was significantly and positively correlated with that in 2005, 2006 and 2007, while Δ in 2006 was not correlated with that in 2007 or 2008. At Guyuan, ΔL_a in 2004 was only related to that in 2006, while ΔL_a in 2005 showed good correlations with that in 2006 and 2007 (Table 8).

TABLE 7. CORRELATIONS† WITHIN LEAF AND GRAIN Δ BETWEEN SITES WITHIN SEASONS

Location	Year	Yinchuan		Huinong		Guyuan	
		ΔL_a	ΔG_m	ΔL_a	ΔG_m	ΔL_a	ΔG_m
Huinong	2004	0.546**	0.507**				
	2005	0.347ns	0.258ns				
Guyuan	2004	0.279ns	0.587**	0.396*	0.433*		
	2005	0.358ns	0.397ns	0.493*	0.140ns		
	2006	0.196ns	0.585**				
	2007	0.125ns	0.117ns				
Pengyang	2004	-0.031ns	0.194ns	0.422*	0.419*	0.414*	0.428*

†ns, not significant; *, $P<0.05$; **, $P<0.01$.

TABLE 8. CORRELATIONS† WITHIN LEAF AND GRAIN Δ BETWEEN SEASONS WITHIN SITES

Location	Year	ΔL_a				ΔG_m			
		2005	2006	2007	2008	2005	2006	2007	2008
Yinchuan	2004	0.524*	0.451*	0.658**	0.406ns	0.817**	0.477*	0.553*	0.406ns
	2005		0.440ns	0.444*	0.113ns		0.554**	0.634**	0.527*
	2006			0.235ns	0.313ns			0.172ns	–0.047ns
	2007				0.498*				0.420ns
Huinong	2004	0.633**					0.126ns		
Guyuan	2004	0.299ns	0.481*	0.043		0.451*	0.450*	–0.376ns	
	2005		0.612**	0.468*			0.549**	–0.245ns	
	2006			0.225				0.529*	

†ns, not significant; *, $P<0.05$; **, $P<0.01$.

The relationships between ΔL_a, ΔG_m, and yield were variable in different sites and different years (Table 9). Positive and significant correlations between Δ and yield were found under limited irrigation, while there was weak or no correlation between the two traits under rain fed conditions (Fig. 1 and Fig. 2). When the data were pooled, positive correlation was found between yield and Δ across 11 environments from 2004 to 2008 (Fig. 3).

TABLE 9. CORRELATIONS† BETWEEN Δ AND GRAIN YIELD WITHIN SITES AND YEARS

Location	Year	Leaf-Δ	Grain-Δ	Early stem-Δ
Guyuan	2004	0.065ns	0.174ns	
	2005	0.086ns	–0.004ns	
	2006	0.284ns	0.301ns	
Pengyang	2004	0.276ns	0.584**	
Yinchan	2004	–0.086ns	0.224ns	
	2005	0.275ns	0.743***	
	2006	0.212ns	0.083ns	
	2007	0.209ns	0.460*	0.714**
	2008	0.503*	0.611**	0.561**
Huinong	2004	0.033ns	0.027ns	
	2005	0.433ns	0.381ns	

†ns, not significant; *, $P<0.05$; **, $P<0.01$.

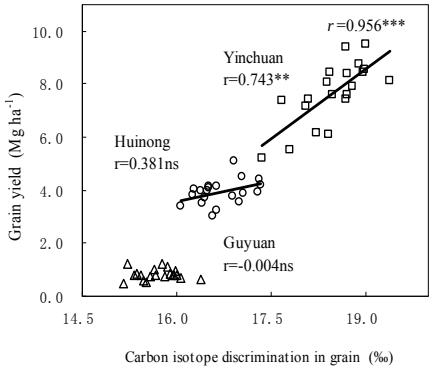

FIG. 1. Relationships between grain yield and CID in grain at three sites in 2005 (Yinchuan, post-anthesis water stress; Huinong, post-anthesis water stress + salinity; Guyuan, pre-anthesis water stress).

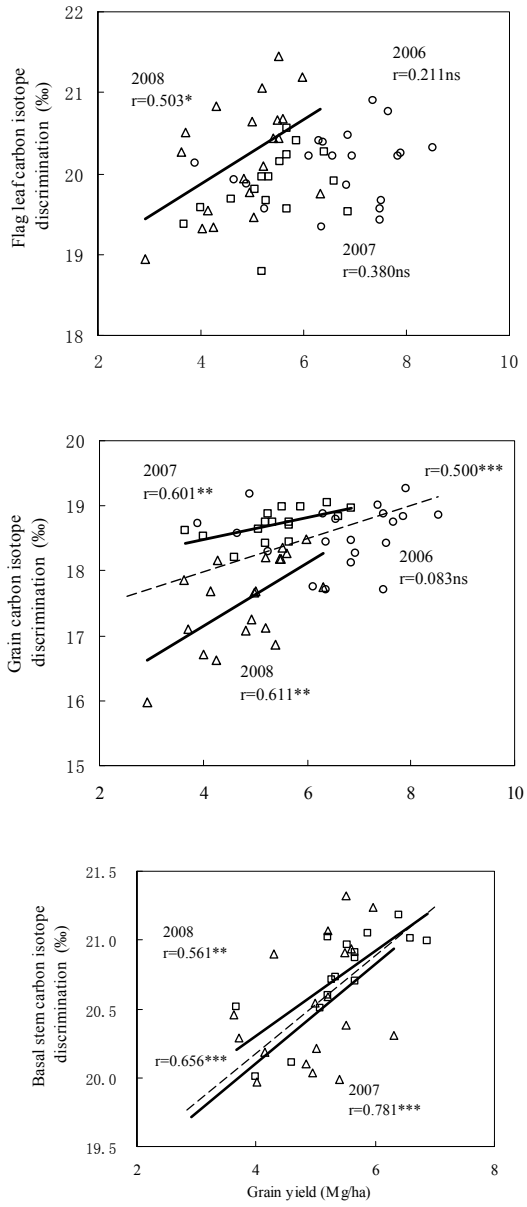

FIG. 2. Relationships between CID and grain yield in (a) flag leaf at anthesis (b) grain and (c) basal stem at maturity in 20 genotypes in Yinchuan in 2008.

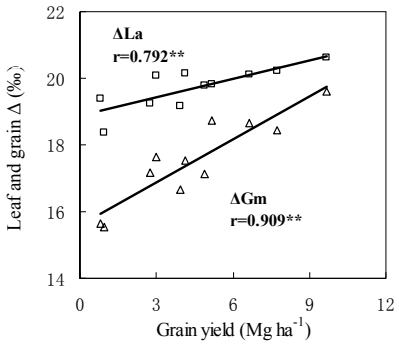

FIG. 3. Relationships between leaf and grain Δ and grain yield across 11 environments.

ΔL_a was positively and significantly correlated with yield in F_4 populations that originated from Yong 3119/03S111 (high Δ × high Δ) and from Ningchun 32/Ningchun 27 in 2007 (Table 10). A positive and significant correlation was found between ΔG_m and yield in the F_6 population of crossing combination Yong 3119/03S111 (high Δ × high Δ), and between ΔL_a and yield in F_6 populations of crossing combination Ningchun 4/Ningchun 27 (high Δ × low Δ) in 2008.

TABLE 10. CORRELATIONS† BETWEEN CID AND YIELD IN PROGENIES GROWN IN YINCHUAN IN 2007 AND 2008

Combinations of F_6	Arrangement of CID	Replicate	2007	2008	
			ΔL_a	ΔL_a	ΔG_m
Yong 3119/03S111	High-Δ/High-Δ	1	0.470**	–0.117ns	–0.022ns
		2		0.136ns	0.408**
Ningchun 4/Ningchun 27	High-Δ/Low-Δ	1	0.176ns	0.270*	–0.046ns
		2		0.132ns	0.085ns
Ningchun 32/Ningchun 27	Low-Δ/Low-Δ		0.428**		

†ns, not significant; *, $P<0.05$; **, $P<0.01$.

3.2. Relationships between CID and leaf morpho-physiological traits

The highest harvest index (HI) was obtained in Yinchuan and the lowest in Guyuan in 2005 and 2006. HI correlated positively with grain Δ in Yinchuan in 2005 and 2006 and with yield in Yinchuan and Guyuan in 2005. The highest ash content in leaf and whole plant was found in Yinchuan and the lowest in Guyuan, while the converse was found for ash content in grain in the two years (Table 11). Ash content in the whole plant was much lower than in the leaf in 2005 and 2006. Ash contents of leaves at heading in Guyuan in 2006 were significantly lower than those in 2005. Leaf ash content of mature leaves (m_aL_m) was significantly correlated with ΔG_m and ΔL_a in Guyuan and Yinchuan in 2005 and 2006. The highest and lowest LDM at heading and during grain filling were noted in Guyuan and Yinchuan in 2005, respectively. In Guyuan, LDM at heading (LDM_h) was significantly positively correlated with ΔL_a. Chlorophyll content (Chl) was significantly higher in Guyuan than in the other two locations, and significantly correlated with ΔL_a in Huinong and with ΔG_m in Yinchuan. RWC at anthesis (RWC_a) was significantly lower in Huinong than in the other locations. During grain filling, there was a stronger decrease in RWC values in Guyuan (23.9%) and Huinong (16.5%) than in Yinchuan (5.9%). RWC_a was significantly negatively correlated with both ΔL_a and ΔG_m in Huinong. In Yinchuan, both RWC_a and RWC_f were significantly correlated with ΔG_m. There was also a significant correlation between RWC_a and RWC_f and grain yield at this location.

TABLE 11. MEAN VALUES OF PHENOLOGICAL TRAITS AND SURROGATES OF CID AND CORRELATIONS WITH LEAF AND GRAIN Δ AT 3 SITES IN 2005

Trait‡	Mean#			Correlation† with leaf Δ			Correlation† with grain Δ		
	Guyuan	Yinchuan	Huinong	Guyuan	Yinchuan	Huinong	Guyuan	Yinchuan	Huinong
HI	0.147c	0.403a	0.338b	0.423ns	0.174ns	0.569**	0.132 ns	0.637**	0.391 ns
DH	56.2ab	58.4a	55.9b	–0.670***	–0.301ns	–0.441*	–0.611**	–0.628**	–0.136ns
DA	95.5a	96.6a	86.8b	–0.553**	–0.288ns	–0.562**	–0.591**	–0.560**	–0.135ns
m_aL_h	7.49a	7.32ab	7.49a	0.222ns	–0.048ns	–0.057ns	0.449*	0.430*	0.339ns
m_aP_h	5.93c	7.72a	6.34b	0.217ns	–0.151ns	–0.335ns	0.474*	0.359ns	–0.060ns
m_aL_m	7.40c	14.03a	11.63b	0.435*	0.524*	0.125ns	0.545*	0.501*	0.135ns
m_aP_m	4.13c	5.05a	4.55b	0.085ns	0.205ns	0.304ns	0.079ns	0.421*	–0.281ns
m_aG_m	1.81a	1.28c	1.58b	0.465*	0.367ns	0.194ns	0.390ns	0.446*	–0.068ns
Gs_f	634.9c	1362a	855.4b	0.087 ns	0.363 ns	0.023 ns	–0.069 ns	0.550*	0.121 ns
Pn_f	2.40b	21.6a	21.1a	–0.126 ns	–0.197 ns	–0.221 ns	–0.182 ns	–0.293 ns	–0.242 ns
Ci_f	256.4b	298.0a	308.2a	–0.138 ns	0.474*	0.211 ns	0.318 ns	0.405 ns	0.075 ns
Ci/Ca_f	0.689a	0.253b	0.773a	–0.189 ns	0.259 ns	0.219 ns	0.240 ns	0.591**	0.020 ns
WUE_f	2.02b	2.64a	1.81c	–0.212 ns	–0.345 ns	–0.346 ns	–0.069 ns	–0.320 ns	–0.259 ns
LDM_h	579.7a	483.9c	531.5b	0.412*	–0.058ns	0.111ns	–0.119ns	–0.221ns	–0.278ns
LDM_f	690.4a	526.3c	566.1b	–0.074ns	–0.213ns	–0.231ns	0.077ns	–0.294ns	0.095ns
Chl_h	52.25a	47.66b	51.78a	–0.186ns	0.214ns	0.513*	0.079ns	0.222*	–0.037ns
Chl_f	360.9a	230.3c	292.9b	–0.162ns	–0.106ns	0.171ns	0.092ns	–0.151ns	0.024ns
RWC_a	89.37a	86.32a	80.98b	–0.399*	0.003ns	–0.134ns	–0.737***	0.422*	–0.159ns
RWC_f	68.05b	81.24a	67.62b	0.159ns	0.165ns	–0.269ns	0.354ns	0.543*	–0.080ns

†ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.
‡HI, harvest index; DH, DA, days from emergence to heading and maturity, respectively; subscripts h, f, m, a = heading, grain filling, maturity and anthesis, respectively; m_aL, m_aP and m_aG, ash content of flag leaf, plant and grain, respectiuvely; Gs_f, stomatal conductance; Pn_f, photosynthetic rate; Ci_f, internal leaf CO_2 concentration; Ci/Ca_f, ratio of internal (flag leaf) to ambient CO_2 concentration; WUE_f; water use efficiency LDM_h, LDM_f specific leaf mass; Chl_h, Chl_f total chlorophyll; RWC_a, RWC_f relative water content.
#Means within a row followed by a different letter are significantly different at $P<0.05$ (Duncan test).

Shorter DH and DA were noted in Huinong in 2005 than in Yinchuan in 2005 and 2006. The duration of the phase from sowing to heading represented 58.8, 60.5 and 64.4% of the phase from sowing to anthesis in Guyuan, Yinchuan and Huinong in 2005, respectively (Table 11). Phenology, in turn, also significantly influenced Δ values. DH and DA correlated negatively with ΔL_a in Guyuan in 2005 and 2006 and Huinong in 2006, and to ΔG_m in Guyuan and Yinchuan in 2006. In 2005, ΔG_m was found to be positively correlated with C_i/C_a and g_S in Yinchuan (Fig. 2). A positive correlation between the length of the coleoptile and yield, and a negative correlation between the length of the coleoptile and ΔL_a were found in Guyuan in 2006 (Table 12).

Significant differences were found between water treatments for CID and ash content in a 2007 pot experiment (Table 13). The highest values of ΔL_a and ΔG_m were recorded under moderate stress in T_3. The difference in the mean ΔL_a at anthesis between T_3 and T_1 was 0.77‰. For ΔG_m this difference was 0.60‰. m_aL_a and m_aL_m were 7.27% and 6.37% higher in T_3 than in T_1, while m_aG_m was 3.72% lower in T_3 than in T_1.

The three sampling parts differed significantly for Δ, m_a and the measured minerals, and were higher in the flag leaf than in grain in all three treatments. The ash content in the

flag leaf at maturity was higher than at anthesis, while the concentration of K in the flag leaf was higher at anthesis than at maturity. Higher Ca and Mg contents in leaf were noted at maturity than at anthesis.

Correlations between Δ and the mineral contents in the flag leaf and grain at anthesis and maturity are presented in Table 14. At anthesis, ΔL_a was found to be positively correlated with m_aL_a in T_1, with Mg content in T_2 and T_3, and with the Ca content in T_3. A negative correlation was found between ΔL_a and K content in T_2. At maturity, ΔG_m was positively correlated with m_aL_m, and negatively correlated with the K content in the flag leaf in T_1, and to the Mg content in the flag leaf in all treatments. Significant negative correlations were found between ΔG_m and m_aG_m in T_2 and T_3 treatments. A negative correlation was also noted between ΔG_m and Ca content in the grain in T_3.

3.3. Evaluation of genetic resource with high or low water use efficiency

Local varieties, which originated in southern Ningxia rain fed regions, exhibited lower Δ values than improved varieties and advanced lines, while some genotypes bred under irrigation displayed higher Δ values. The Australian variety Quarry (winter wheat) had consistently low Δ values during 2007 and 2008, while Drysdale (an Australian spring wheat) had lower Δ values among 20 genotypes planted in 2007, and intermediate Δ values in 2008 (Table 15).

TABLE 12. AVERAGE VALUES OF PHENOLOGICAL TRAITS AND SURROGATES OF CID AND THEIR CORRELATION WITH LEAF AND GRAIN Δ AND YIELD AT TWO SITES IN 2006

Trait‡	Mean#		Correlation† with					
			ΔL_a		ΔG_m		Yield	
	Guyuan	Yinchuan	Guyuan	Yinchuan	Guyuan	Yinchuan	Guyuan	Yinchuan
HI	0.478a	0.301b	0.494*	0.240ns	0.240 ns	0.447*	0.401 ns	0.507*
DH	56a	52b	–0.801**	–0.325ns	–0.374ns	–0.365ns	–0.122 ns	–0.400 ns
DA	94a	88b	–0.619**	–0.226ns	–0.283ns	–0.341ns	0.122 ns	0.004 ns
PH	61b	95a	–0.222ns	–0.451*	–0.298ns	–0.624**	0.675**	0.122 ns
CL	3.0		–0.105ns	–0.584**	–0.096ns	–0.239ns	0.520 *	0.317 ns
WC_a	180.2b	256.5a	0.006ns	0.204ns	0.517*	0.482*	0.334ns	–0.22 ns
WC_f	129.5b	225.6a	0.143ns	0.517*	0.457*	0.500*	0.449*	0.152 ns
m_aL_h	6.04b	6.95a	0.223ns	–0.286ns	0.202ns	–0.164ns	–0.191 ns	0.389 ns
m_aL_m	7.4b	10.78a	0.713**	0.005ns	0.328ns	0.508*	0.001 ns	–0.112 ns
m_aP_m	4.68a	4.10b	–0.069ns	–0.059ns	–0.059ns	0.068ns	–0.180 ns	–0.360 ns
m_aG_m	1.55a	1.22a	–0.508*	–0.015ns	–0.515*	–0.017 ns	–0.024 ns	–0.142 ns
Gs_f	544.6b	3624a	0.340ns	0.355ns	0.231ns	0.415ns	0.277 ns	0.133 ns
TL_f	27a	25.1b	–0.326ns	–0.437*	–0.162ns	–0.432*	–0.324 ns	–0.129 ns
Pn_f	5.9b	19.7a	–0.028ns	–0.086ns	0.0031ns	0.049ns	0.502*	–0.073 ns
Ci_f	322.5a	316.4a	0.647**	0.119ns	0.306ns	0.150ns	–0.039 ns	–0.084 ns
Ci/Ca_f	0.8a	0.8a	0.547*	0.025ns	0.090ns	0.061ns	–0.217 ns	–0.062 ns
WUE_f	0.7b	2.2a	–0.288ns	–0.100ns	0.076ns	0.028ns	0.544**	0.063 ns

†ns, not significant; *, $P<0.05$; **, $P<0.01$.
‡(see footnote Table 11); PH, plant height at maturity; CL, coleoptile length; WC_a, WC_f, water content of leaves at anthesis and filling, respectively; TL_f, temperature of leaves at filling.
#Means within a row followed by a different letter are significantly different at $P<0.05$ (Duncan test).

TABLE 13. MEAN AND STANDARD DEVIATION (SD) OF ASH AND MINERAL (K, MG, CA) CONTENTS IN THE FLAG LEAF (ANTHESIS AND MATURITY) AND IN MATURE GRAIN UNDER 3 WATER REGIMES IN POT CULTURE IN 2007

Plant part	Parameter†	T_1		T_2		T_3		F-test ‡
		Mean #	SD	Mean #	SD	Mean #	SD	(df = 2)
	ΔL_a (‰)	17.73	0.59	18.18	0.67	18.50	0.31	8.59**
	ΔG_m (‰)	17.71b	0.47	17.73b	0.35	18.30a	0.26	29.68***
Flag leaf at anthesis	m_aL_a	114.79b	7.50	112.05b	9.81	120.84a	9.11	9.83**
	K	29.82b	3.80	29.01b	2.63	34.14a	1.84	15.75***
	$\%m_a$	25.98		25.89		28.25		
	Mg	3.47a	0.51	3.18ab	0.64	3.09b	0.34	4.71*
	$\%m_a$	3.02		2.84		2.56		
	Ca	5.55a	1.28	5.69a	1.33	5.43a	0.80	0.27ns
	$\%m_a$	4.83		5.07		4.50		
Flag leaf at maturity	m_aL_m	189.80b	16.21	198.75a	23.26	202.71a	21.91	7.95**
	K	17.22b	5.62	23.10a	4.56	20.67ab	4.97	3.86*
	$\%m_a$	9.07		11.62		10.20		
	Mg	4.73b	1.75	5.27a	1.38	4.81ab	1.30	3.23ns
	$\%m_a$	2.49		2.65		2.37		
	Ca	9.78a	1.30	10.94a	1.14	10.17a	1.76	2.21ns
	$\%m_a$	5.15		5.51		5.02		
Grain at maturity	m_aG_m	26.41a	0.82	25.97b	0.92	25.46c	0.92	17.71***
	K	4.18a	0.44	4.27a	0.23	3.98a	0.27	2.23ns
	$\%m_a$	15.83		16.46		15.62		
	Mg	2.02b	0.17	2.26a	0.21	1.90b	0.12	10.18**
	$\%m_a$	7.65		8.70		7.45		
	Ca	0.63a	0.12	0.61a	0.07	0.58a	0.07	1.82ns
	$\%m_a$	2.37		2.36		2.28		

†Units of K, Mg and Ca are mg g^{-1} DM

‡*, P<0.05; **, P<0.01; ***, P<0.001; ns, not significant.

#Means within a row followed by a different letter are significantly different at P<0.05 (Duncan test).

T_1, T_2, T_3 represent moderate, intermediate and severe drought stress obtained by maintaining soil humidity at 75, 55 and 45% of the humidity at field capacity, respectively; m_aL_a, ash content in flag leaf at flowering; m_aL_m, ash content in flag leaf at maturity; m_aG_m, ash content in grain at maturity.

TABLE 14. CORRELATIONS† BETWEEN FLAG LEAF OR GRAIN CID WITH ASH (M_A) AND MINERAL CONTENTS UNDER 3 WATER REGIMES

Trait	ΔL_a			ΔL_m			ΔG_m		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
m_a	0.654*	0.394ns	0.057ns	0.838**	0.292ns	0.296ns	–0.437ns	–0.695*	–0.721*
K	–0.575ns	–0.813**	–0.617ns	–0.824**	0.528ns	–0.226ns	–0.395ns		
Mg	0.527ns	0.725*	0.826**	–0.753**	–0.668*	–0.695*	0.175ns	0.250ns	–0.332ns
Ca	0.311ns	0.567ns	0.708*	–0.561ns	–0.400ns	–0.612ns	–0.623ns	–0.476ns	–0.649*

†ns, not significant; *, P<0.05; **, P<0.01.

T_1, T_2, T_3 represent moderate, intermediate and severe drought stress obtained by maintaining soil humidity at 75, 55 and 45% of the humidity at field capacity, respectively.

TABLE 15. LEAF AND GRAIN Δ (‰) OF LOCAL VARIETIES, ADVANCED LINES AND RELEASED VARIETIES ACROSS FOUR SITES AND SEASONS 2004-08

Location	Year	Leaf or grain Δ	Local varieties	Improved varieties	Advanced lines	Drysdale	Quarry
Guyuan	2004	ΔL_a	19.29	19.17	19.08		
		ΔG_m	16.88	17.16	17.25		
	2005	ΔL_a	19.60	19.40	19.20		
		ΔG_m	15.67	15.72	15.69		
	2006	ΔL_a	17.76	18.50	18.33		
		ΔG_m	15.20	15.52	15.52		
	2007	ΔL_a	17.90	17.47	17.78	17.93	18.25
		ΔG_m	15.38	15.53	15.46	14.34	15.58
Pengyang	2004	ΔL_a	20.41	20.01	20.05		
		ΔG_m	17.57	17.50	17.75		
Yinchuan	2004	ΔL_a	20.15	20.76	20.61		
		ΔG_m	18.89	19.61	19.86		
	2005	ΔL_a	20.00	20.30	20.90		
		ΔG_m	17.57	18.74	18.39		
	2006	ΔL_a	19.49	20.15	20.15		
		ΔG_m	18.29	18.45	18.70		
	2007	ΔL_a	19.63	19.96	19.69	19.37	19.31
		ΔG_m	18.37	18.81	18.68	18.79	18.73
	2008	ΔL_a	19.44	20.22	20.38	20.44	
		ΔG_m	17.20	17.64	17.56	18.18	16.23
Huinong	2004	ΔL_a	19.62	19.90	19.68		
		ΔG_m	17.20	17.34	17.21		
	2005	ΔL_a	19.10	19.20	18.90		
		ΔG_m	16.41	16.72	16.75		

TABLE 16. AGRONOMIC, PHYSIOLOGICAL AND PHOTOSYNTHETIC PARAMETERS OF LANDRACES, ADVANCED LINES AND RELEASED VARIETIES AT THREE SITES IN 2005

Locations	Phenotypic characteristics †							
	GY	HI	$SLDW_{gf}$	RWC	g_s	TL	DH	PH
Yinchuan								
Landraces	0.89b	30.10b	53.16a	84.99b	829.01b	24.35a	63.50a	130.00a
Advanced lines	2.33a	40.91a	48.46b	87.05a	1290.5ab	24.12b	58.11b	105.96b
Released varieties	2.34a	41.89a	44.92c	86.05a	1503.75a	23.95c	57.56b	103.85b
F-test ‡	33.07*	40.85*	164.1**	121.0**	18.98*	936.9***	32.12*	243.32**
Huinong								
Landraces	1.30c	27.37b	54.44a	82.48a	622.34b	27.80a	60.50a	107.00a
Advanced lines	1.69a	34.89a	59.30a	82.48a	1135.34a	27.48a	55.00b	83.33b
Released varieties	1.60b	34.25a	52.86a	84.19a	1015.29a	27.57a	55.78b	80.37b
F-test ‡	1882***	73.40*	6.08ns	0.37ns	37.34*	0.50ns	35.02*	294.84**
Guyuan								
Landraces	0.81a	17.40a	56.95b	83.96a	1309.25a	24.62a	59.50a	71.99a
Advanced lines	0.78a	15.66a	73.31a	88.19a	1438.32a	24.89a	55.44b	59.89ab
Released varieties	0.60a	15.01a	71.73a	84.40a	1094.85a	24.43a	56.33ab	55.15b
F-test ‡	0.95ns	0.32ns	15.04ns	0.48ns	1.17ns	3.69ns	11.83ns	41.29*

†GY, grain yield; HI, harvest index; $SLDW_{gf}$, specific leaf dry weight at grain filling stage; RWC, relative water content of flag leaf at anthesis stage; g_s, stomatal conductance; TL, temperature of flag leaf at grain filling stage; DH, days from emergence to heading; PH, plant height at maturity.

‡*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant.

Mean values within a column and site followed by the same letter are not significantly different ($P<0.05$, Duncan test).

The three groups of genotypes, i.e. local varieties, advanced lines and improved varieties differed significantly for GY, HI, $SLDW_{gf}$, RWC_a, g_S, LT, DH and PH in Yinchuan, while most of those traits did not differ among the three groups except for PH in Guyuan. Significant differences were found among the three groups for GY, HI, g_S, DH and PH in Huinong. In Yinchuan and Huinong, the lowest ΔG_m, GY, HI, RWC_a and g_S were observed in landraces, while the highest LT was recorded in this group. Landraces were taller and headed later compared with the other two groups in all locations (Table 16).

3.4. Development of population from progenitors and sets of elite isomorphic lines

200 F_6 lines derived from three crossing combinations, i.e. Yong 3119 × 03S111 (high Δ × high Δ), Ningchun 4 × Ningchun 27 (high Δ × low Δ), and Ningchun 32 × Ningchun 27 (low Δ × low Δ) were obtained. Some elite lines were found to out-yield their parents. 32 progenies were also obtained by crossing Drysdale, Quarry and high or low Δ varieties. These lines were developed and used in breeding programmes. A F_2 group (Ningchun 4/Drysdale) has been used for molecular studies.

ΔL_a and ΔG_m values of F_6 progenies grown in 2008 of the high Δ × high Δ combination were higher than the high Δ × low Δ combination (Tables 17 and 18). For the high Δ × high Δ combination, the mean values of ΔL_a and ΔG_m of the F_6 lines were close to that of their high Δ parent, and some F_6 individuals were higher than their high Δ parent, while for the high Δ × low Δ combination, ΔL_a and ΔG_m of all F_6 individuals were higher than their low Δ parent.

For the combination Yong 3119 × 03S111 (high Δ × high Δ), there were two F_6 lines whose yield exceeded the high yielding parent by more than 10%, and 29 lines whose yield exceeded Ningchun 4 by more than 20% (Table 19). The yield of 08YJ19, 08YJ14, 08YJ51, 08YJ3 and 08YJ42 exceeded Ningchun 4 by more than 40%. For the combination Ningchun 4 × Ningchun 27 (high Δ × low Δ), there were 7 F_6 lines whose yield exceeded the high yield parent by more than 20% and 30 F_6 lines whose yield exceeded Ningchun 4 by more than 20%. The yield of 08YJ87, 08YJ70, 08YJ111, 08YJ76 and 08YJ104 exceeded the high yield parent by more than 40% (Table 20).

TABLE 17. ΔL_A AND ΔG_M (‰) OF F_6 LINES AND YONG 3119 × 03S111 (HIGH Δ × HIGH Δ) PARENTS IN YINCHUAN IN 2008

F_6 lines	ΔL_a	ΔG_m	F_6 lines	ΔL_a	ΔG_m	F_6 lines	ΔL_a	ΔG_m	F_6 lines	ΔL_a	ΔG_m
3	21.15	18.84	16	20.9	17.72	29	20.65	18.34	42	20.55	18.33
4	20.55	18.41	17	20.37	18.13	30	20.37	18.27	43	20.66	17.58
5	20.69	18.40	18	21.05	18.47	31	20.55	19.16	44	21.81	18.15
6	21.17	18.57	19	20.46	18.3	32	20.59	18.2	45	20.4	18.27
7	20.49	18.32	20	20.73	18.46	33	20.49	17.66	46	20.69	18.07
8	20.58	18.38	21	20.82	18.47	34	20.65	18.38	47	20.37	18.55
9	20.78	18.59	22	20.72	18.71	35	20.37	18.38	48	20.59	18.06
10	20.59	18.02	23	20.92	18.75	36	20.41	18.68	49	21.54	17.91
11	20.46	18.46	24	20.74	18.11	37	20.21	18.32	50	20.47	18.17
12	20.92	18.59	25	20.92	18.36	38	20.58	18.95	51	20.34	17.99
13	20.67	18.46	26	-	-	39	20.54	18.21	52	20.73	17.94
14	20.62	18.44	27	-	-	40	21.03	18.09	53	20.4	17.63
15	20.44	18.23	28	20.65	18.15	41	20.35	18.49	54	20.45	18.16
Parents			Yong 3119	20.65	18.20	03S111	20.51	18.37	Parental mean	20.58	18.28

TABLE 18. ΔL_A AND ΔG_M (‰) OF F_6 LINES AND NINGCHUN 4 × NINGCHUN 27 (HIGH Δ × LOW Δ) PARENTS IN YINCHUAN IN 2008

F_6 lines	ΔL_a	ΔG_m	F_6 lines	ΔL_a	ΔG_m	F_6 lines	ΔL_a	ΔG_m	F_6 lines	ΔL_a	ΔG_m
59	20.64	17.7	72	20.53	18.08	85	20.47	17.91	98	20.70	17.85
60	20.6	17.84	73	20.25	18.34	86	20.91	17.88	99	21.05	18.17
61	20.36	17.76	74	20.25	18.19	87	20.71	17.7	100	20.59	17.08
62	20.67	17.92	75	20.83	18.02	88	20.48	18.08	101	20.18	17.12
63	20.39	18.07	76	20.53	18.07	89	20.40	17.6	102	20.64	17.22
64	20.62	18.48	77	20.68	18.09	90	20.30	17.76	103	20.29	17.83
65	20.51	18.34	78	20.01	18.29	91	20.14	18.25	104	20.21	17.68
66	20.28	18.8	79	20.78	18.28	92	20.49	18.25	105	20.45	17.94
67	20.32	18.2	80	21.17	18.22	93	20.29	17.96	106	20.24	17.65
68	20.9	17.97	81	20.58	17.77	94	20.51	17.81	107	19.78	18.01
69	20.79	18.59	82	20.59	17.70	95	20.73	17.51	108	20.64	17.24
70	20.89	18.66	83	-	-	96	21.34	17.79	109	20.43	18.33
71	21.04	18.2	84	-	-	97	20.55	17.99	110	20.05	18.23
									111	20.72	18.12
Parents			Ningchun 4	20.50	18.72	Ningchun 27	19.33	16.82	Parental mean	19.91	17.77

TABLE 19. YIELD OF F_6 LINES AND THEIR PARENTS IN YINCHUAN IN 2008

Yong 3119 × 03S111(high Δ × high Δ)				Ningchun4 × Ningchun27 (high Δ × low Δ)			
Parents	Yield (kg ha^{-1})	F_6 population	Yield (kg ha^{-1})	Parents	Yield (kg ha^{-1})	F_6 population	Yield (kg ha^{-1})
Yong3119	5663.5	Mean	5877.6	Ningchun4	4814.4	Mean	5929.1
03S111	6487.5	Max.	8718.6	Ningchun27	5571.3	Max.	7720.6
Mean	6075.5	Min.	2584.4	Mean	5192.85	Min.	3960.8
F_6 lines		F_6 lines		F_6 lines		F_6 lines	
3	6887.8	30	6004.2	59	4901.1	86	5969.7
4	5468.3	31	5524.2	60	5553.1	87	7720.6
5	5983.9	32	6553.3	61	5529.4	88	5506.4
6	5521.4	33	5986.9	62	6202.8	89	5229.7
7	4432.2	34	5541.4	63	6190.3	90	5807.5
8	6280	35	6709.7	64	6119.4	91	6407.5
9	5046.7	36	6060.3	65	5717.5	92	6405.3
10	5561.4	37	6303.6	66	5612.5	93	6157.5
11	4748.9	38	6646.4	67	5555.8	94	6262.2
12	5524.4	39	6061.1	68	5253.3	95	6381.4
13	5701.1	40	4121.9	69	5920.3	96	5565.3
14	7764.4	41	6183.9	70	7710.0	97	4773.9
15	5170.3	42	6820.6	71	6491.7	98	6739.2
16	4869.7	43	6036.1	72	5381.4	99	5392.8
17	5333.3	44	6100.8	73	6404.7	100	6307.8
18	5961.4	45	5516.9	74	6564.2	101	4441.9
19	8718.6	46	6002.2	75	5456.9	102	6009.7
20	6362.8	47	6076.9	76	6953.3	103	5966.7
21	6301.4	48	6057.9	77	6448.9	104	6942.8
22	6667.2	49	6156.9	78	5889.2	105	4797.8
23	6427.5	50	5381.9	79	5680.3	106	4853.1
24	5535.6	51	7105.8	80	3960.8	107	5810
25	5857.8	52	4849.4	81	6024.7	108	6728.3
28	5696.7	53	5677.8	82	6200.6	109	5352.5
29	5991.1	54	2584.4	85	6581.7	110	5245.8
						111	7305.6

TABLE 20. YIELD OF SOME ELITE F_6 LINES AND THEIR PARENTS IN YINCHUAN IN 2008

Parents	Yield (kg ha^{-1})	Exceeding high yield parent (%)	Exceeding average of parents (%)	Exceeding Ningchun 4 (%)
Yong 3119	5663.5			17.64
03S111	6487.5			34.75
Ningchun 4	4814.4			
Ningchun 27	5571.3			15.72
Yong 3119/03S111				
08YJ 38	6646.4	2.45	9.40	38.05
08YJ 22	6667.2	2.77	9.74	38.48
08YJ 35	6709.7	3.43	10.44	39.37
08YJ 42	6820.6	5.13	12.26	41.67
08YJ 3	6887.8	6.17	13.37	43.07
08YJ 51	7105.8	9.53	16.96	47.59
08YJ 14	7764.4	19.68	27.80	61.27
08YJ 19	8718.6	34.39	43.50	81.09
Ningchun 4/Ningchun 27				
08YJ85	6581.7	18.14	26.75	36.71
08YJ108	6728.3	20.77	29.57	39.75
08YJ98	6739.2	20.96	29.78	39.98
08YJ104	6942.8	24.62	33.70	44.21
08YJ76	6953.3	24.81	33.90	44.43
08YJ111	7305.6	31.13	40.69	51.74
08YJ70	7710.0	38.39	48.47	60.14
08YJ87	7720.6	38.58	48.68	60.36

For the combination Ningchun 4 × Ningchun 27 (high Δ × low Δ) in Guyuan, there were five elite F_6 lines, 08GJ68, 08GJ94, 08GJ67, 08GJ99 and 08GJ65 whose yield exceeded the high yield parent. There was no line whose yield exceeded the high yield parent in Ningchun 32 × Ningchun 27 (low Δ × low Δ) (Table 21).

3.5. Genetics study of CID

The broad sense heritability in the stabilized genotypes grown from 2004 to 2008 was high and ranged from 70.4% to 95.4% (Table 6). In 2006, the h_B^2 of ΔL_a was higher than that of ΔG_m whether in parents or F_3 populations, and the highest Δ value for F_3 descendants was found in the high Δ × high Δ combination, followed by the high Δ × low Δ combination, and the low Δ × low Δ combination. Higher h_B^2 of F_3 generations were noted in Guyuan than in Yinchuan (Table 22).

In Yinchuan in 2008, higher h_B^2, ranging from 65.3% to 82.1%, was found in the two F_6 population originating from two combinations. No significant difference in h_B^2 was found between ΔL_a and ΔG_m. A higher h_B^2 of the high Δ × low Δ combination was found than the high Δ × high Δ combination. ΔL_a and ΔG_m of F_6 progenies originating from the high Δ × high Δ combination was higher than the high Δ × low Δ combination. For the high Δ × high Δ combination, the F_6 lines' mean values of ΔL_a and ΔG_m were close to that of their high Δ parent; while for the high Δ × low Δ combination, the F_6 individuals' ΔL_a and ΔG_m were all higher than their low Δ parent (Table 23).

TABLE 21. YIELD OF F_6 LINES AND THEIR PARENTS IN GUYUAN IN 2008

Ningchun 32 × Ningchun 27 (low Δ × low Δ)				Ningchun 4 × Ningchun 27 (high Δ × low Δ)			
Parents	Yield (kg ha^{-1})	F_6 population	Yield (kg ha^{-1})	Parents	Yield (kg ha^{-1})	F_6 population	Yield (kg ha^{-1})
Ningchuan 27	2500	Mean	1755	Ningchun 4	2055.6	Mean	1945.7
Ningchun 32	1888.9	Max.	2194.4	Ningchun 27	2500	Max.	2800.6
Mean	2194.4	Min.	1055.6	Mean	2277.8	Min.	1055.6
F_6 lines		F_6 lines		F_6 lines		F_6 lines	
1	1750	26	1055.6	51	2444.4	76	1944.4
2	2000	27	1805.6	52	1888.9	77	2055.6
3	1944.4	28	1694.4	53	1750	78	1722.2
4	1555.6	29	2166.7	54	1805.6	79	1472.2
5	1666.7	30	1833.3	55	1777.8	80	1361.1
6	2166.7	31	2166.7	56	2138.9	81	1638.9
7	1750	32	1472.2	57	2416.7	82	1694.4
8	2055.6	33	2000	58	1361.1	83	2277.8
9	1944.4	34	1944.4	59	2083.3	84	1833.3
10	1722.2	35	2111.1	60	2055.6	85	1694.4
11	1861.1	36	1277.8	61	2138.9	86	1333.3
12	1527.8	37	2083.3	62	1972.2	87	1666.7
13	1444.4	38	1944.4	63	1361.1	88	1750
14	1944.4	39	2194.4	64	1888.9	89	1527.8
15	1666.7	40	2083.3	65	2650.6	90	1833.3
16	1500	41	1638.9	66	2111.1	91	1666.7
17	1833.3	42	1694.4	67	2600.7	92	1333.3
18	2000	43	1888.9	68	2800.6	93	2055.6
19	1805.6	44	1833.3	69	1861.1	94	2722.2
20	1750	45	1416.7	70	2111.1	95	1055.6
21	1750	46	1694.4	71	1861.1	96	2027.8
22	1861.1	47	1750	72	2055.6	97	2305.6
23	1611.1	48	1500	73	1972.2	98	2138.9
24	1666.7	49	1222.2	74	2166.7	99	2650.2
25	1444.4	50	1055.6	75	2305.6		

TABLE 22. GENETIC ANALYSIS FOR ΔL_A AND ΔG_M OF SELECTED F_3 AND PARENTS IN 2006

Combinations of F3	Pairing of Δ	Sites	Average	Max.	Min.	CV (%)	AP	h_B^2 (%)
ΔL_a (‰)								
Parents		Yinchuan	20.08	20.89	19.35	0.424	94.2	94.2
		Guyuan	18.35	19.12	16.98	0.577	89.3	89.3
Yong 3119/03S111	High/High	Yinchuan	20.36	21.38	19.39	2.41	20.58	60.0
		Guyuan	18.69	20.11	17.51	2.98	18.76	40.6
Ningchun 4/Ningchun 27	High/Low	Yinchuan	19.64	20.46	18.84	2.01	19.80	54.7
		Guyuan	18.46	20.08	16.88	3.64	18.67	66.5
Ningchun 32/Ningchun 27	Low/ Low	Yinchuan	19.54	20.69	18.21	2.66	19.63	50.1
		Guyuan	17.59	18.76	16.03	3.47	18.37	65.7
ΔG_m (‰)								
Parents		Yinchuan	18.55	19.26	17.70	0.461	82.6	82.6
		Guyuan	15.49	16.47	14.74	0.455	83.3	83.3
Yong 3119/03S111	High/High	Yinchuan	18.60	19.48	17.71	2.28	18.81	47.0
		Guyuan	16.03	17.14	14.99	3.04	15.94	68.5
Ningchun 4/Ningchun 27	High/Low	Yinchuan	18.00	19.29	16.38	3.52	18.49	37.7
		Guyuan	15.90	17.16	14.69	3.75	15.52	51.9
Ningchun32/Ningchun 27	Low/ Low	Yinchuan	18.34	19.46	16.98	2.82	18.15	35.1
		Guyuan	15.37	16.91	14.32	3.74	15.80	40.2

TABLE 23. THE GENETIC ANALYSIS OF Δ FOR PROGENIES IN YINCHUAN IN 2007 AND 2008

Year	Δ (‰)	Combinations of F_6 †	Average	Max.	Min.	CV (%)	Ave. of parents	h_B^2
2007	ΔL_a	Yong 3119/03S111 (High-Δ/High-Δ)	20.42	20.97	19.72	1.21	20.27	
		Ningchun 4/Nc 27 (High-Δ/Low-Δ)	20.55	21.24	19.48	1.78	19.88	
		Ningchun 32/Nc 27 (Low-Δ/Low-Δ)	20.23	20.78	19.42	1.68	19.74	
2008	ΔL_a	Yong 3119/03S111 (High-Δ/High-Δ)	20.66	21.81	20.21	2.07	20.58	65.3
		Ningchun 4/Nc 27 (High-Δ/Low-Δ)	20.54	21.53	19.80	2.36	19.91	79.8
	ΔG_m	Yong 3119/03S111 (High-Δ/High-Δ)	18.31	19.16	17.58	2.47	18.28	68.1
		Ningchun 4/Nc 27 (High-Δ/Low-Δ)	17.98	18.80	17.08	3.51	17.77	82.1

†Nc = Ningchun.

3.6. Different strategies for using CID as a selection tool in rain fed and limited irrigated cropping systems

According to the yield of F_6 population of different crossing combinations obtained in 2007 and 2008, the ranking of yield in Yinchuan is high Δ × high Δ combination > low Δ × low Δ combination > high Δ × low Δ combination in 2007 and high Δ × low Δ combination > high Δ × high Δ combination in 2008. In addition, positive correlations between yield and Δ

were noted in 2007 and 2008. Therefore, matching crossing combinations composed of high Δ parents and selecting high Δ lines in progenies would be beneficial to yield gain in the Ningxia limited irrigation region.

In Guyuan, the yield of the high Δ × low Δ combination was higher than the low Δ × low Δ combination whose yield was higher than the high Δ × high Δ combination. In rain fed regions, the high Δ × low Δ combination is expected to integrate high yield characters in high Δ varieties (derived from irrigated regions) with high TE (transpiration efficiency) characters in low Δ (derived from rain fed regions), which would create high yielding progenies.

4. DISCUSSION

4.1. Effect of environment condition on Δ

The higher Δ values and grain yield in Yinchuan than in the rain fed trials of Pengyang and Guyuan revealed that irrigation had a greater influence on grain yield and Δ values than the higher evapo-transpiration and vapor pressure deficit that occurred in northern locations. The difference in Δ and grain yield between Huinong and Yinchuan is likely to be due to the effects of soil salinity, which reduces Δ [46]. Salt stress induced a stronger reduction of Δ and grain yield in the driest year, probably due to an increase in the salt concentration in the soil solution and/or the cumulative effects of drought and salt stress. Drought stress of different severities, which occurred after anthesis [47] corresponded to a ME4A (Mediterranean-type) drought [48]. Conversely, in the present study, water availability during the different development phases differed highly with the environment. Drought stress was moderate in Yinchuan because of irrigation during the dry period of the growth cycle. In contrast, the wheat crop experienced a severe drought stress before anthesis in Guyuan and Pengyang. Moreover, as shown by the soil water profiles measured during the growth cycle, soil moisture at sowing was very low, as a consequence of the low rainfall in autumn and early winter [49] and the high runoff/rainfall ratio of the soils [50, 51]. This winter drought situation corresponds to the mega-environment ME4B [48], also present in the Southern Cone of Latin America and estimated to cover a total wheat area of 3 million ha worldwide. Effects of drought stress are likely to depend, in this situation, on the quantity of water stored in the soil [3, 10, 17] or on water supply at critical stages of the growth cycle. For example, a single irrigation of 60 mm at the jointing stage in Guyuan can lead to a yield increase of 75% [52]. As suggested by rainfall before sowing and soil moisture at sowing, water stored in the soil at sowing was lowest in Guyuan in 2006, compared to 2004 and 2005. This could explain the lowest yield and ΔL_a and ΔG_m values observed in this location in 2006. ΔG_m was lower than ΔL_a in all environments. Similarly, lower Δ values in the mature grain than in the leaf at anthesis was reported [10], whatever the water regime. In the present study, the difference between ΔL_a and ΔG_m was much higher under rain fed conditions than under irrigated conditions. Lower grain Δ in these pre-anthesis water stress conditions may reflect closure of stomata [15] as a consequence of less water stored in the soil at anthesis, and remobilization of pre-anthesis reserves accumulated during the pre-anthesis period and having lower Δ values [10].

4.2. Relationship between Δ and yield across different environments

Positive correlations between Δ and yield in Yinchuan and Huinong are in agreement with those reported for bread wheat and barley under both water-stressed and well-watered conditions [1, 3, 8, 14, 15, 17, 23, 53, 54, 55]. The positive correlations between Δ and yield suggested that variation in C_i/C_a and Δ were driven more by stomatal conductance than by

photosynthetic capacity. This hypothesis was fully confirmed in Yinchuan by the significant positive correlation between g_S and Δ.

Conversely, non-significant correlation was found between Δ and grain yield in the rain fed Guyuan site, which is characterized by a pre-anthesis stress. The lack of correlation between Δ and grain yield in Guyuan could be related to the very severe stress in this environment. Severe drought conditions that affect flowering and grain formation can drastically reduce sink size and break the relationship between stomatal conductance, Δ and grain yield [3, 56].

4.3. Relationship between Δ and its surrogates

The lower ash content of vegetative parts of the plants and the higher ash content of the grain, observed in Guyuan and Huinong, compared to Yinchuan in both 2005 and 2006, reflected the more severe stress experienced by the crop. Also, the higher ash content of vegetative parts and grain in 2005 corresponded to more rainfall and higher transpiration in 2005 than 2006. The mineral accumulation in leaves and kernels primarily depends on re-mobilization of C products from leaves and stems [57]. Under drought stress, re-mobilization is less affected than photosynthesis [58]. The contribution of re-mobilization to final yield is consequently higher, leading to a decrease of m_aL_m and an increase of m_aG_m [59, 60]. The relationship between ash content at heading and ΔG_m, observed in Guyuan and Yinchuan has not been reported elsewhere. It can be explained by the strong limitation of stomatal conductance at this stage that may have affected both transpiration and Δ. In these two locations, m_aL_m was significantly and positively correlated with both ΔL_a and ΔG_m. A significant positive correlation was reported between m_aL_m and ΔG_m under post-anthesis water stress [10]. Ash content in the mature leaf consequently appeared to be a useful indirect selection criterion for Δ. In disagreement with previously published results [20], no correlation was found between m_aG_m and Δ [8, 10, 59]. Mineral accumulation in kernels depends highly on genotype and environment [59].

Leaf specific dry matter increased under stressed conditions. An increase in LDM as a response to water stress has been reported in durum wheat [29, 61]. Such changes in LDM are probably associated with an increase in the cell perimeter to cell area ratio and in a higher accumulation of hemi-cellulose in the cell walls [29]. A positive correlation was noted between LDM (at heading) and ΔL_a under rainfed conditions. In peanut (*Arachis hypogea* L.), a significant positive correlation was found between LDM and leaf Δ [62]. Such a relationship may reflect a covariant effect of crop phenology on these parameters [29]. The later flowering genotypes tend to have leaves with lower LDM and lower Δ. In addition, LDM, rather than just reflecting variations in leaf structure, may also be affected by differences in the accumulation of carbohydrates [29]. Under water limited conditions, genotypes with high LDM may be able to maintain higher stomatal conductance and re-mobilize more high Δ C products to the grain, and may have consequently higher ΔG_m. A positive correlation between LDM and m_aG_m was reported under rain fed conditions [29], but no correlation was found in the present study.

Some new surrogates for Δ, e.g. K, Mg, and Ca contents in leaf and grain, were evaluated and identified in 2007. ΔL_a was negatively correlated with K content and positively correlated with Mg content in the flag leaf at anthesis. At maturity, Δ in grain was negatively correlated with Mg content in the flag leaf and negatively correlated with Ca content in grain. As a main osmoticum in the plant, leaf K plays a key role in regulating stomatal movement. Under drought conditions, high K content in the flag leaf may lead to high stomatal sensitivity to water stress and smaller stomatal aperture (low Δ). Moreover, K in the plant is also

associated with photosynthesis [63]. High K content in the leaf, related to high photosynthetic rate, could lead to low C_i/C_a (i.e. the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration) and a low Δ value. Mg plays an important role in activating enzymes and in protein, RNA and DNA synthesis. In our study, the positive correlation between Δ and Mg content in the flag leaf at anthesis may suggest a higher metabolic activity for high Δ genotypes [64]. High-Δ genotypes often grow faster than do low Δ genotypes in environments where a high soil water status has been maintained [14, 21, 55, 64]. Ca is transported via the xylem rather than via the phloem. The movement of Ca in plant xylem is mainly driven by the transpiration flow [40]. After its deposition in tissues, Ca often remains in a stable form and is seldom redistributed and removed. Ca content (expressed in % or in proportion of total ash) is consequently high in the organs that have high transpiration rates and have a long growing period [41]. In our study, the higher leaf Δ that was associated with higher stomatal conductance and stronger transpiration was possibly related to the high Ca content in the leaf.

K and Mg are easily removed in the plant from senescing organs to the leaflets or grain via the phloem [40, 65]. Potassium concentration in the phloem has some effect on the loading of photosynthetic products from source organs to the phloem. The transport of assimilates from source organs to sink organs via the phloem is coupled with the movement of potassium [36, 37]. In the present study, the lower K content in flag leaves at maturity than at anthesis suggested that a quantity of K was removed out of leaves during the late growing period and recycled by the crop. Genotypes that are able to sustain greater transpiration losses (and thus high grain Δ) during grain filling are more efficient in dry matter partitioning to the grain [22]. The high ΔG_m genotypes would transport more assimilates together with K from leaves to grain than low ΔG_m genotypes and have lower mature flag leaf K content. In the present study, the negative correlation between the Mg content in mature flag leaves and ΔG_m may be accounted for by the different phenology of the genotypes. Strong negative correlations have been found between Δ and days to heading or anthesis in several studies conducted with cereals in Mediterranean, terminal-drought environments [23, 55]. We also found significant negative correlations between ΔG_m and days to heading and maturity with low ΔG_m genotypes, heading and maturing later than did high ΔG_m genotypes. Mg plays an important role in reducing active oxygen and malondialdehyde (MDA), which can delay senescence of the plant [38, 39]. The high Mg content in mature leaves of low ΔG_m genotypes may explain their longer growth period and later maturity date. A negative relationship between Δ and Ca content in grain was noted in our study. Differing from K and Mg, Ca is not easily removed in the plant and there is limited Ca movement between leaf and grain through the phloem [66]. In our study, the lower Ca content in grain than in leaf supported this theory. In addition, more efficient translocation of C products to the grain for high ΔG_m genotypes tended to dilute Ca in the grain, which accounted for the negative correlation between Ca content and ΔG_m in T_3.

4.4. Differences among landraces, advanced lines and released varieties for morpho-physiological characters

The three groups of genotypes, i.e. landraces, advanced lines and released varieties, did not differ significantly for ΔL_a in the present study, while a significant difference was found between the three groups for ΔG_m in Yinchuan. As the net photosynthetic rate was not correlated with either ΔL_a or ΔG_m in this study, the significant difference for stomatal conductance in Yinchuan may explain the variation in ΔG_m between the three groups. The latest flowering date for the landraces compared with the other two groups was also partly responsible for their lowest ΔG_m. The slightly higher (non-significant) yield found in landraces than the other two groups in Guyuan (pre-anthesis condition) may be attributed to

their longer growth period, later flowering date and 'conservative' water use (lower g_s). However, these characteristics would become disadvantageous under Ningxia irrigated conditions, explaining the lower yield for the landraces in Yinchuan and Huinong. Besides, lower HI for landraces in Yinchuan and Huinong reflected their weaker capacity in terms of partitioning C to the grain than the advanced lines and released varieties under post-anthesis water stress condition. The highest leaf temperature of the landraces in Yinchuan may be related to their stomatal conductance and lower transpiration rate, which was unfavourable to heat avoidance during the later growth period. The order of $SLDW_{gf}$ among the three groups varied with sites. Lower SLDW during the mid-grain filling stage in the released varieties, being registered by higher ΔG_m, than in the landraces in Yinchuan, was likely to reveal the higher efficiency of the released varieties in remobilizing C from leaf to grain [22, 67].

4.5. Genetic analysis for Δ

In 2006, the highest Δ value for F_3 descendants was found in the high Δ × high Δ combination, followed by the high Δ × low Δ combination, and the low Δ × low Δ combination. In 2008, the ΔL_a and ΔG_m of F_6 progenies originated from high Δ × high Δ combination was higher than high Δ × low Δ combination. For the high Δ × high Δ combination, the F_6 lines' mean values of ΔL_a and ΔG_m were close to that of their high Δ parents; while for the high Δ × low Δ combination, the F_6 individuals' ΔL_a and ΔG_m were all higher than their low Δ parent, suggesting high heritability of Δ and that high Δ progenies would be easy to generate from the combination composed of high Δ parents.

Higher h_B^2 and lower CV were found for ΔL_a than ΔG_m in F_3 populations in 2006, while similar h_B^2 of ΔL_a and ΔG_m was found in 2008, although higher CV was noted for ΔG_m than ΔL_a, indicating the complexity of grain filling. The carbohydrate in grain comes from current assimilates in leaves and C retranslocated either before or after anthesis. CID would be variable in C assimilated in different growth periods. Moreover, the proportion of retranslocated C is likely to vary with genotype and with stress intensity [68]. Therefore, differences in ΔG_m would be higher than ΔL_a. The grain is likely to yield a more ambiguous assessment of intrinsic variation in leaf transpiration efficiency than that obtained by using vegetative organs [45].

4.6. Evaluation of different strategies for using CID as a selection tool in rain fed and limited irrigated systems

Significant correlations between Δ and either HI or GY in Yinchuan and Huinong, which are characterized as post-anthesis water stress, confirmed the hypothesis that higher Δ is related to higher efficiency of C partitioning to the kernel, which favours yield gain. High-Δ is proposed as the selection target in the Ningxia irrigation regions for higher yield, which can result in lesser competition of vegetative organs against reproductive organs and higher HI. The ΔL_a and ΔG_m values of F_6 lines originating from high Δ × high Δ in Yinchuan were higher than those from high Δ × low Δ, suggesting high h_B^2 for Δ. High-Δ progenies were found in the high Δ × high Δ crossing combination. It seems that the parents with high Δ values would generate still higher Δ progenies. The high Δ × low Δ combination was found to have higher h_B^2 of ΔL_a and ΔG_m than the high Δ × high Δ combination.

Conversely, non-significant correlation was found between Δ and grain yield under rain fed condition Guyuan. In this environment, characterized by a pre-anthesis water stress, the magnitude of the correlation between Δ and yield is likely to depend on the quantity of water stored in the soil at sowing [3, 10]. Nevertheless, the local varieties Maohuomai and Hongmangmai and the improved variety Ningchun 27 were found to exhibit low Δ. Several

lines were found to exceed their high yielding parent in the high Δ × low Δ combination, suggesting that pairing crossing combination involving low Δ parents bred under rain fed conditions and high Δ parents bred under irrigation may create more opportunities to breed high yielding progenies.

5. CONCLUSIONS

Significant variation in Δ was found across different wheat genotypes, locations, and years. The Δ of local varieties was significantly lower than that of improved varieties and advanced lines. Lower Δ was found under rain fed than under limited irrigated condition. In the limited irrigation areas, leaf Δ and grain Δ were positively correlated with yield. In rain fed areas, grain Δ was weakly and negatively correlated with yield.

Leaf ash content at maturity was significantly and positively correlated with ΔG_m and ΔL_a. LDM at heading was significantly and positively correlated with ΔL_a under rain fed conditions. K and Mg content in the flag leaf and Ca content in grain seem to be potentially useful surrogates for Δ.

200 F_6 lines derived from three crossing combinations, i.e. Yong 3119 × 03S111 (high Δ × high Δ), Ningchun 4 × Ningchun 27 (high Δ × low Δ), and Ningchun 32 × Ningchun 27 (low Δ × low Δ), were obtained. 08YJ19, 08YJ14, 08YJ51, 08YJ3, 08YJ42, 08YJ87, 08YJ70, 08YJ111, 08YJ76 and 08YJ104 were outstanding lines which were found to demonstrate substantially higher yield than their high yielding parents or Ningchun 4. 31 progenies were also generated by crossing between Drysdale and Quarry and high or low Δ varieties. These lines have been developed and are being used in breeding programmes. An F_2 group (Ningchun 4/Drysdale) has been used for molecular studies.

High broad sense heritability (h_B^2) of Δ was found under both rain fed and irrigated conditions. The h_B^2 of ΔL_a was higher than that of ΔG_m in genotypes and F_3 populations. Higher h_B^2 of F_3 generations was shown under rain fed than under limited irrigated conditions.

The relationship between grain yield and Δ did not appear very stable across environments and water regimes. It depended greatly on the quantity and distribution of rainfall, the occurrence of irrigation and consequently the water status of the crop during the growth cycle. The use of Δ as a yield-predictor should only be recommended under conditions of moderate pre-anthesis water stress and limited irrigation, and not in environments characterized by very severe pre-anthesis drought stress.

On the basis of the results obtained in 2007 to 2008, high Δ × high Δ seems to be the optimal crossing combination, and the selection of progenies with a high Δ value would lead to yield gain under limited irrigation (Yinchuan), while high Δ × low Δ would be a better crossing combination in breeding programmes in rain fed systems (Guyuan).

Because of the high cost of Δ analysis, some surrogates, identified in our study, should be integrated in the selection programme. Higher ash content in the flag leaf at maturity, lower specific leaf dry weight at the later grain filling stage, shorter plant height, lower TE (larger stomatal aperture), and early heading date would be desirable selection characters in the limited irrigation region. Early vigor, high biomass and plant height at anthesis, higher specific leaf dry weight at anthesis, higher TE (smaller stomatal aperture), and longer growth period are likely to be the best selection characters in the rain fed region.

Further studies should be established in other Wheat Production Zones of China, especially Northwest China, to describe the effect of environmental factors on the relationship between Δ and yield, and to examine the potential of Δ and/or its potential surrogates as indirect selection criteria for yield in the corresponding climatic zones. In addition, attempts should be made to find molecular markers related to CID and WUE.

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RELATIONSHIPS AMONG WATER USE EFFICIENCY, GRAIN YIELD, CARBON ISOTOPE DISCRIMINATION AND ASH CONTENT IN WHEAT UNDER DIFFERENT MEGA-ENVIRONMENTS AND WATER REGIMES IN CHINA AND INDIA

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Abstract

Positive correlations have been repeatedly reported between grain yield, carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) and ash content in wheat cultivated under Mediterranean-type environments (characterized by post-anthesis water stress). The relationships among these traits have been much less analyzed under other wheat mega-environments. The present study examined the relationships between grain yield, $\Delta^{13}C$ and ash content in wheat in Northern China (characterized by pre-anthesis water stress) and in the Peninsular Zone of India (characterized by residual moisture stress). In both mega-environments, wheat was grown under rain fed and irrigated conditions. The relationships between grain yield, Δ and ash content were less stable than under post-anthesis water stress, and were highly dependent on the quantity of water stored in the soil at sowing, which in turn depends on out-of-season rainfall. This considerably limits the use of Δ and ash content as indirect selection criteria for yield in these mega-environments. In India, the relationships between Δ, ash content and components of water use efficiency (WUE) (the latter were estimated from a soil water balance model) were also investigated. Significant correlations were found across environments between ash content in leaf and grain and the model estimates of the quantity of water transpired during the growth cycle. WUE was significantly negatively correlated with ash content in leaf and grain. Additional analyses of the relationships among grain yield, Δ and ash content, including soil moisture measurements over the growing period and/or estimation of water balance components, are needed in these mega-environments to define precisely the range of conditions leading to significant correlations and allowing the use of Δ and ash content as indirect selection criteria for yield.

1. INTRODUCTION

Carbon isotope discrimination has been proposed by several authors as a predictive selection criterion for grain yield under drought stress [1]. Wheat grain yield was found to be positively correlated with grain Δ under the conditions of southern Australia [2], Morocco [3], Spain [4], the south of France [5], Greece [6] and Algeria [7]. The positive correlation between grain Δ and yield was quite stable under Mediterranean-type climatic conditions [1], characterized by post-anthesis water deficit and referred to as wheat mega-environment ME4A [8]. Exceptions were reported in Algeria [9] and in Spain [10], under very severe drought conditions. In some cases, grain yield was also positively correlated with leaf Δ

assessed at anthesis [5, 11, 12]. However, the correlations between grain yield and Δ were weaker and less stable under pre-anthesis water stress [13] and residual soil moisture conditions [14]. In these two environments, referred to as mega-environments ME4B and ME4C, respectively [8], the correlation between grain yield and Δ depends largely on the quantity of water stored in the soil before sowing and on the evaporative demand during the growth cycle [1].

Similarly, leaf ash content (m_a), proposed as a surrogate for $\Delta^{13}C$ [11, 15], was found to correlate significantly with grain yield in the mega-environment ME4A [6, 16], but not under residual moisture stress [1, 14]. Grain m_a correlated negatively with grain yield under post-anthesis water stress [5] and under residual soil moisture [14]. A more precise characterization of the environment is consequently needed before using Δ and m_a as secondary traits for yield, particularly under pre-anthesis and residual moisture stress.

Several authors [17, 18] have proposed that Δ can be used to estimate or predict water use efficiency (WUE). WUE of a crop has been variously defined as the ratio of total biomass, or above ground biomass, or harvested yield, against total available water, or evapo-transpiration, or plant transpiration [17]. The ratio of total biomass produced against total available water may be expressed as [19]:

$$WUE_B = M/(E_s + T + R + D) \quad (1)$$

where M, E_s, T, R and D are the biomass produced, the evaporation from the soil surface, the transpiration during the growing season, the runoff, and the drainage below the root zone, respectively.

Equation (1) can be rearranged to give:

$$WUE_B = (M/T)/\{1+ [(E_s + R + D)/T]\} \quad (2)$$

where M/T is the ratio of biomass produced at the plant level to water consumed.

Agronomic water use efficiency on a grain basis, the ratio of production of total biomass to total available water can be expressed as:

$$WUE_G = HI \times (M/T)/\{1 + [(E_s + R + D)/T]\} \quad (3)$$

where HI is the harvest index, i.e., the ratio of grain yield to above ground biomass.

Equation (3) shows that improved WUE can be obtained by either an increase of M/T, a reduction of E_s/T, or an increase of HI.

Under drought conditions Δ was shown to be negatively correlated with transpiration efficiency at the leaf level [20] and was expected to be negatively related to M/T and WUE under water stress conditions. Several authors attempted to experimentally verify this hypothesis. A significant negative correlation was obtained in wheat between Δ and M/T [21, 22]. A negative correlation between leaf Δ and WUE was found in rice [18]. However, due to the difficulty of directly assessing WUE components, these studies were carried out in pot experiments. Runoff and drainage were negligible, and the soil surface was covered by a plastic film to minimize soil evaporation. WUE was consequently close to M/T (see Eq. 2). A highly significant negative correlation between Δ and WUE in durum wheat was reported in

Austria [23], by measuring soil water with a neutron probe and using a water balance approach [24] to estimate evapo-transpiration. However, there was no significant correlation between Δ and WUE in bread wheat cultivated in Australia [25]. WUE was estimated by using a soil water balance model and neglecting run off and drainage. The evaluation of water balance components, in addition to allowing a better definition of the testing environment, permits estimation of WUE components (runoff, drainage, soil evaporation and transpiration) that cannot be easily measured over the whole growing season. Several crop growth models have been proposed for these purposes, some allowing estimation of expected yield for several growing conditions [26]. Most models, however, require a large set of input data that are generally not available to the plant breeder. They also demand an extensive site-specific calibration and are not convenient for site comparisons. In the present study, we used the soil water balance model "Budget" [26], which is more accurate for evaluation purposes with limited data and comparison of environments.

The first objective of the present study was to analyze the relationships among $\Delta^{13}C$, ash content and grain yield under different mega-environments and water regimes. It was carried out in two important wheat growing areas, northern China (Ningxia Province) and Peninsular India, the first being characterized by in-season rainfall (with drought stress at the beginning of the growth cycle) and the second by out-of-season rainfall (wheat growing under residual soil moisture).

Ningxia Province is located on the Loess plateau at an altitude of 1000–2000 m above sea level, decreasing from south to north. Arable land represents 13.1% of the total area of the region, of which 20.5% is irrigated. Spring wheat covers 38% of the cultivated area. The Ningxia region has been classified by the Chinese Academy of Agricultural Sciences as Wheat Production Zone VIII (north-western spring-sown wheat) [27]. In this production zone, drought is the main limiting factor for rain fed wheat production, and an important part of the irrigated area is affected by soil salinity. Drought and salinity tolerance are consequently the two major objectives for wheat breeders in this region [28].

In the Peninsular zone of India that accounts for one third of the total wheat area in India [29], wheat production is limited mainly by inadequate availability of irrigation water and by high temperatures [30]. Durum wheat is largely cultivated, typically from November to April (rabi season), under residual soil moisture, full irrigation (when possible) or limited irrigation (irrigation until anthesis). It is generally exposed to extremely high temperatures during the grain filling period [30]. In China and India, the relationships between Δ, ash content and grain yield were investigated under both rain fed and irrigated conditions.

The second objective of this study was to analyze the relationships between water use efficiency (and its components), grain yield, Δ and ash content. This was carried out across three water regimes (residual soil moisture, limited irrigation and full irrigation) in India.

2. MATERIALS AND METHODS

2.1. Experimental

2.1.1. China

In China, a set of 20 spring bread wheat (*Triticum aestivum* L.) genotypes was used in the experiments. It included some local varieties from irrigated and rain fed areas, cultivars and advanced lines. Experiments were carried out in the field, under rain fed conditions in Guyuan, and under irrigated conditions in Huinong and Yinchuan, during two consecutive

years (2003–2004 and 2004–2005), and thereafter referred to as seasons 1 and 2, respectively. The main characteristics of these locations are given by Xu et al. [13]. Guyuan experimental site is located in southern Ningxia on a dark loess soil with a columnar structure, rich in potassium and poor in nitrogen and phosphorus, and with a moderate water-holding capacity (around 20%). Yinchuan and Huinong are located in central and northern Ningxia, respectively, on warping dams that have been built across gullies for the purpose of creating newly arable land by silt deposition. Such soils cover more than 30 million ha on the Loess Plateau [31], and are rich in clay and loam. The Huinong experimental site is saline.

2.1.2. India

In India, 20 durum wheats (*T. turgidum* L. var. durum), widely cultivated in the Peninsular Zone, were used in this study. Trials were conducted at the Experimental Farm of Agharkar Research Institute, located in the Hol District, Pune, India, during two consecutive seasons (2004–2005 and 2005–2006), hereafter referred to as seasons 1 and 2, respectively. The soil, known as 'black cotton' or 'gilgai' soil is a Vertisol with a high proportion of clay, a high water holding capacity of 65% and a pH value of 8.1. The twenty durum wheat cultivars were grown under three water regimes, namely residual soil moisture, limited irrigation (leading to post-anthesis water stress) and full irrigation. The different treatments mimicked the main situations in which wheat is cultivated in the Peninsular Zone. Residual soil moisture stress is the most common type of water stress experienced by rain fed wheat in this zone [30]. Full-irrigation is practiced when water availability permits. However, inadequate availability of irrigation water is common in the Peninsular Zone [29], leading to limited irrigation that generally translates in post-anthesis water stress. The experimental design was a randomized complete block with three replicates per genotype. Sowing density, fertilization and chemical control of pests and diseases were as recommended locally.

2.2. Measurements

Days to heading and days to anthesis were recorded in each plot, and at maturity plants were harvested for the determination of grain yield. Rainfall, air temperature, relative humidity and solar radiation were recorded. Total water input (W_i) was calculated as the sum of rainfall and irrigation (when applicable) during the wheat growth cycle.

2.2.1. Carbon isotope discrimination

Twenty flag leaves were harvested per plot at anthesis. At maturity, a 10 g grain sample was collected from each plot. Leaf and grain samples were dried at 60 ^{o}C for 48 h and ground to a fine powder. Because of the high cost of $\delta^{13}C$ analysis, samples of the three replicates were carefully mixed in equal weights, to constitute a bulk sample. $\delta^{13}C$ was measured on a 1 mg sample with an isotope ratio mass spectrometer(Model Thermo Finnigan, Bremen, Germany) at the Plant Research Institute of the Chinese Academy of Sciences, Beijing (Chinese samples), and at the University of Agricultural Sciences, Bangalore (Indian samples).

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{reference}) - 1] \times 1000 \quad (4)$$

where R, the isotope ratio = $^{13}C/^{12}C$. A secondary standard (potato starch), calibrated against the primary standard, Pee Dee Belemnite (PDB) fossil carbonate, was used as the reference.

$\Delta^{13}C$ in the flag leaf at anthesis (ΔL_a) and in grain at maturity (ΔG_m) were calculated as [32]:

$$\Delta^{13}C\ (‰) = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 1000 \quad (5)$$

where $\delta_p = \delta^{13}C$ of the plant sample and $\delta_a = \delta^{13}C$ of atmospheric $CO_2 = –8‰$.

2.2.2. Ash content

Ash content (m_a) was determined in seasons 1 and 2 in India. This trait was measured in the whole plant at heading, in the last fully expanded leaf (with the ligule present), and in the whole plant, the flag leaf and the grain at maturity [33]. Approximately 1.0 g of dry material was incinerated at 575 ^oC for 16 h (until light grey ash was obtained). Ash content (%) was expressed on dry mass basis. Ash content in the flag leaf at anthesis, the flag leaf at maturity and grain at maturity were referred to as m_aL_a, m_aL_m and m_aG_m, respectively.

2.3. Estimation of soil water balance and water use efficiency components

Soil water balance components were estimated from the 'Budget' model [26].

2.4. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Effects of genotype, environment and their interaction were determined for yield and yield components. The Duncan test was used to establish significant differences between environments. Analysis were made using SAS, version 8.1. [34]. Pearson phenotypic correlations were calculated to determine the relationship between traits and grain yield.

3. RESULTS

3.1. China

Rainfall varied across locations and between years (Table 1). In Yinchuan and Huinong the crop, irrigated twice during tillage and elongation phases and receiving rain after anthesis, did not experience a strong drought stress. Conversely, in Guyuan, the crop faced a severe water stress before anthesis. Grain yield varied greatly with year and location. Higher yields were obtained under irrigated (Yinchuan, Huinong) than under rain fed conditions (Guyuan). Under irrigation, yield was lower in Huinong (saline) than in Yinchuan (non-saline).

TABLE 1. SOWING DATE, ANTECEDENT MOISTURE, WATER INPUTS AND GRAIN YIELD OVER TWO SEASONS AT THREE LOCATIONS IN NORTHERN CHINA

Location	Season	Sowing date	Soil water (%)	Rainfall (mm)	Irrigation (mm)	W_i † (mm)	GY † (t ha^{-1})
Guyuan	1	20 March	19.6	219.0	–	219.0	2.76d
	2	20 March	14.2	212.3	–	212.3	0.83d
Yinchuan	1	27 Feb	21.2	58.7	180	238.7	9.69a
	2	01 March	24.4	24.2	180	204.2	7.73a
Huinong	1	28 Feb	26.0	88.3	180	268.3	4.89b
	2	05 March	25.2	29.6	180	209.6	3.92b

†W_i = total water input (rainfall and irrigation), GY = grain yield; GY means without a common letter are significantly ($P<0.05$) different (Duncan test).

Both ΔG_m and ΔL_a were strongly and positively correlated with grain yield across environments (r = 0.924, P<0.001 and r = 0.789, P<0.001, respectively). The relationship between grain yield, ΔG_m and ΔL_a varied greatly with the environment (Table 2). In Guyuan, grain yield was positively correlated with ΔG_m in year 1, but not in year 2. In Yinchuan, grain yield was positively correlated with ΔG_m in year 2, but not in year 1, and only one significant correlation was noted between grain yield and ΔL_a over three sites and two seasons (Table 2).

TABLE 2. CORRELATIONS BETWEEN GRAIN YIELD AND CARBON ISOTOPE DISCRIMINATION OVER TWO SEASONS AT THREE LOCATIONS IN NORTHERN CHINA

Location	Season	ΔL_a †	ΔG_m †
Guyuan	1	0.382ns	0.674***
	2	0.505*	0.412ns
Yinchuan	1	0.066ns	0.134ns
	2	0.232ns	0.731***
Huinong	1	0.179ns	0.209ns
	2	0.429ns	0.413ns

†Carbon isotope discrimination in the flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m),
*, significant at P<0.05; ***, significant at P<0.001; ns, not significant

3.2. India

Rainfall during the 2005 monsoon (614.4 mm) was higher than during the 2004 monsoon (519.2 mm). Because of the lack of rain during the growing season (15.3 and 15.8 mm in seasons 1 and 2, respectively), the quantity of water available for the irrigated crop, as estimated by the model, was close to the quantity of irrigation water (Table 3). As expected, both infiltration and runoff were much higher under irrigated (full and limited) than under rain fed conditions. Conversely, drained water was similar under the three water regimes. Soil evaporation was lower under rain fed than under irrigated conditions but did not differ significantly between full and limited irrigation. The quantity of water transpired was highly correlated with the quantity of water available to the crop (r = 0.982, P<0.001). Estimated yield was, on average, 18.5, 32.5 and 49.5% of the potential yield, under residual moisture, post-anthesis water stress and full irrigation, respectively. Grain yield was, on average, 37.7 and 20.2% lower under residual moisture and limited irrigation, respectively, than under full irrigation. Experimental yield was significantly correlated with the yield predicted by the model (r = 0.883, P<0.01).

TABLE 3. WATER INPUTS, COMPONENTS OF SOIL WATER BALANCE AND EXPECTED YIELD (Y_E), ESTIMATED BY THE 'BUDGET' MODEL, UNDER THREE MOISTURE REGIMES OVER TWO SEASONS IN PENINSULAR INDIA

Season/water regime	Water (mm) †								Y_e‡
	I_w	W_i	I_n	R	D	E_s	T	ET	
Season 1 (2004–2005)									
Residual soil moisture	30	45.3	34.8	10.5	15.1	49.6	153.9	204.1	19
Limited irrigation	210	225.3	94.2	130.7	14.9	70.3	187.8	258.1	38
Full irrigation	330	345.3	134.3	211.0	14.9	70.5	206.9	277.4	50
Season 2 (2005–2006)									
Residual soil moisture	30	45.8	35.0	10.8	19.2	45.5	149.3	194.7	18
Limited irrigation	210	217.8	87.6	130.2	20.6	66.6	174.7	241.3	27
Full irrigation	330	337.8	127.4	210.4	20.4	66.6	200.3	266.9	49

†I_w = irrigation, W_i = total water inputs (irrigation + rainfall), I_n = infiltration, R = runoff, D = drainage, E_s = soil evaporation, T = transpiration, ET = evapotranspiration
‡Data are % of potential yield

As in China, highly significant correlations were noted between grain yield and grain Δ across water regimes in both seasons (r = 0.708, $P<0.001$ and r = 0.574, $P<0.001$, respectively). The correlations between grain yield and ΔL_a, ΔG_m, m_aL_a, m_aL_m and m_aG_m are given in Table 4. A significant correlation was found between grain yield and ΔL_a under residual soil moisture, both in seasons 1 and 2. A significant correlation was noted between grain yield and ΔG_m under full irrigation in season 1, and under residual moisture in season 2. In addition, under limited irrigation, grain yield was significantly and negatively correlated with m_aG_m in season 1, and positively correlated with m_aL_a in both seasons.

TABLE 4. CORRELATIONS BETWEEN GRAIN YIELD, δ AND ASH CONTENT IN THE FLAG LEAF AND GRAIN UNDER THREE MOISTURE REGIMES OVER TWO SEASONS IN PENINSULAR INDIA

Season/water regime	ΔL_a †	ΔG_m †	m_aL_a ‡	m_aL_m ‡	m_aG_m ‡
Season 1 (2004–2005)					
Residual soil moisture	0.423*	0.273ns	0.344ns	0.286ns	–0.371ns
Limited irrigation	–0.306ns	0.383ns	0.424*	–0.007ns	–0.441*
Full irrigation	0.039ns	0.693**	0.079ns	0.302ns	–0.393ns
Season 2 (2005–2006)					
Residual soil moisture	0.492*	0.621**	0.157ns	–0.092ns	–0.253ns
Limited irrigation	–0.334ns	–0.144ns	0.483*	0.335ns	0.055ns
Full irrigation	0.157ns	0.189ns	–0.204ns	–0.153ns	–0.365ns

†Carbon isotope discrimination in the flag leaf at anthesis (ΔL_a) and grain at maturity (ΔG_m),
‡Ash content in flag leaf at anthesis (m_aL_a) and at maturity (m_aL_m), and in grain at maturity (m_aG_m).
*, significant at $P<0.05$; **, significant at $P<0.01$; ns, not significant.

The different components of water use efficiency, estimated from the model, also varied with the water regime (Table 5). In season 1, M/T was much lower (around 30%) under residual soil moisture than under irrigated conditions, while in season 2, similar values were noted under the three water regimes. The component $1 + [(E_s + R + D)/T]$ was much lower under residual moisture than under irrigation in the two seasons. This was mainly due to the lower E_s and R values estimated under this water regime that largely compensated for the slightly higher T values. Significant correlations were found between the average values of grain and leaf Δ for a given environment and T, the quantity of water transpired during the growth cycle, estimated by the model (r = 0.951, $P<0.001$ and r = 0.804, $P<0.05$ for grain and leaf Δ, respectively). WUE was significantly and negatively correlated with m_aL_m (r = –0.900, $P<0.01$) and m_aG_m. (r = –0.773, $P<0.05$).

TABLE 5. WUE AND ITS COMPONENTS, ESTIMATED BY THE 'BUDGET'MODEL, UNDER THREE MOISTURE REGIMES OVER TWO SEASONS IN PENINSULAR INDIA

Season/water regime	HI †	M/T † ($g\ kg^{-1}$)	$1+ [(E_s + R + D)/T]$ †	WUE
Season 1 (2004–2005)				
Residual soil moisture	0.35	4.28	1.49	1.01
Limited irrigation	0.32	6.03	2.15	0.90
Full irrigation	0.36	6.22	2.43	0.92
Season 2 (2005–2006)				
Residual soil moisture	0.35	6.38	1.51	1.48
Limited irrigation	0.32	6.65	2.24	0.95
Full irrigation	0.36	6.39	2.48	0.93

†HI = harvest index, M = biomass produced, E_s = evaporation from the soil surface, T = transpiration during the growing season, R = runoff, D = drainage below the root zone.

4. DISCUSSION

4.1. Relationships between grain yield, $\Delta^{13}C$ and m_a under different water regimes

4.1.1. China

Grain yield varied markedly with season and location. The differences in grain yield and Δ observed under irrigated conditions between Huinong and Yinchuan are likely to be due to the effects of soil salinity, which reduces both traits [35]. The relationships between grain yield, ΔG_m and ΔL_a varied greatly with the environment. Because of irrigation during the dry (pre-anthesis) period, drought stress was moderate and delayed in Yinchuan and Huinong. In contrast, the wheat crop experienced a severe drought stress before anthesis in Guyuan. Moreover, soil water at sowing was very low as a consequence of the low rainfall in autumn and early winter [36], and the high runoff to rainfall ratio of soils [37]. In Guyuan, grain yield was correlated with ΔG_m in season 1, but not in season 2. The winter drought encountered in the Ningxia region corresponds to the mega-environment ME4B [8], which is also present in the southern cone of Latin America, and is estimated to cover a wheat area of 3 million km^2 worldwide. In such environments, where in-season rainfall and its proportion corresponding to the pre-anthesis period are very low, a limited water supply before anthesis led to a dramatic yield increase. For example, in Guyuan a single irrigation of 60mm at the jointing stage led to a yield increase of 75% [38]. The significant correlation between ΔG_m and grain yield observed in Guyuan in season 1, and the absence of a relationship in season 2 (the driest environment), are likely to be due to differences in the quantity of water stored in the soil at sowing [1]. The absence of a significant correlation between ΔG_m and grain yield in Guyuan in season 2 could also be related to the very low yields in this environment. The lack of correlation between grain yield and ΔG_m has also been reported in poor-yielding environments in Spain and Algeria [4, 9]. Severe drought conditions that affect flowering and grain formation, can drastically reduce sink size and break the relationships between stomatal conductance, Δ and grain yield [9]. In Yinchuan, grain yield was significantly correlated with ΔG_m in season 2, but not in season 1, and no correlation was noted between grain yield and ΔL_a. The lack of correlation between ΔG_m and grain yield under well-watered conditions (Yinchuan, season 1) is in full agreement with Monneveux et al. [1, 39]. Under well-watered conditions, stomatal conductance is likely to be high in all cultivars resulting in increased C_i/C_a and Δ values [40], while increased photosynthetic capacity potentially decreases C_i/C_a. The decrease in C_i/C_a associated with increased photosynthetic capacity is consequently offset by the C_i/C_a increase resulting from stomatal aperture, hence reducing the possibility of a relationship between Δ and grain yield. When irrigation does not fully meet water requirements, and the plant experiences a subtle water stress, or when less water is stored in the soil (as in Yinchuan in season 2), stomatal conductance becomes the factor driving the variation of C_i/C_a and the correlation between Δ and yield becomes significant [39]. In Huinong, the relationship between Δ and grain yield was positive, but not significant. Salinity induced a stronger reduction in grain yield and Δ in the driest season, probably due to an increase in the salt concentration in the soil solution and/or the cumulative effects of drought and salt stress. One explanation could be that salt stress not only affects stomatal conductance but also photosynthetic capacity [41]. The decrease in C_i/C_a induced by stomatal closure could be partially offset by the lower use of CO_2 by Rubisco.

4.1.2. India

In India, a significant correlation was noted in both seasons between grain yield and leaf Δ under residual soil moisture. Grain yield correlated with grain Δ in season 2, but not in

season 1. Significant correlations were reported between grain yield and grain Δ in an experiment simulating residual soil moisture conditions during the dry season in northwest Mexico [1], but not in India [14]. Again, these discrepancies are likely to be due to differences in soil water content at sowing. High-Δ genotypes tend to grow faster than low Δ genotypes [42]. By covering the ground more quickly, they could be more efficient in reducing soil evaporation. Their higher biomass at anthesis and greater reserves enable them to translocate larger amounts of stored assimilates to the grain. High $\Delta^{13}C$ may also reflect higher stomatal conductance, particularly after anthesis, when soil moisture decreases and water stress becomes stronger. At anthesis, soil water depletion may have been greater for high Δ genotypes than for low Δ genotypes, which are more conservative in their water use. Excessive soil water depletion by high Δ genotypes can however offset the advantages described above [42]. This could explain the absence of correlation observed in India [14] under conditions where a low quantity of water was available in the soil at sowing. Conversely, a significant negative correlation between grain yield and m_aG_m that was not observed in the present study was reported under rain fed conditions in India [14]. Under limited irrigation, grain yield was significantly and positively correlated with m_aL_a in both seasons, as observed in another study [16]. Grain yield was significantly and negatively correlated with m_aG_m in season 1, while such a relationship was not reported in India [14]. Finally, significant correlations reported between grain yield and m_aL_m, under both residual soil moisture and full irrigation [14] were not observed in the present study. Taken together, these results indicate that, under out-of-season rainfall conditions (i.e., growth cycle not including the rainy period of the year), most of the relationships between grain yield and the indirect selection criteria such as Δ and m_a, are quite unstable over years, probably because of a high inter-annual variation in the quantity of water stored in the soil. This situation contrasts markedly with the Mediterranean environment (rainy period overlapping with part of the growth cycle), where stable relationships were repeatedly found between grain yield and grain Δ [1, 2, 3, 4, 5, 6, 7], grain yield and leaf Δ [1, 5,11, 12] and grain yield and leaf m_a [1, 6, 16].

4.2. Variation of WUE components with water regimes

As observed previously [26], the soil water balance model 'Budget' provided a good prediction of grain yield. The different components of the soil water balance (i.e. infiltration, runoff, drainage, soil evaporation and transpiration) varied markedly with the water regime. The components of WUE calculated from them, showed some differences between water regimes, but not between seasons, except for M/T, which was much more affected by the absence of irrigation in season 1 (driest season) compared with season 2. The component $1 + [(E_s + R + D)/T]$ was much lower under residual moisture than under irrigated conditions in the two seasons. These results suggest that under rain fed conditions T is less reduced than biomass and the other components of WUE. The increase in WUE under residual soil moisture compared with full irrigation, was not explained by the variation of harvest index or M/T and appeared to be largely driven by a reduction in the quotient $1 + [(E_s + R + D)/T]$, that represented only 34% of the value registered under full irrigation. The reduction of $1 + [(E_s + R + D)/T]$ itself was not explained by an increase in T, but rather by a strong decrease in the quantity of water lost by evaporation, runoff and drainage. The quantity of water lost by runoff was estimated by the model to be twenty times less than under full irrigation. Water use efficiency was similar under limited irrigation and full irrigation, because of similar reductions in grain yield and available water. The reduction in harvest index was compensated by an increase in M/T and a decrease of $1 + [(E_s + R + D)/T]$. This decrease was again mainly explained by a decrease of $(E_s + R + D)$ rather than by an increase in T. The component $(E_s + R + D)$ that drives most of the variation in WUE across environments can be reduced mainly by management practices. Runoff can be reduced by zero-tillage [19]. There are several

technologies available to reduce E_s, such as the use of regulated deficit irrigation and partial root zone drying [43]. E_s can also be minimized through improved early vigour and growth of the crop [42]. Any reduction of E_s will make more water available for the plant, thus decreasing E_s/T and increasing overall WUE.

4.3. Relationship between $\Delta^{13}C$, m_a and water use efficiency components

Significant correlations were found across environments between m_aL_a, m_aG_m and T, the simulated quantity of water transpired during the growth cycle. T was also significantly correlated with m_aL_m. Variations in M/T (the transpiration efficiency at the whole plant and growth cycle levels) and HI are mainly driven by genetic factors. In wheat, it has been suggested that the variation in M/T was related to the variation in instantaneous leaf transpiration efficiency (TE) [32]. A negative correlation between Δ and M/T was reported in pot experiments [22]. In the present study, no relationship was found between WUE and HI and M/T. The negative but not significant correlation between WUE and $1 + (E_s + R + D)$ was largely due to a negative relationship between WUE and D. A negative, but not significant correlation was found between WUE and Δ. WUE was significantly and negatively correlated with m_aL_m and m_aG_m. There was, in summary, a negative trend in the relationships between WUE and yield components, Δ and m_a.

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INTERACTIVE EFFECTS OF WATER STRESS AND SALINITY ON ^{13}C ISOTOPE DISCRIMINATION IN RICE, WHEAT AND MAIZE CULTIVARS

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Abstract

Crop yields in salt-affected areas in semi-arid environments are frequently limited by salinity and low moisture availability. Three greenhouse pot experiments were conducted to assess the potential of carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) to select superior wheat, rice and maize varieties in saline and water-scarce environments. Four wheat varieties from Kazakhstan, two upland rice varieties from Sierra Leone and two maize varieties from Austria (ES Beatle, and DK 315) were used. Six treatments included (i) control (water applied at field capacity (FC) throughout the experiment (ii) drought 1 (D1, water applied at 70% of FC at day 8 after thinning (iii) drought 2 (D2, water applied at 30% FC at 50% booting (iv) salt (S, 50 mM or 10 dS m^{-1} applied 8 and 10 days after thinning in 2 splits (v) drought 1 × salt interaction (D1 × S) and (vi) drought 2 × salt (D2 × S), where water stress and salinity were applied at 50% booting for wheat, but at 60 days after thinning for rice and maize. For wheat, D1 and (D1 × S) treatments resulted in a more drastic reduction in dry matter (DM) of spikes than all the other treatments, suggesting that pre-anthesis water stress contributed more to a reduction in DM than salt stress. The combined effect of water and salt stress resulted in less ^{13}C discrimination or low Δ values compared with the control (well-watered and no salt), and the individual water and salt stress treatments. For rice, whereas water stress (D1 and D2) resulted in a decrease in Δ compared with the control (19.5‰ to 18.0‰), the application of salt resulted in higher Δ values compared with the control (19.5‰ to 20.4‰). A negative correlation between shoot DM and Δ was observed for maize. Variations in Δ ranged from 4.84‰ (control) to 5.17‰ (D1) for ES Beatle, and from 5.77‰ (control) to 5.89‰ (D1 × S) for DK 315. High DM was associated with high Δ, suggesting that Δ provides an integrated measure of crop water productivity under water limited conditions. However, this relationship for rice was greatly affected by combined salinity and water stress**,** suggesting that selecting crops for greater agronomic water use efficiency under such conditions is complex, and further study in the field for different crops and soil fertility status is warranted.

1. INTRODUCTION

Water and salt stress are major abiotic constraints contributing to the low productivity of cereals (rice, wheat and maize) in semi-arid and Mediterranean environments. Identifying a related plant trait would be invaluable in selecting and evaluating genotypes tolerant to low moisture availability and high salt concentrations. Plants discriminate against the heavy isotope of carbon (^{13}C) during photosynthesis, and hence a measure of this discrimination in plant tissues can be used as a surrogate of water use efficiency (WUE), the production of biomass per mm of water consumed. The technique commonly referred to as CID, is increasingly being used to evaluate C_3 plants for increased WUE in water-scarce and saline environments.

The correlation between intrinsic water use efficiency (mole C fixed mole^{-1} water transpired) and Δ has been extensively studied in several crops including common bean (*Phaseolus vulgaris* L.) [1], wheat (*Triticum aestivum* L.) [2, 3, 4], peanut (*Arachis hypogaea* L.) [5, 6, 7, 8], barley (*Hordeum vulgare* L.) [9] and cowpea (*Vigna unguiculata* L. Walp.) [10]. These studies suggest that genetic variation in Δ may be sufficient to be useful as a selection criterion for improved WUE. Water use efficiency may be estimated from measurements of dry matter (DM) accumulation over time relative to the amount of water transpired (transpiration efficiency, TE) or by measurements of gas exchange (instantaneous water use efficiency, WUEi).

The use of Δ as a potential tool to evaluate the impact of water and salt stress on rice and other crops has received much attention. A reduction in $\Delta^{13}C$ as salinity increases suggests that salinity induces a greater degree of stomatal resistance that provides less opportunity for discrimination against ^{13}C, indicating that CID could be used as a selection criterion in breeding efforts to develop salt tolerant crops [11]. $\Delta^{13}C$ of plant DM integrates transpiration, the ratio of net photosynthesis to water transpired, over a period during which the DM is assimilated. Under saline conditions it has been hypothesized that high Δ values (maximum discrimination) are positively correlated with increased salt tolerance in rice varieties [12]. Poss et al. 2004 [12] concluded that high Δ values in leaves of salt stressed rice appear to be influenced by the increased contribution of respired C to the total C budget of rice leaves.

Although the relationship between CID, water and/or salt stress have been well studied and documented for C_3 plants, few studies have looked at the interactive effects of salt and water stress in C_3 and C_4 plants. The objective of the present investigation was to assess the potential of Δ to quantify the interactive effects of water and salt stress in two rice (C_3), four wheat (C_3) and two maize (C_4) varieties.

2. MATERIALS AND METHODS

Three pot experiments were conducted in a glasshouse at the International Atomic Energy Agency's Laboratories, Seibersdorf, Austria, using wheat, rice and maize varieties. The soil used was a Typic Eutrocrepts with a clay loam texture. Plastic pots each filled with 4 kg of a mixture of air dried, sieved soil (<2 mm) and sand (1: 1 ratio) were placed in a naturally lit glasshouse with a day/night temperature regime of 34/21 ^{o}C and a relative humidity of 40–75%. Some of the physical characteristics of the soil were particle size distribution (in g kg^{-1}soil, 130 clay, 156 silt and 714 sand), permanent wilting point 70.8% (volume), bulk density (1.6 g cm^{-3}). The soil pH (KCl) was 7.50 and organic and total C were 7.91 and 17.5 g kg^{-1} soil, respectively. The soil had a total N concentration of 0.60 g kg^{-1}, total P of 906 mg kg^{-1}, available P of 233 mg kg^{-1} (Bray 2) and 26.1 g kg^{-1} (Olsen P), electrical

conductivity (EC) at 25 ^{o}C of 130 μS cm^{-1}, and a cation exchange capacity (CEC) as measured by the cobalt hexamine method of 17.5 cmol (+) kg^{-1}. δ^{13}C of the soil was −25.7‰.

Six treatments including (i) control (C, water applied at field capacity throughout the experiment (ii) drought 1 (D1, water applied at 70% FC 8 d after thinning) (iii) drought 2 (D2, water applied at 30% FC at 50% booting) (iv) salt (S, 50 mM or 10 dS m^{-1} applied 8 and 10 days after thinning, in 2 splits to prevent shock to the plants) (v) drought 1 × salt interaction (D1 × S) and (vi) drought 2 × salt (D2 × S, water stress and salt applied at 50% booting for wheat and at 60 days after thinning for rice and maize). Plants were thinned five days after emergence. The watering of rice and wheat plants was on a weight basis, with the daily supply of water equal to the evapo-transpiration loss, maintaining as much as possible at constant soil water content. Soil moisture in each pot in the maize experiment was monitored by time domain reflectometry (TDR), and water was supplied according to the growth of the plants and the TDR readings.

Four spring wheat varieties of varying drought tolerance obtained from Kazakhstan (Saratovskya-29, Severyanka, Stepnaya-15 and Otan-1), two upland rice varieties (WAB 5650 and ROK 3) from Sierra Leone, and two maize varieties from Austria (ES Beatle and DK 315) were arranged in a randomized complete block design with 3 replications. There were 72 pots for wheat and 36 each for rice and maize. Each pot contained three plants and received fertilizer equivalent to 100 kg N/ha^{-1} ($^{15}NH_4NO_3$ at 2 atom % ^{15}N excess) in two split applications, 40 kg P ha^{-1} (as triple superphosphate) and 50 kg K ha^{-1} (muriate of potash). Plants were harvested at 85 days after sowing (DAS) at physiological maturity for wheat, 80 DAS for rice (late vegetative growth stage) and 70 DAS for maize (late vegetative growth stage). The rice varieties did not flower.

At harvest, plants were separated into spikes (if any), shoots and roots, oven dried at 70 ^{o}C to a constant weight, weighed and then finely ground, and portions of the ground samples were analyzed for δ^{13}C, C, N and atom % ^{15}N using a Europa Scientific ANCA 20-20/GSL isotope ratio mass spectrometer, with a precision in δ^{13}C of ± 0.15‰. δ^{13}C was determined according to Eq. 1 using Vienna PDB as the standard.

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R = ^{13}C/^{12}C. The δ^{13}C composition of the plant material is related to ^{13}C isotope discrimination (Δ) according to Eq. 2.

$$\Delta^{13}C\ (‰) = [(\delta^{13}C_{air} - \delta^{13}C_{plant})/(1 + \delta^{13}C_{plant})] \times 1000 \quad (2)$$

where $\delta^{13}C_{air} = -8‰$ and $\delta^{13}C_{plant}$ is the measured value of the plant material.

3. RESULTS

3.1. Combined effects of water and salt stress on wheat, rice and maize biomass

3.1.1. Wheat

Drought and salt treatments and their interactive effects caused a decrease in dry matter (DM) of spikes and shoots in wheat (Fig. 1). The water stress treatment applied at the initial growth stage (D1) and the combined effect of early water and salt stress (D1 × S) resulted in a more drastic reduction in DM of spikes than all the other treatments (Fig. 1). The % reduction in DM compared with the control was less in the combined early water stress and salt (D1 × S) than the salt treatment without water stress (S) and water stress applied at anthesis (D2). The % reduction in DM of spikes by the treatments compared to the control was most severe in Otan-1 and less severe in Saratovskay-29.

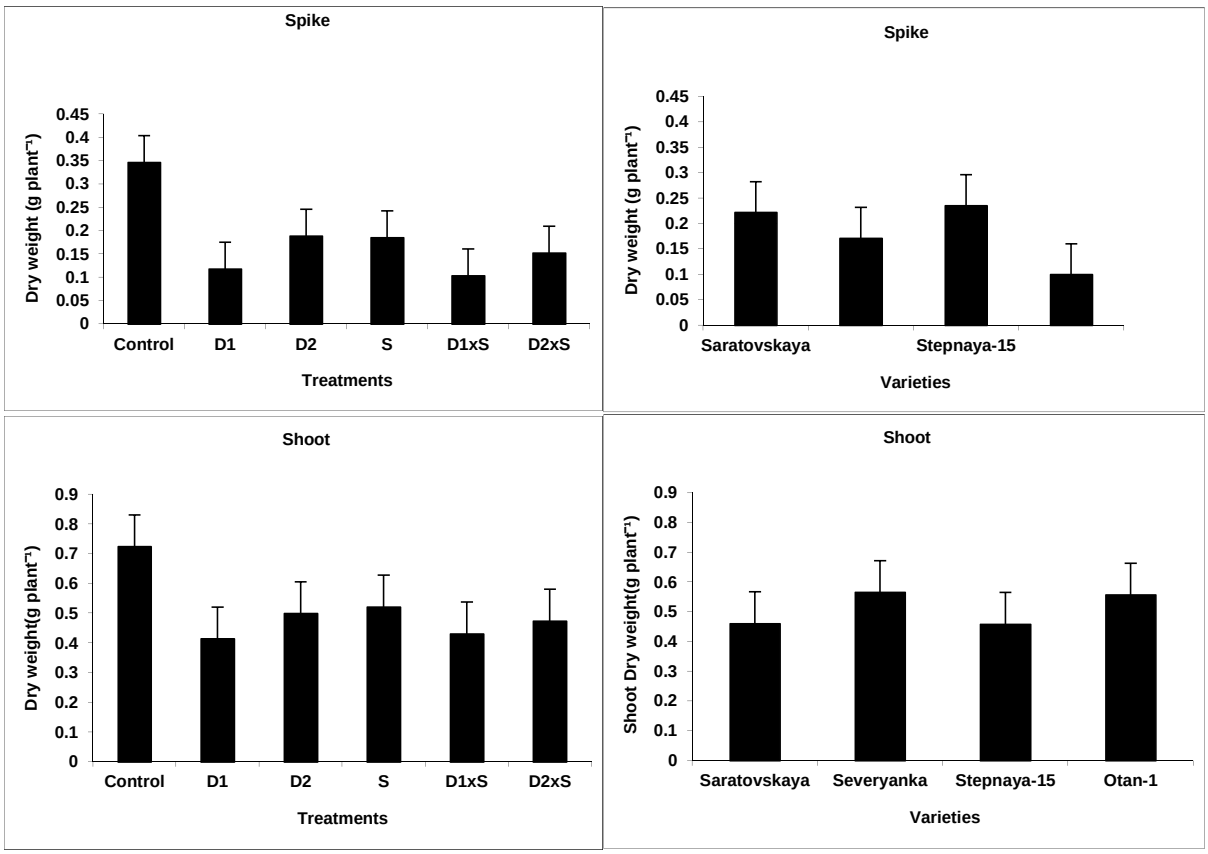

FIG. 1. Mean DM of shoots and spikes of four wheat varieties under water and salinity stress.

3.1.2. Rice

There was a more drastic reduction in shoot DM in the salt treatments (S, D1 × S and D2 × S) than in the water stress treatments (D1 and D2) (Fig. 2). The relative % shoot biomass compared with the control (100) were 56% (D1), 60% (D2), 40% (S), 20% (D1 × S) and 28% (D2 × S), indicating that the rice varieties used were more susceptible to salt stress than to drought stress. A combination of water and salt stress had a more severe effect on the shoot than the other treatments (Fig. 2). Shoot and root DM yields of WAB 5650 were slightly higher than those for ROK 3 in the control and water stress treatments, but slightly lower in the salt treatments.

3.1.3. Maize

The water stress treatments resulted in a greater reduction in shoot DM than the salt stress treatment (Fig. 3). The shoot weights (g plant^{-1}) of the two varieties were 8.95(C), 5.0 (D1), 7.05 (D2), 6.55 (S), 3.55 (D1 × S) and 6.70 (D2 × S), suggesting that the effect of the early water stress treatment had a more severe effect that the salt treatment. The % shoot weights compared with the control (100%) of the two varieties were 21% (D1) and 10% (D1 × S), and were less than that in leaves of 56% (D1) and 40% (D1 × S). The variety ES Beatle had significantly higher total DM under salt stress (S) and a combined salt and water stress (D2 × S) than DK 315 (Fig. 3).

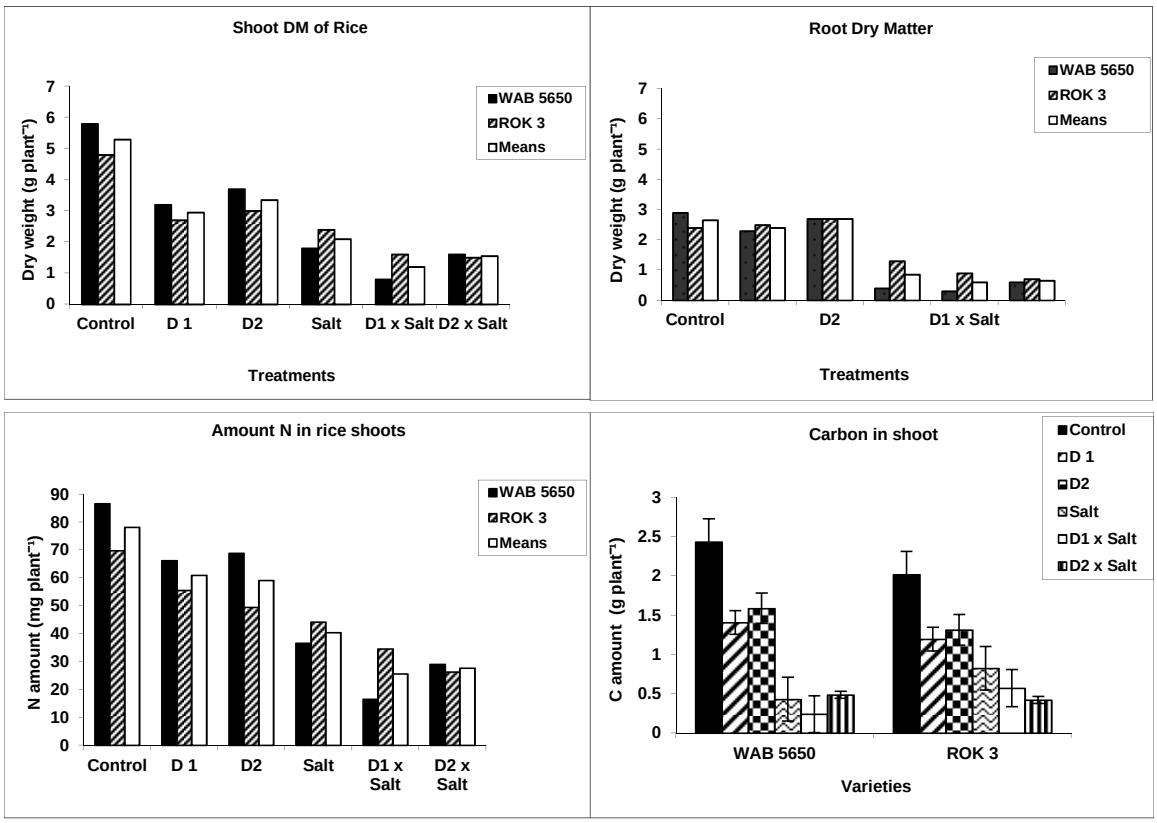

FIG. 2. Shoot and root DM and amounts of N and C in two rice varieties under water and salinity stress.

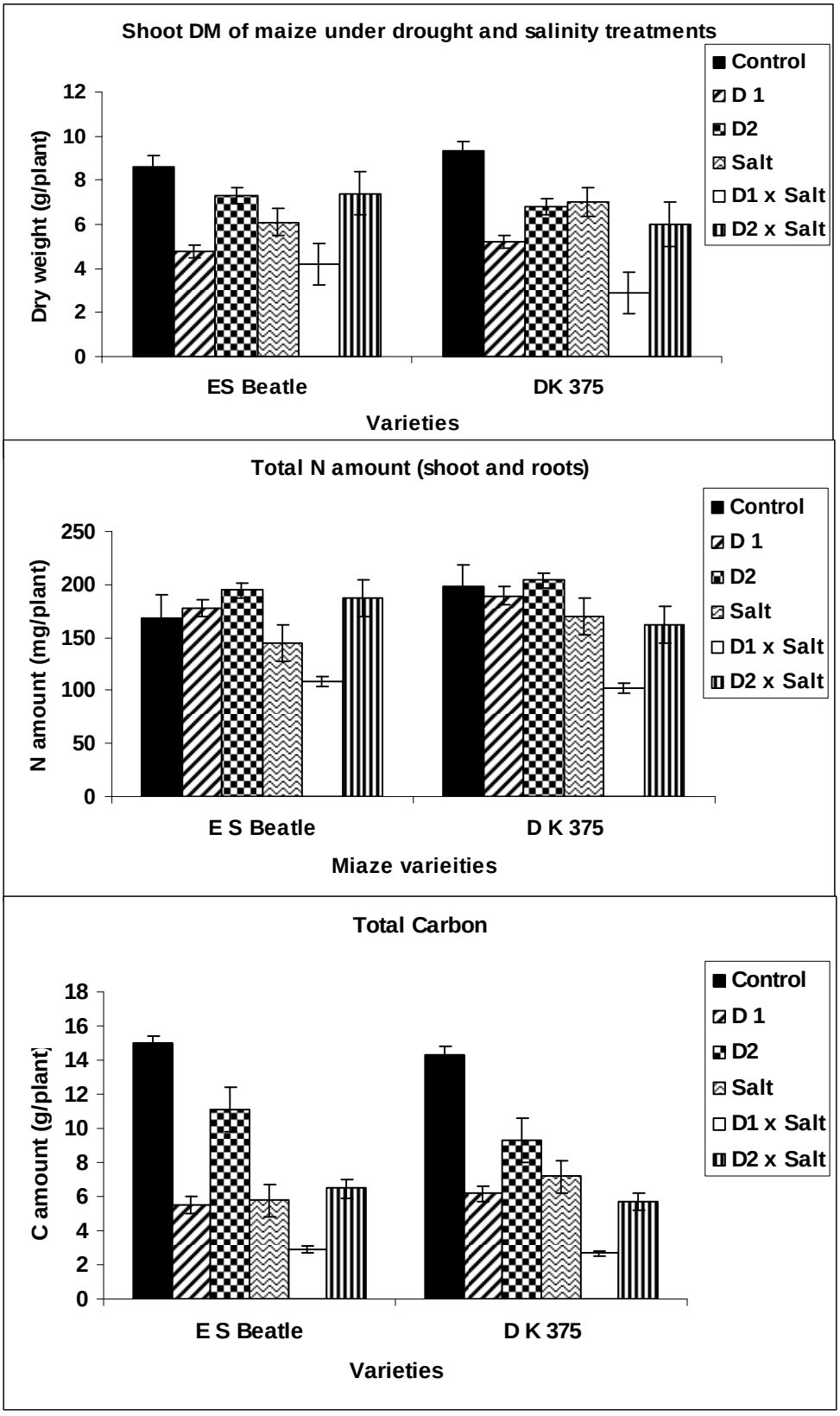

FIG. 3. Shoot DM and total amounts of N and C in two maize varieties under water and salinity stress.

3.2. Combined effects of water and salt stress on CID

Significant variations in mean Δ values due to the treatments were observed for wheat varieties ranging from 21.3 ‰ for the control to 17.6 ‰ for the D1 x S treatment (Fig. 4). A significant reduction in Δ values in wheat compared with the control occurred in all treatments. Water stress during early crop growth had a more severe penalty on the DM and Δ in wheat than the effect of salt (Fig. 4). Among the four varieties tested Severyanka had the highest Δ value. For rice, whereas water stress resulted in a decrease in Δ values compared with the control (19.5 ‰ to 18.0 ‰), salt stress resulted in higher Δ values compared with the control (19.5 ‰ to 20.4 ‰) (Fig. 4). The CID values in shoots were higher for the WARDA variety WAB 5850 than the local variety ROK 3.

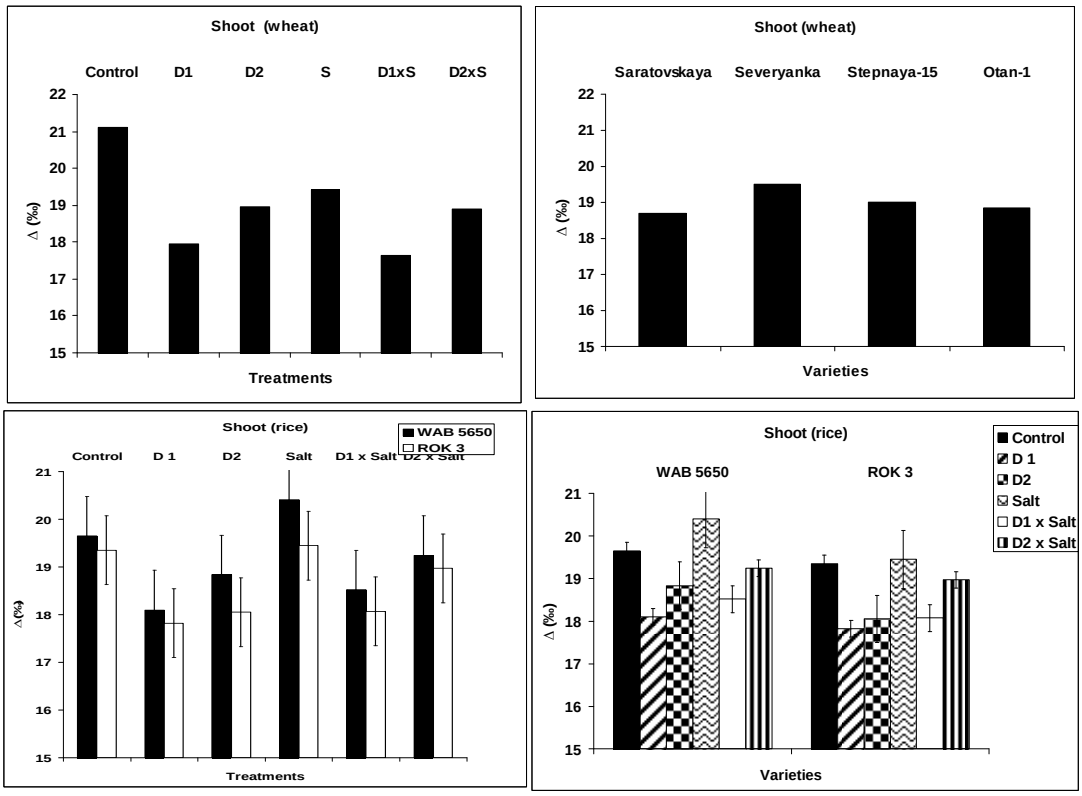

FIG. 4. CID of shoots of four wheat and two rice varieties under water and salinity stress.

The Δ for maize increased in all the treatments compared with the control and this trend was reversed in wheat (Fig. 5). Variations in Δ ranged from 4.84 ‰ (control) and 5.17 ‰ (D1) for ES Beatle, and 5.77 ‰ (control) and 5.89 ‰ (D1 x S) for DK 315. The highest Δ was recorded for the early water stress treatment (D1) and (D1 x S), suggesting a negative correlation between DM and Δ for maize irrespective of the treatments. No significant differences in Δ between the leaves and shoots and the varieties were observed.

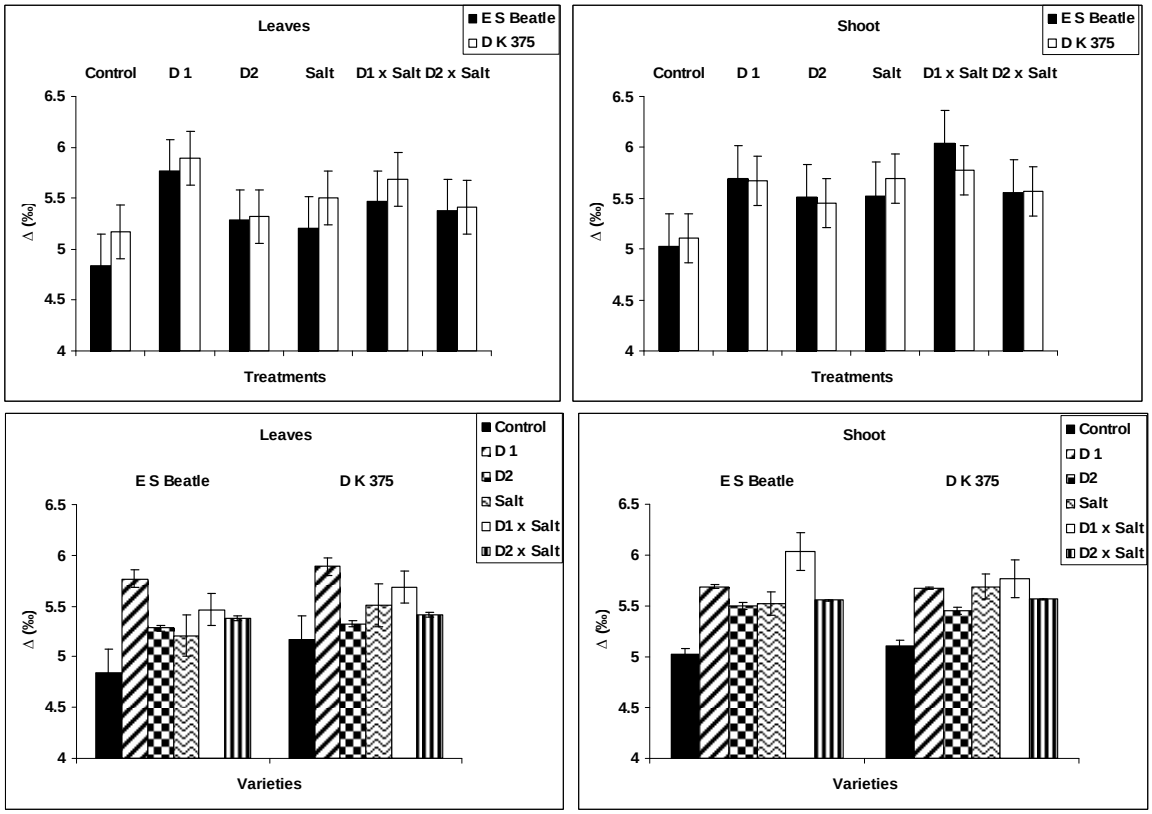

FIG. 5. CID of leaves and shoots of two maize varieties under water and salinity stress.

3.3. C and N accumulation in plants

The amounts of total N (mg plant^{-1}) followed a similar trend to that of the DM, with mean values in wheat being 19.1 (C), 13.1 (D1), 15.8 (D2), 13.7 (S) 12.7 (D1 x S) and 13.2 (D2 x S). Among the four varieties tested, Otan-1 had the highest amount of N in shoots (Fig. 6).

The percentage of N derived from fertilizer (%Ndff) was highest in shoots and lowest in roots (Fig. 7). The water stress treatments had a relatively lower %Ndff than the salt stress treatments. For rice, there was a significant reduction in the mean value of N of the two varieties, from 79 mg N in the control to 26.0 mg N in the D1 x S treatment (Fig. 2). The amounts of N in rice were not significantly different in the water stress and salt treatments. The N accumulation in maize was less in the water stress than the salt stress treatments. The water and salt treatments resulted in a decrease in the amount of C in plant parts compared with the control (Fig. 2 and Fig. 3).

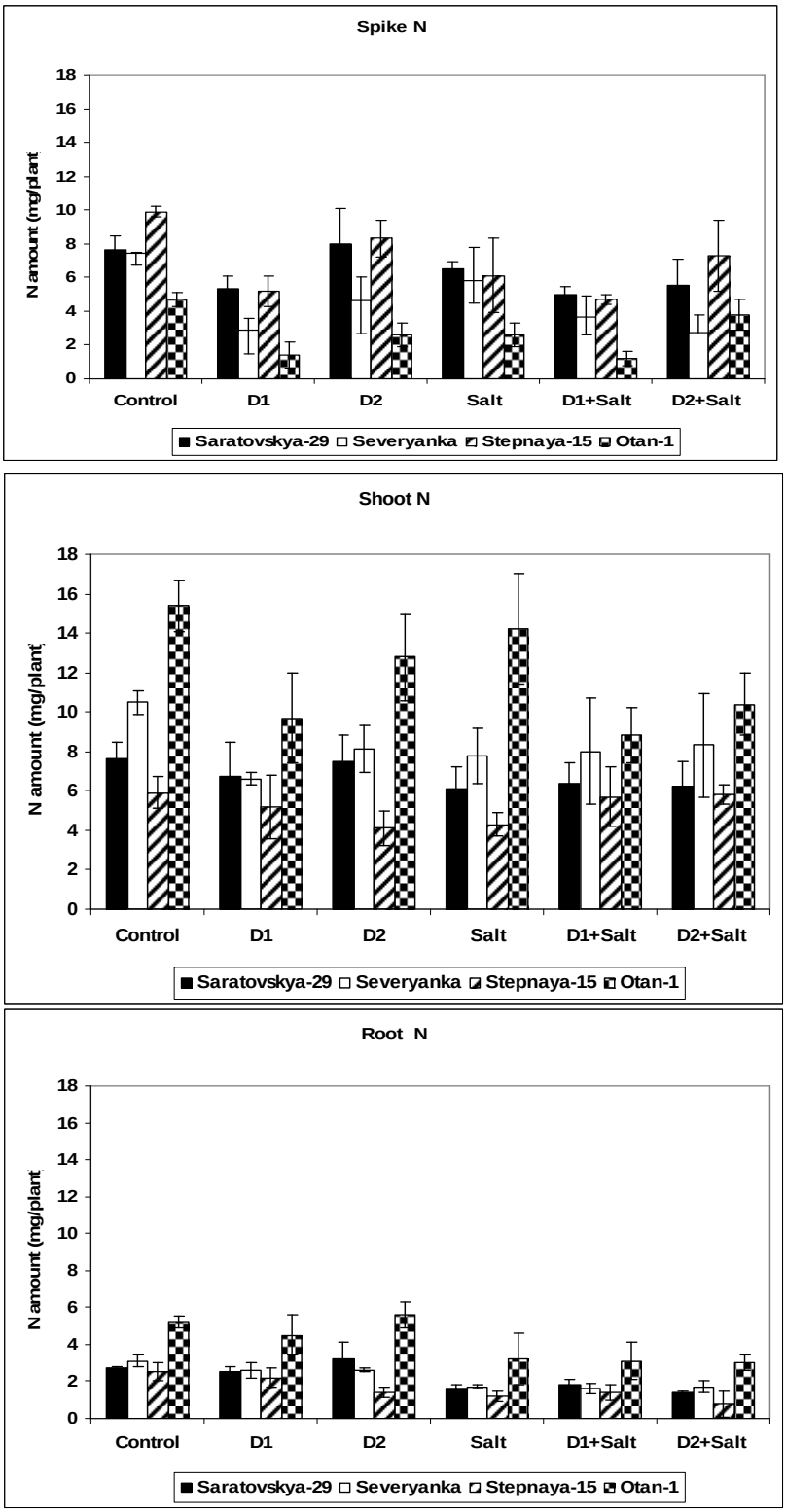

FIG. 6. Amounts of N in spikes, shoots and roots of four wheat varieties under water and salinity stress.

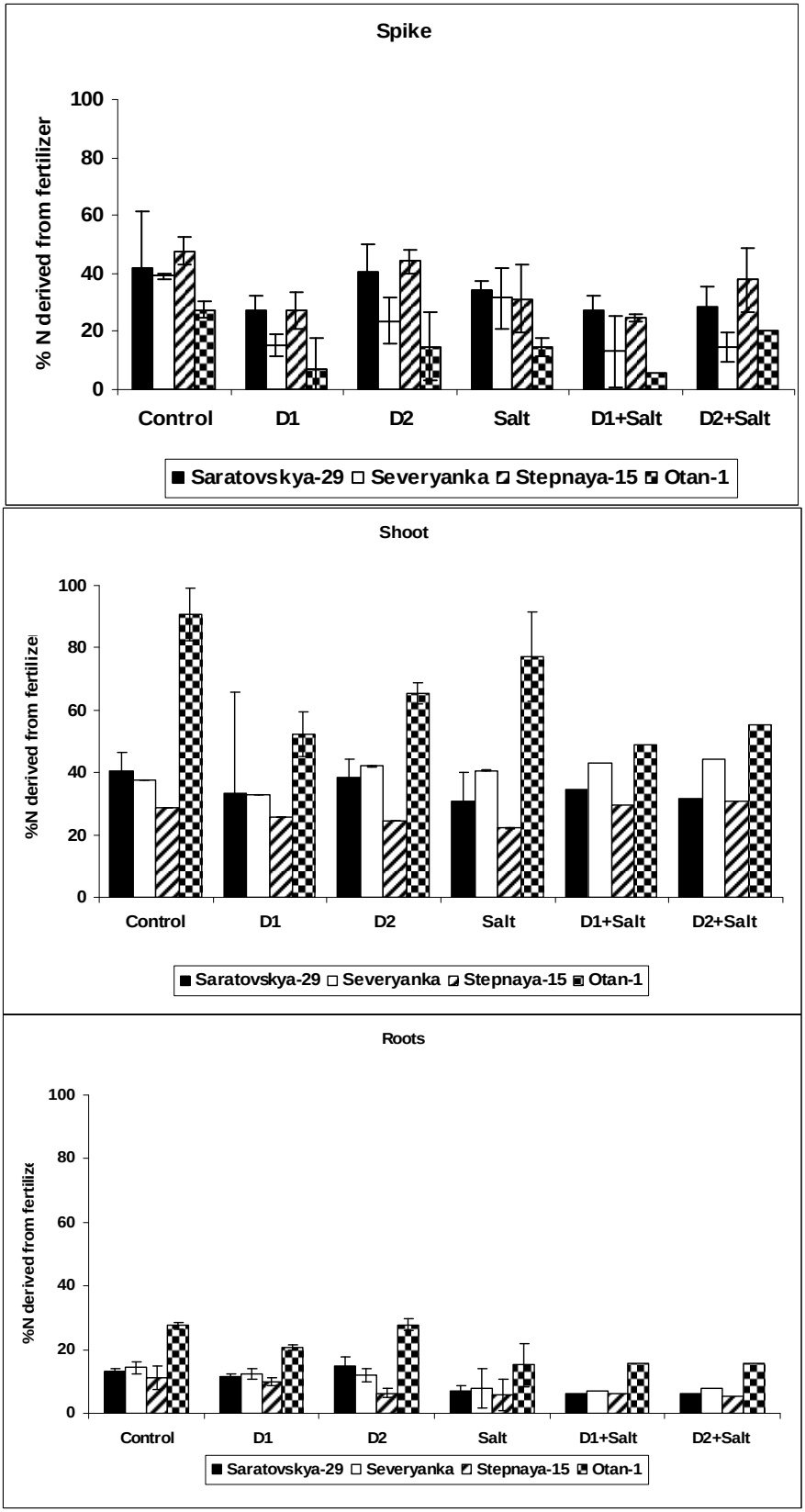

FIG. 7. %Ndff in spikes, shoots and roots of four wheat varieties under water and salinity stress.

4. DISCUSSION

4.1. Relationships between yield and Δ under water and salt stress

Crops grown in salt-affected areas are frequently limited not only by high salt content but also low moisture availability. For wheat, the water stress treatment applied at the initial growth stage (D1) and the combined effect of early water and salt stress (D1 × S) resulted in a more drastic reduction in DM of spikes (data not shown) than all the other treatments, suggesting that the pre-anthesis water stress contributed more to a reduction in DM than the salt stress treatment. Our results showed that the combined effect of water and salt stress resulted in less ^{13}C discrimination or lower Δ values in comparison with the control (well-watered and no salt treatment) and the individual water and salt stress treatments. Similar results were reported by Shaheen and Hood-Nowortny [13] for wheat. Lower Δ was observed for the early water stress treatment compared with the water stress after flowering in all four wheat cultivars.

Cultivar differences in the mean Δ (average Δ values of the six treatments) were observed with Saratovskay-29 having a higher discrimination of ^{13}C compared with the other three varieties. High DM was associated with high Δ (Fig. 1 and Fig. 4) suggesting a positive correlation between shoot DM and Δ as observed by Araus et al. [14] and Monneveux et al. [15] in durum wheat, although Hafsi et al. [16] reported a lack of correlation between grain yield and grain Δ**.** It is known that severe drought conditions that affect flowering and grain formation can drastically reduce sink size and break the relationship between stomatal conductance (and Δ) and grain yield. Alternatively, under well-watered conditions, stomatal conductance is likely to be high resulting in an increased ratio of the mole fraction of intercellular to ambient CO_2 (C_i/C_a) and Δ values, while increased photosynthetic capacity potentially decreases C_i/C_a. The decrease in C_i/C_a associated with increased photosynthetic capacity is consequently offset by the increase in C_i/C_a resulting from opening of stomatal apertures, hence reducing the possibility of a relationship between Δ and grain yield [3].

In wheat, salt stress caused a lower reduction of yield (shoots and spikes) and Δ compared with the effect of water stress. This could be due to a lower increase in the concentration of salt in the soil solution and/or cumulative effects, suggesting that salt stress not only affects stomatal conductance but also photosynthetic capacity [17]. Salt tolerant varieties are able to maintain high transpiration losses (and thus high Δ) under well-watered conditions. They are therefore more efficient in DM partitioning to the grain which could explain the positive correlation between DM and Δ in wheat. Generally, there was less ^{13}C discrimination in spikes compared with shoots and leaves.

For rice, since the two cultivars did not flower (probably because they are photosensitive), the D2 and D2 × S treatments were applied at 60 DAS. Low DM was associated with high Δ, and Δ increased with salt stress while it decreased with water stress (Fig. 3). Contrary to wheat, salt stress induced a stronger reduction in DM, but an increase in Δ in rice. However, cultivar differences in shoot yields were associated with Δ. Thus WAB 5650 had a slightly higher DM and Δ compared with ROK 3. The type of stress had varying effects on the performance of the cultivars.

For C_4 maize, a negative relationship between shoot DM and Δ was contrary to the results for wheat. The water and salt stress treatments resulted in higher Δ values in both the leaves and shoots than the control (Fig. 4). In any case, the Δ values could not be used to explain the level of tolerance of the cultivars to the treatments since the differences were not

significant. There are extremes where positive relationships between grain CID and grain yield will not hold. e.g. weather conditions that are too dry, too cold or too wet [18].

4.2. N and C accumulation in plant parts

The water stress at the early growth stage (D1) and its combined effect with salt (D1 × S) resulted in less accumulation of N and C in spikes and in shoots than the salt treatments (Fig. 2 and Fig. 6). However, the salt treatments resulted in a significant reduction in the amounts of N and C in roots compared with spikes and shoots. The salt treatment caused a lower root: shoot ratio than the water stress, indicating that under salt stress the wheat allocated more C and N into shoots and less into roots, in contrast to water stress and the control (well-watered and no salt applied). Mineral accumulation in vegetative plant parts and in mature kernels has been proposed as an additional or surrogate indirect selection criterion for Δ, to predict grain yield of temperate cereals in Mediterranean areas.

5. CONCLUSIONS

The combined effect of water and salt stress resulted in less ^{13}C discrimination or low Δ values compared with the control (well-watered and no salt), and the individual water and salt stress treatments. For rice, whereas water stress (D1 and D2) resulted in a decrease in Δ compared with the control (19.5‰ to 18.0‰), the application of salt resulted in higher Δ values compared with the control (19.5‰ to 20.4‰). A negative correlation between shoot DM and Δ was observed for maize. High DM was associated with high Δ, suggesting that Δ provides an integrated measure of crop water productivity under water limited conditions. Our results indicate that selecting crops for greater agronomic water use efficiency under the combined effect of water and salt stress conditions is complex, and further study in the field for different crops and soil fertility status is warranted.

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EFFECT OF N AND P AVAILABILITY ON ^{13}C ISOTOPE DISCRIMINATION IN WHEAT AND RICE

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Abstract

Nutrient deficiencies (particularly N and P) contribute to low yields of wheat and rice in drought-prone (semi-arid and arid) environments. Although carbon isotope discrimination (CID, $\Delta^{13}C$ or Δ) has been proposed as a useful trait for selecting C_3 cereal crops in drought and salinity stressed environments, the effect of plant nutrition on the efficacy of using Δ as a selection tool for high yield in nutrient-stressed environments is unknown. Two greenhouse pot experiments were conducted to assess the effect of N and P supply and their combined effects on Δ in shoots (ΔSh) and in spikes (ΔSp) of wheat grown under well-watered conditions, and of rice grown under saline and non-saline conditions. ΔSp and ΔSh of wheat significantly decreased (P<0.001) with P fertilization and the effect was more pronounced in spikes (20.63‰ at P0 to 19.36‰ at P20) than in shoots (21.70‰ at P0 to 20.80‰ at P40). No significant differences in Δ were observed among the wheat varieties tested. For rice, P supply significantly reduced the value of Δ in the control treatment, but the effect was not seen in the saline treatment. Our results showed that both N and P significantly affected Δ in well-watered wheat, suggesting that soil nutrient status must be considered in future research on the relationship between yield and CID of C_3 cereals.

1. INTRODUCTION

The ^{13}C isotope discrimination technique has become a powerful research tool to identify and select C_3 and C_4 species (rice, wheat, and maize) with improved water use efficiency (WUE), i.e. the ratio of the biomass produced to the water consumed. Farquhar et al. [1] proposed that for C_3 plants Δ is related to a diffusional fraction (a = 4.4‰), discrimination against $^{13}CO_2$ by ribulose diphosphate carbolylase or Rubisco (b = 30‰) and p_i/p_a, the ratio of the intercellular to the ambient partial pressure of CO_2, and can be expressed as Eq. 1.

$$\Delta C_3 = a + (b - a)\, p_i/p_a \quad (1)$$

Similarly for C_4 plants the photosynthesis-induced Δ is described by Eq. 2.

$$\Delta C_4 = a + (b_4 + \varphi\, b_3 - a)\, p_i/p_a \qquad (2)$$

where a is the ^{13}C discrimination due to CO_2 diffusion in air (4.4‰), b_4 is the fraction of the dissolution of CO_2 to HCO_3^- and fixation by phosphoenolpyruvate or PEP (–5.7‰ at 30 ^oC), b_3 is the ^{13}C discrimination due to Rubisco (30‰), and φ is the fraction of CO_2 fixed by PEP carboxylase.

The two equations suggest that factors other than water such as phosphorus (P) and nitrogen (N) that play a major role in photosynthesis could hamper the use of CID as an effective tool to select crops with improved WUE. In most water limited environments nutrients (particularly N and P) are also limiting, and the Δ values under these conditions may be affected by the availability of these elements. Shangguan et al. [2] reported that Δ of wheat was reduced by increased drought intensity and increased N deficiency. It was also demonstrated that WUE was negatively correlated with Δ so WUE increased with decreasing N supply and increasing water stress. Yin and Raven [3] reported that variations in N source resulted in small but significant variations in CID in wheat and maize. Dercon et al. [4] observed that Δ values decreased with increasing water stress but increased with increasing N availability, and that higher N supply favoured CID because diffusion of air from outside to inside the leaf is not sufficiently fast to keep up with the CO_2 demand generated by increased N availability. Maranville and Madhavan [5] observed that under N stress conditions when water is non-limiting, the stomata remain open and little or no restriction to atmospheric CO_2 occurs. Therefore the increase in Δ with N stress resides in the differential fixation of CO_2 by PEPcase and Rubisco which reside in different physical proximities within the bundle sheet/mesophyll area. Pansak et al. [6] observed that the relationship between available NO_3^- and Δ values suggest that differences in CID were more related to availability of N than to the differences in water availability. Tsialtas et al. [7] reported that addition of urea as the N source had inconsistent effects on $\Delta^{13}C$.

Although CID has been proposed as tool in breeding programmes for improving the performance of wheat and rice in water limited and saline environments because it provides an integrative assessment of genotypic variation in leaf transpiration efficiency, there are inconsistent reports on the effects of N and P on the relationships between CID and yield of wheat and rice. The agricultural ecosystems in developing countries where rice, maize and wheat are produced are frequently limited by salinity, lack of soil moisture and low soil N and P availability. If $\Delta^{13}C$ can be shown to reliably predict salinity tolerance in upland rice under low availability of N and P, it would provide a useful tool for rapid evaluation of upland rice germplasm for salt tolerance in this ecosystem.

Leaf-level water use efficiency generally increases in response to increasing leaf N concentration in C_3 plants [8]. This is because more leaf N allows greater photosynthesis at a given rate of water loss. Variation in plant N status could have important implications for the coupling between C and water exchange [9]. Clay et al. [10] and Zhao et al. [11] reported that both water and N significantly affected the relationships between yield and CID of spring wheat, and that environmental conditions such as water and fertilizer must thus be considered in future research on the relationship between yield and CID of spring wheat.

A wealth of work has been published showing a strong negative relationship between $\Delta^{13}C$ and salinity tolerance by crops, but the combined effect of N and P levels on the relationship is not well understood. Although Edbon et al. [12] and Tsialtas et al. [7] reported that P had no significant effect on $\Delta^{13}C$, but caused a strong depletion of $\delta^{15}N$, Brück et al.

[13] reported decreased $\Delta^{13}C$ when P was applied on pearl millet. The carbon costs associated with N and P acquisition by crops [14] and its effects on crop biomass and yield suggest that $\Delta^{13}C$ may be affected by a combination of N and P stress. The purpose of this study was to assess the effect of N and P supply and their interactive effects on CID under well-watered (wheat) and saline (rice) conditions.

2. MATERIALS AND METHODS

Two pot experiments were conducted in a glasshouse at the International Atomic Energy Agency's Laboratories at Seibersdorf, Austria, to assess the interactive effect of N and P supply on $\Delta^{13}C$ discrimination in wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) under well-watered and saline conditions, respectively.

An acid soil from Hungary, classified as a Dystric Eutrocrepts, was air dried and sieved to pass a 2-mm screen. The soil was a loamy sand with 82, 88 and 830 g kg^{-1} clay, silt and sand, respectively. The soil pH (KCl) was 5.4 and contained 7.91 g organic C kg^{-1}. The total N was 0.83 g kg^{-1}, total P 233 mg kg^{-1}, available P 6.5 mg kg^{-1} (Bray 2) and 7.8 g kg^{-1} (Olsen). The cation exchange capacity as measured by the cobalt hexamine method was 2.66 cmol (+) kg^{-1} and $\delta^{13}C$ was −25.7‰ ($\Delta^{13}C$ = 17.26‰). Plastic pots containing 1 kg of soil-sand mixture (1: 1 ratio) were placed in a naturally lit glasshouse with a day/night temperature regime of 34/21°C and a relative humidity of 40–75%.

2.1. ^{13}C isotope discrimination of wheat varieties at varying P supply

The experiment was a factorial design with four P levels (0, 20, 40 and 60 kg P ha^{-1}), four wheat varieties and three replications. The wheat varieties obtained from Kazakhstan (Saratovskya-29, Severyanka, Stepnaya-15 and Otan-1) had varying tolerance to drought. The pots received a basal fertilizer application equivalent to 100 kg N/ha^{-1} ($^{15}NH_4NO_3$ at 2 atom % ^{15}N excess) and 50 kg K/ha^{-1}. Six seeds were planted in each pot and were thinned to three per pot and grown till physiological maturity. Soil water content was monitored by time domain reflectometry (TDR) and was maintained at 70% of field capacity. Plants were/harvested at physiological maturity, separated into spikes (if any), shoots and roots, oven dried at 70 °C to constant weight, weighed and ground. A portion of the ground samples was used for analysis of C, N, $\delta^{13}C$ and atom % ^{15}N concentrations using an isotope ratio mass spectrometer (Europa Scientific ANCA 20-20/GSL) with a precision of 0.15‰.

2.2. ^{13}C isotope discrimination of rice at varying N and P supply and two salinity levels

The treatments were four P levels (0, 20, 40 and 60 kg P ha^{-1}), three N levels (0, 50 and 100 kg N/ha^{-1}) and two salt treatments (1 and 10 dS m^{-1}), arranged in a split-split plot design with three replicates. The salt treatments w
plots and the P treatments the sub-sub plots. N was applied as $^{15}(NH_4)_2SO_4$ at 5 atom % ^{15}N excess in 2 splits, and P as triple superphosphate. Each pot received a basal application of KCl at 60 kg K ha^{-1}. Five upland rice seeds (cv. TDS 5 from Vietnam) were sown pot^{-1} and were thinned to three plants, 7 days after germination. All the P and the first split of ^{15}N were applied at planting. The second split of N was applied at 30 days after sowing (DAS).

Each pot was initially supplied with 25 mL of water per day but was progressively increased to 50 mL and to 100 mL depending on the growth stage and water uptake by the

plants as monitored by the TDR. At 65 DAS, 36 of the pots were supplied with approximately 100 –150 mL of 10 dS m^{-1} NaCl solution (salinity treatment) and grown till final harvest at 106 DAS. At sampling, the above ground biomass was cut, chopped into small pieces and oven dried at 70 ^{o}C to constant weight. Roots were thoroughly washed with tap water and then with distilled water, and oven dried. The above- and below ground biomass were weighed, ground and analyzed for C, N, $\delta^{13}C$ and atom % ^{15}N concentrations with an isotope ratio mass spectrometer (VG Instruments Isoprime). The CO_2 concentration in the glasshouse during the entire experimental period varied from 400 to 480 micromoles $mole^{-1}$.

The $\delta^{13}C$ concentration of the plant sample was calculated from the isotope ratio measurements ($R = {}^{13}C/{}^{12}C$) of the sample and a standard by Eq. 3.

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (3)$$

where the secondary standard was Vienna PDB.

The ^{13}C isotope discrimination of the plant sample was calculated from $\delta^{13}C$ measurements by Eq. 4.

$$\Delta^{13}C\ (‰) = [(\delta^{13}C_{air} - \delta^{13}C_{plant})/(1 + \delta^{13}C_{plant})] \times 1000 \quad (4)$$

where $\delta^{13}C_{air}$ is the $\delta^{13}C$ value of atmospheric CO_2 (–8‰) and the $\delta^{13}C_{plant}$ is the measured value of the plant material.

Statistical analysis was carried out using ANOVA.

3. RESULTS

3.1. Wheat

3.1.1. Phosphorus supply and biomass production

Dry matter (DM) yield of the spikes and shoots increased with P supply in all the wheat varieties with the maximum yield recorded at P 40, although the increase in above ground DM did not differ much between P20 and P40 (Fig. 1). The mean % increase over the control (P0) in DM of spikes was 247% for P10, 325% for P20 and 302% for P40. Phosphorus supply resulted in an increase in root DM from 0.09 g at P0 to 0.230 g $plant^{-1}$ at P40. Shoot and root DM were highest in Otan-1 and increased steadily with increasing P supply, but this was not reflected in the spike weight (Fig. 2).

3.1.2. Phosphorus supply and $\Delta^{13}C$

The $\Delta^{13}C$ values in spikes (ΔSp) and in shoots (ΔSh) significantly decreased ($P<0.001$) with P fertilization and the effect was more pronounced in spikes (20.63‰ at P0 to 19.36‰ at P20) than in shoots (21.70‰ at P0 to 20.80‰ at P40). No significant differences in Δ were observed among the wheat varieties tested, although ΔSh was higher than ΔSp (Fig. 3 and Fig. 4). High Δ was associated with low DM yield (Fig. 2 and Fig. 4) suggesting that increased P supply resulted in decreased Δ values.

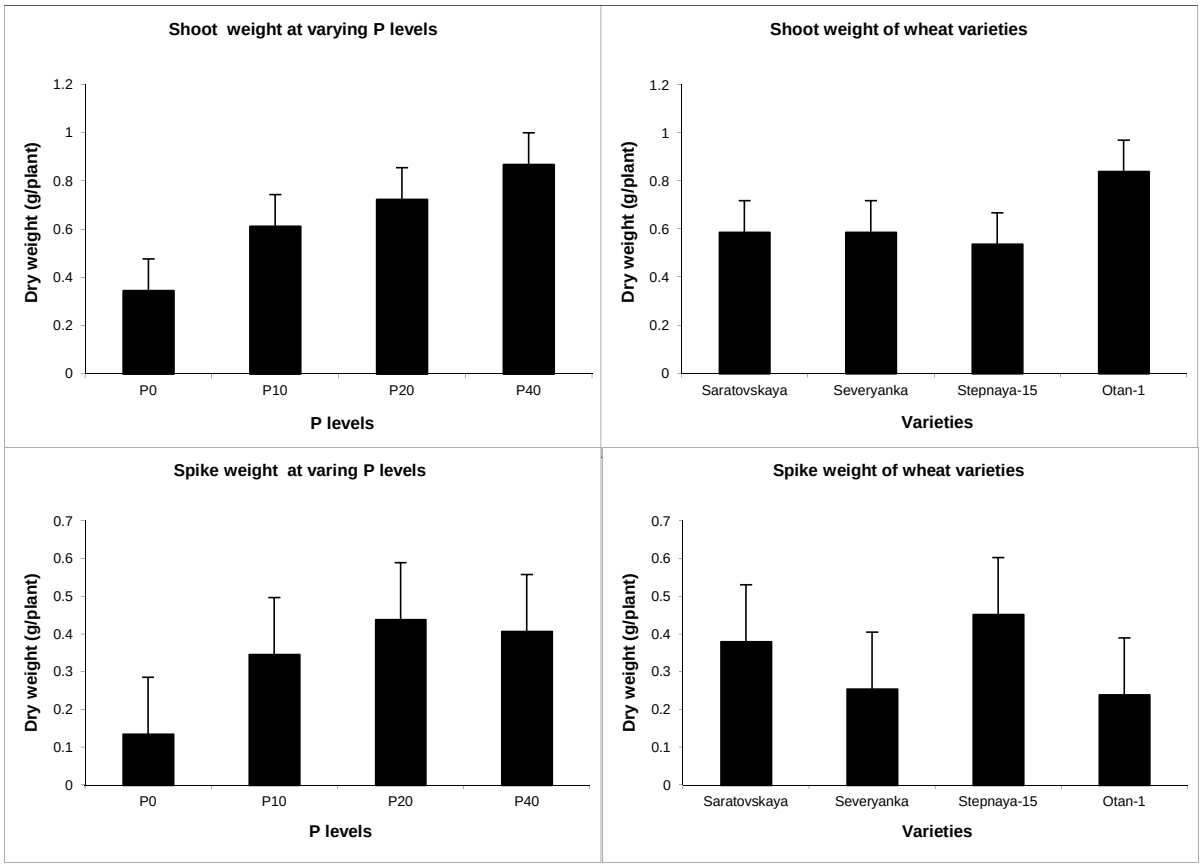

FIG. 1. Mean dry weight of shoots and spikes of four wheat varieties at four phosphorus levels.

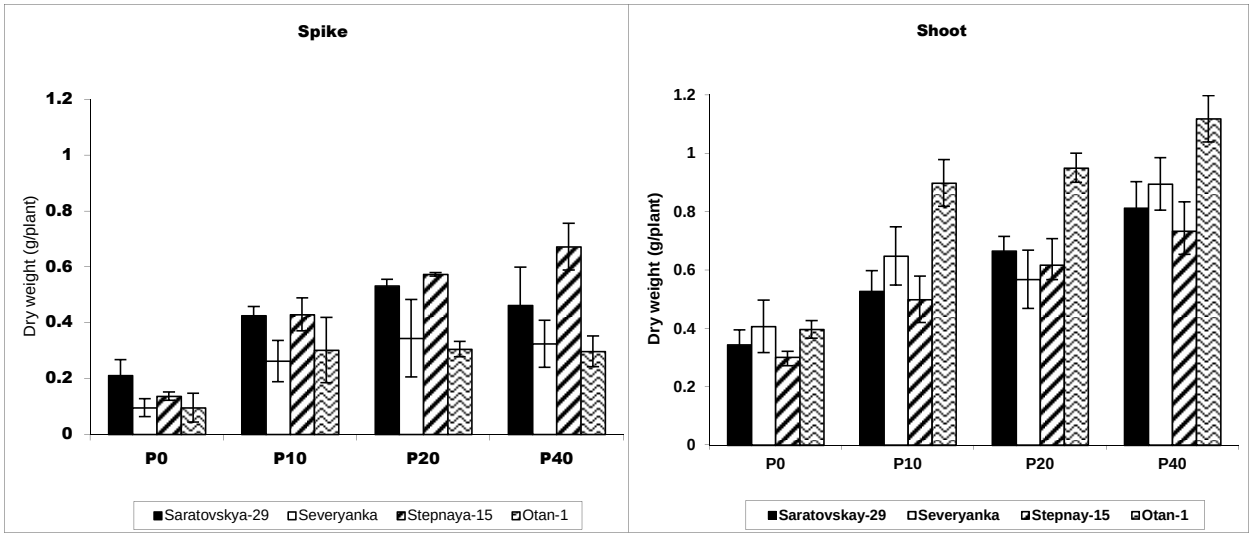

FIG. 2. Shoot and spike dry weight of four wheat varieties at four phosphorus levels.

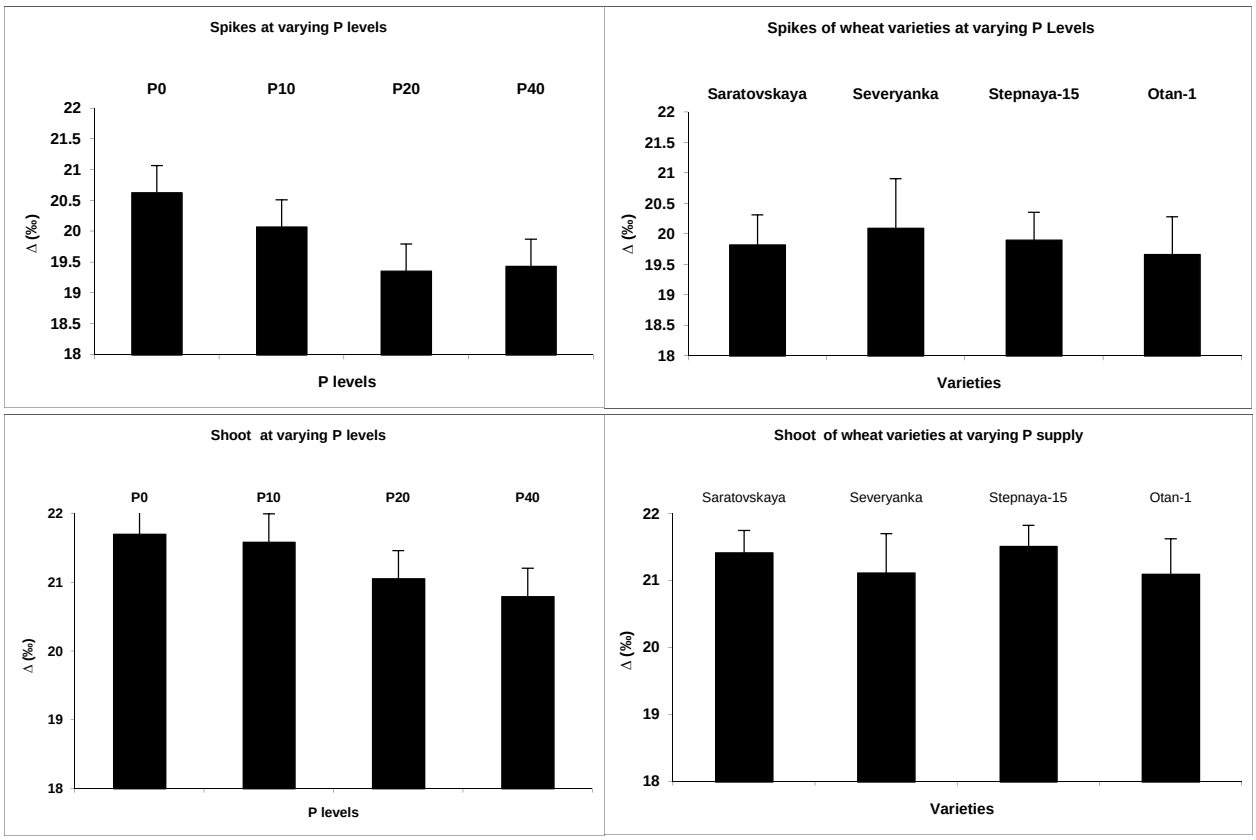

FIG. 3. Mean CID in spikes and shoots of four wheat varieties at four phosphorus levels.

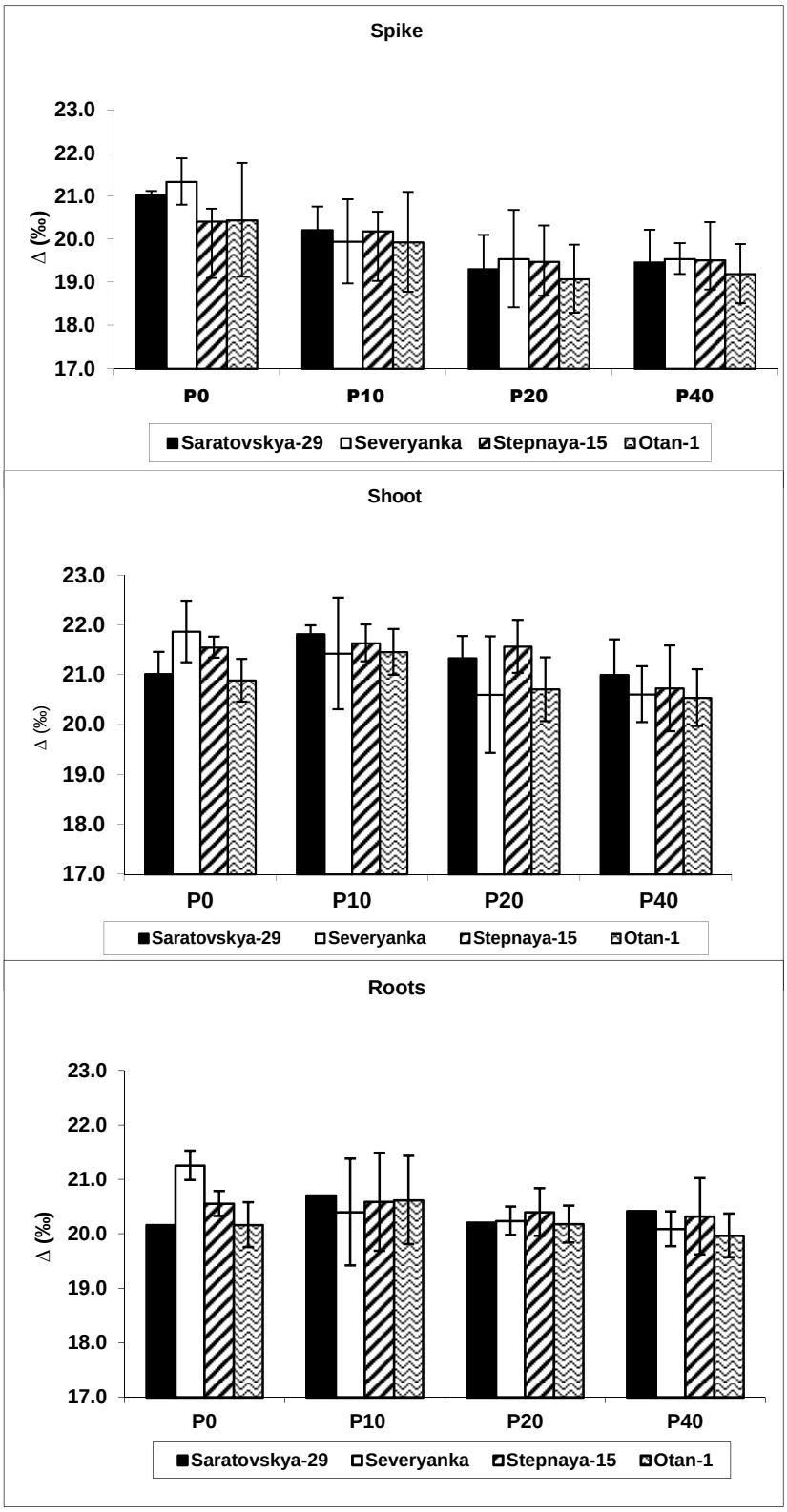

FIG. 4. CID in spikes, shoots and roots of four wheat varieties at four phosphorus levels.

3.1.3. Nitrogen and carbon accumulation

Phosphorus supply resulted in increased N accumulation in the whole plant with the average ranging from 10.8 mg N at P0 to 26.7 mg N plant^{-1} at P40 (Fig. 5). Nitrogen concentrations were higher in spikes than in shoots and roots. Although the N accumulation

followed a similar trend to that of DM, the N concentrations in spikes varied significantly from 22.6 mg g^{-1} in Otan-1 and Severyanka to 24.3 mg g^{-1} in Stepnaya-15. The two varieties, Saratovskay-29 and Stepnay-15, had a higher amount of N in the spike than the other two varieties, whereas Otan-1 had the highest shoot N (Fig. 6). The percentage of N derived from the fertilizer was highest in shoots and lowest in roots, and was higher in Otan-1 than the other three varieties (data not shown). Carbon accumulation in shoots and roots of Otan-1 was the highest among the four varieties irrespective of P supply, and was lowest in spikes (Fig. 7).

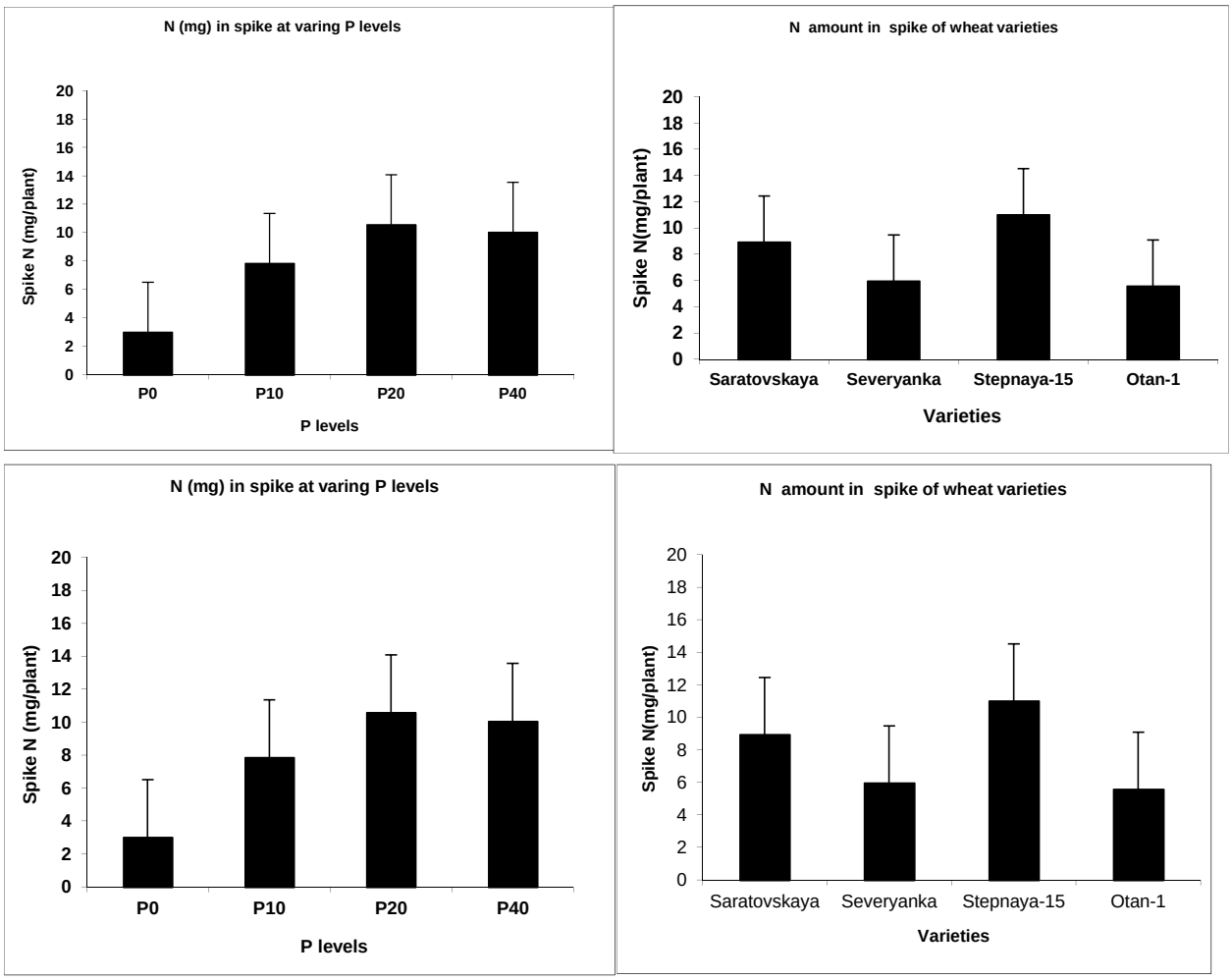

FIG. 5. Mean N accumulation in shoots and spikes of four wheat varieties at four phosphorus levels.

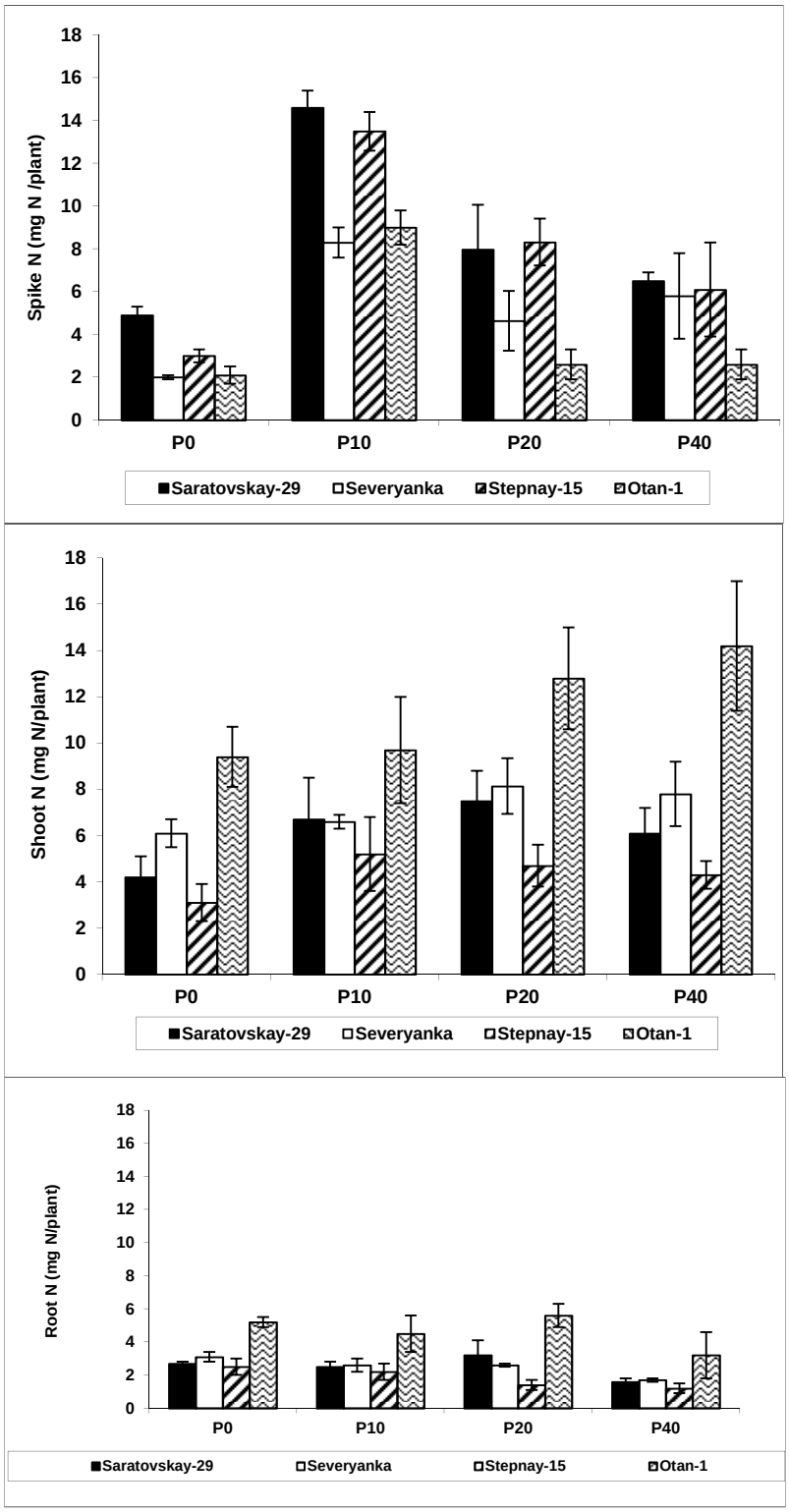

FIG. 6. N accumulation in shoots, spikes and roots of four wheat varieties at four phosphorus levels.

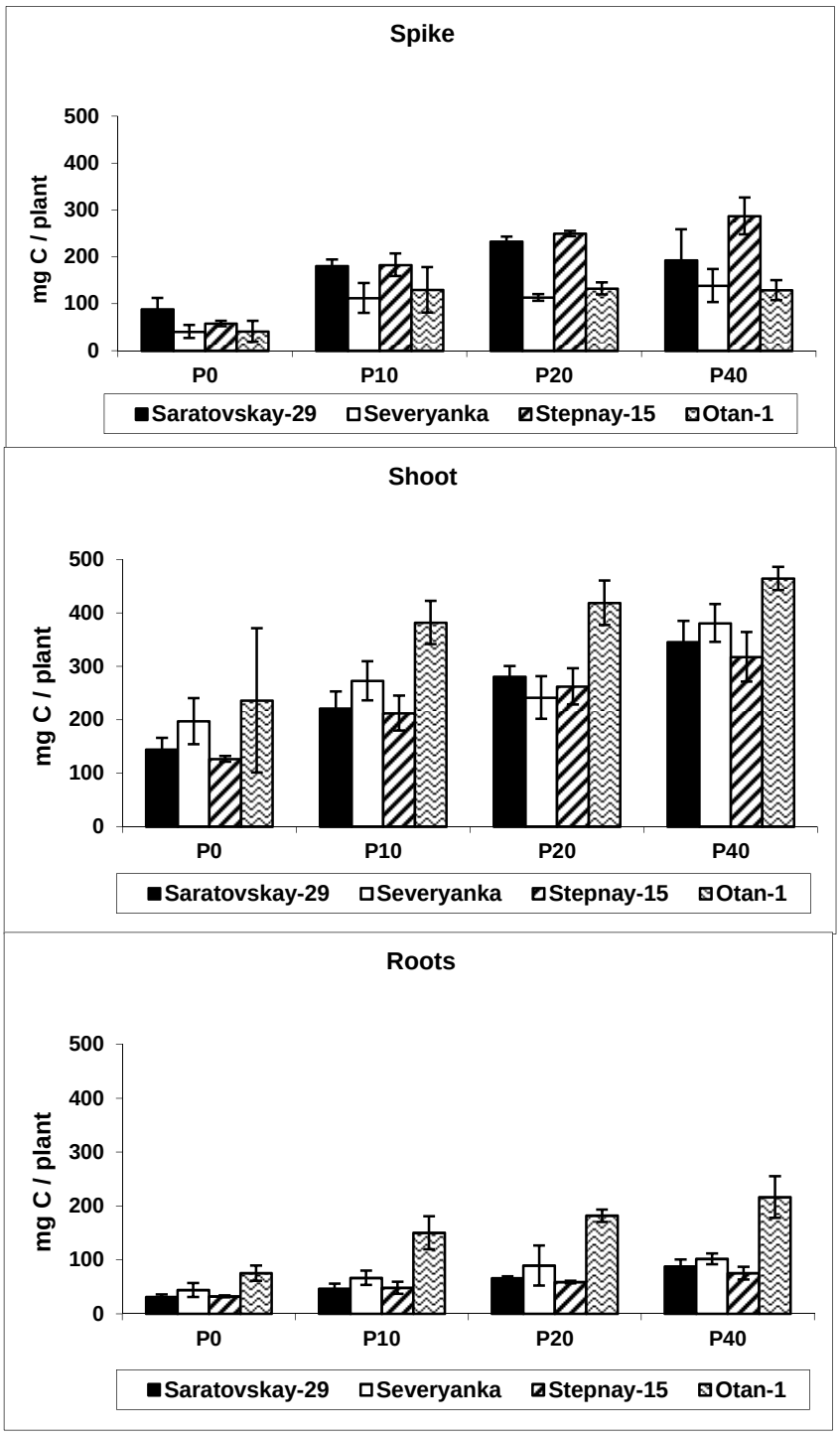

FIG. 7. C accumulation in shoots, spikes and roots of four wheat varieties at four phosphorus levels.

3.2. Rice

3.2.1. Interactive effect of P and N supply with salinity on biomass production

Rice biomass increased sharply with P supply from P0 to P20 but showed no further increase at higher P levels (P40 and P60). However, there was a significant response of biomass to increasing N supply (Fig. 8). A significant decrease in shoot DM occurred at 10 dS m^{-1} compared with the control. The effect of salt stress was less severe on DM with increased application of N and P. Root DM was severely reduced by the salt treatment compared to the shoot.

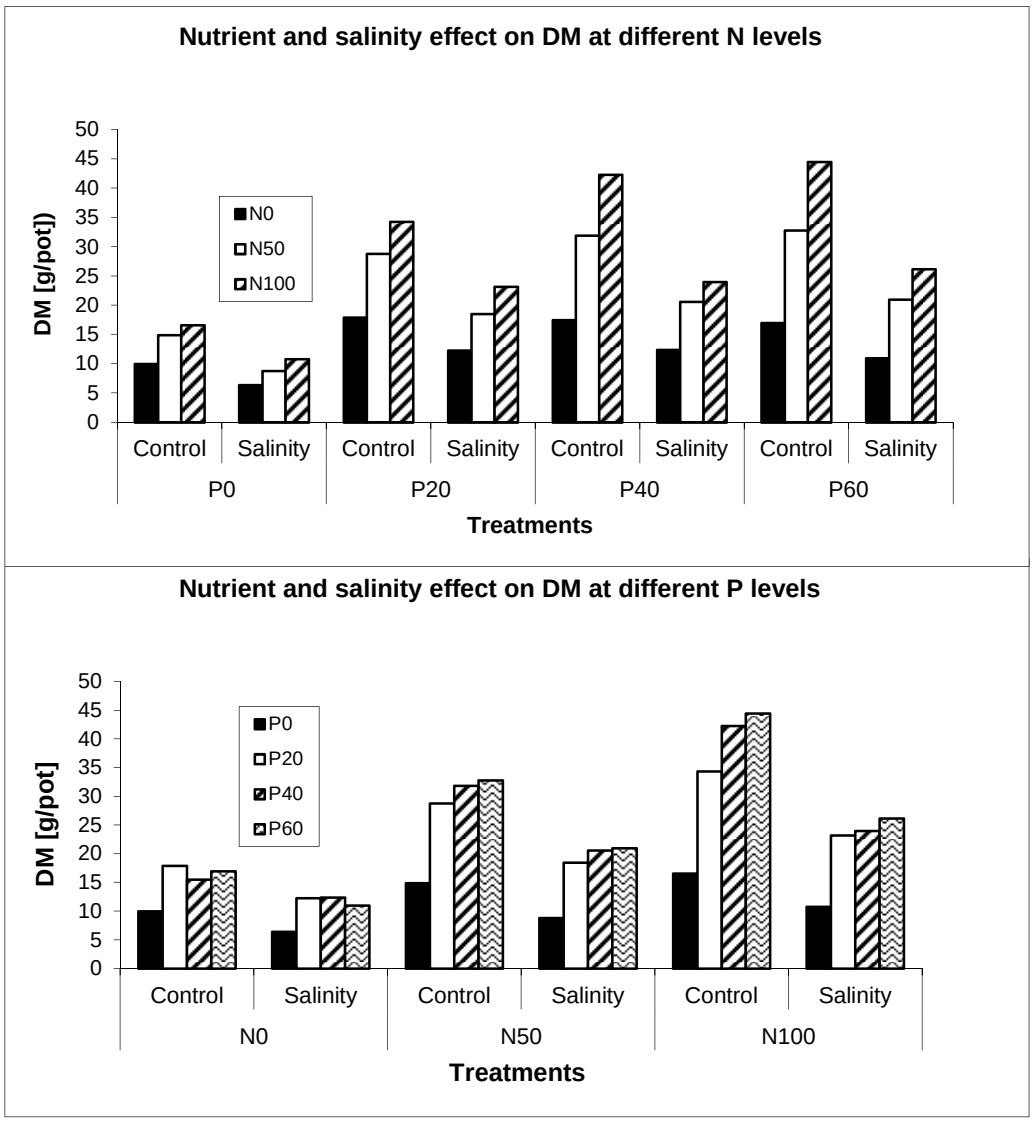

FIG. 8. DM of rice under saline and non-saline conditions at three nitrogen and four phosphorus levels.

3.2.2. Interactive effect of P and N supply with salinity on $\Delta^{13}C$

$\Delta^{13}C$ values decreased with salinity (20.6‰ for the control and 19.6‰ for the saline treatment), but increasing the N supply did not significantly affect Δ. Phosphorus supply significantly reduced Δ in the control treatment, but the effect was not seen in the saline treatment (Fig. 9). N and C accumulation in shoots and roots increased with N and P supply. Salinity resulted in a decrease in the amounts of N and C in plants. The interaction of N and salinity stress resulted in a more severe reduction in N uptake than P stress x salinity.

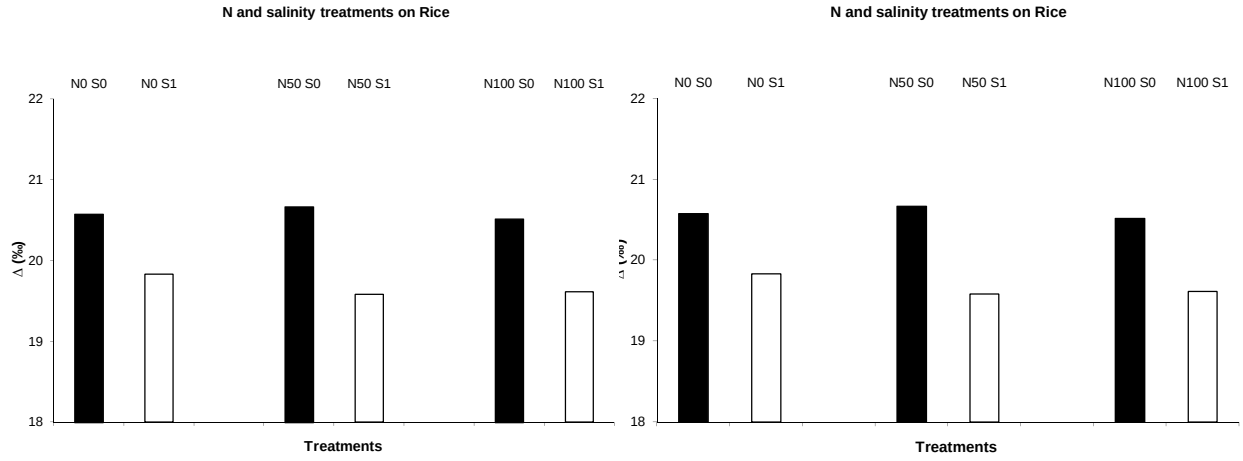

FIG. 9. CID in shoots of rice as affected by salinity and levels of nitrogen and phosphorus supply.

4. DISCUSSION

4.1. P supply and CID in wheat

An increase in P supply (0-40 kg ha^{-1}) resulted in a significant increase in shoot and spike DM but a decrease in Δ, which indicates that P stress leads to more discrimination against ^{13}C. Few attempts have been made to study the effect of P on Δ. Our results agree with Brück et al. [13] who reported a decrease in $\Delta^{13}C$ when P was applied to pearl millet, but Edbon et al. [12] reported that P had no significant effect on $\Delta^{13}C$ although $\delta^{15}N$ was depleted. In grassland species, Tsialtas et al. [7] reported Δ in *Lolium perenne* decreased with P addition, but there was no effect on *Poa pratensis*, *Festuca valida* and *Taraxacum officinale*. It is known that low Δ is associated with high radiation use efficiency, and increasing P supply would lead to less stomatal opening leading to high transpiration efficiency. The results suggest that variation in plant P status may have implications for the coupling of C and water exchange in wheat as observed for N by Cernusak et al. [9]. The carbon costs associated with P acquisition by crops [14] and its effects on crop biomass and yield suggest that $\Delta^{13}C$ may be affected by P stress.

ΔSp was significantly lower than ΔSh, suggesting the remobilization of assimilated C from the leaves to grain. The CID in spikes reflected more on the DM than on the CID in shoots. Differences in ΔSp among the four wheat varieties were not significant, although ΔSh was higher in Stepnaya-15 than the other varieties. This could be attributed to the adequate availability of water, sufficient for the growth of the plants. P supply resulted in an increased N content in plant parts.

4.2. N and P supply and CID of rice under saline conditions

Salinity resulted in a decrease in shoot DM and ΔSh indicating a positive relationship between CID and salinity tolerance in rice [15]. A decrease in Δ due to salinity under different environments has been reported for wheat [16, 17], Kentucky bluegrass [18], barley [19] and rice [15, 20], suggesting that salinity induced a greater degree of stomatal resistance that provided less opportunity for discrimination against ^{13}C. Thus tolerant genotypes are able to maintain higher stomatal conductance and/or photosynthetic capacity in the long term under salt stress.

It is known that under saline conditions, there is less CID resulting in low Δ values. Under saline conditions, increasing N supply from 0 to 100 kg ha^{-1} showed a slight but

significant decrease in ΔSh as reported in wheat [10], maize [4, 21] and rice [15], suggesting that adequate N fertilizer should be applied when using CID as a tool for screening salinity tolerant rice germplasm. Dercon et al. [4] showed that higher N supply favoured ^{13}C isotopic discrimination because the CO_2 diffusion from outside to inside the leaf was not sufficiently fast to keep up with the CO_2 demand generated by the increased N availability.

A review of CID by O'Leary [22] indicated that the nutritional status of a plant might affect Δ, and that well nourished plants showed more positive $\delta^{13}C$ values than plants deficient in N and/or K. As observed for wheat in the present study, increasing P supply from 0 to 20 kg P ha^{-1} resulted in a slight decrease in ΔSh, after which no significant decrease was observed. The combined effect of increasing the nutritional status (N and P supply) and salinity on CID was only observed at high P (60 kg ha^{-1}) and high N (50 and 100 kg ha^{-1}) under saline conditions. The results are in agreement with those of Zhao et al. [11] who reported that water and N both significantly affected the relationships between yield and CID of spring wheat. The CID values in spikes were low compared with the values in shoots. Less discrimination in spikes compared to shoots under nutrient and salinity stress could be attributed to the re-fixation of CO_2 [23].

5. CONCLUSIONS

Nutrient deficiencies (particularly N and P) contribute to low yields of wheat and rice in drought-prone (semi-arid and arid) environments. Although CID has been proposed as a useful trait for selecting C_3 cereal crops in drought and salinity stressed environments, low availability of soil N and P could hinder its accuracy and effectiveness as a selection tool for high yield. Characterization of the environment should not only focus on water availability (pre-anthesis, post anthesis and residual soil moisture stress) but should address N and P availability, since P deficiency could affect CO_2 fixation and the translocation of assimilates from the vegetative to the reproductive organs. Further investigation of the effect of P on CID under well-watered and stressed conditions is warranted.

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RELATIONSHIP BETWEEN YIELD, CARBON ISOTOPE DISCRIMINATION ($\Delta^{13}C$) AND WATER USE EFFICIENCY OF DURUM WHEAT IN THE SYRIAN ARAB REPUBLIC. 3. SIMULATION MODELLING

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Abstract

Six durum wheat genotypes were grown at Tel Hadya during the three seasons 2004–2005, 2005–2006 and 2006–2007, and the data were used to calibrate and validate the simulation model SIMWASER. Simulated and measured soil water status and yield production showed very good conformity, and therefore SIMWASER was able to reproduce the water balance of the site in a realistic manner, as well as yield production throughout the season. The validated model was run for the period between 1980 and 2007 for the Tel Hadya site using daily weather data. Also, different genetic scenarios were used regarding different transpiration values. Carbon isotope discrimination ($\Delta^{13}C$) was used as a closely-related surrogate for transpiration at the plant level. The simulated yields showed weak relationships with measured rainfall. However, closer relationships (higher R^2) were found between simulated yield and plant transpiration. Moreover, when years were grouped according to rainfall, dry years (rainfall <175 mm) and wet years (rainfall >175 mm), stronger relationships were evident between simulated yield and measured rainfall. The dry years produced much higher yields per unit of rainfall (rainfall use efficiency) compared with the wetter years, ranging between 13 and 90 kg ha^{-1} mm^{-1} for the wet and dry years, respectively. The high Δ cultivars transpired more water, had higher yields and therefore had higher water use efficiencies compared with low Δ cultivars.

1. INTRODUCTION

Durum wheat is an important crop worldwide, particularly in the Mediterranean basin which accounts for 75% of total production. Durum wheat is primarily grown under rain fed conditions, where the frequent drought combined with heat stress [1], fragile soils [2], and limited renewable water resources [3] are the major factors limiting grain yield. Today, the sustainability of crop production systems is at risk because of the degradation of agriculturally-productive soil [4] and water resources [5]. For agriculture in the West Asia–North Africa (WANA) region to meet the demands imposed by today's demography requires more efficient use of water within cropping systems [3, 4]. In such water limited environments, the long term consequences of agricultural practices on crop productivity and water-use efficiency are difficult to assess, because annual rainfall variability may mask existing trends. Cropping-systems analysis using simulation techniques that integrate long term climatic records is a complement to traditional field experimentation, which allows the outcomes of agricultural interventions to be quantified across the observed rainfall variability [6].

The climate of a given area is considered to be the average situation of weather over a long period of time, during which it fluctuates in average or exceptional ways. The long term weather cycle is considered to be about 35 years [7]. However, ground temperature has increased by about 0.5 ^{o}C during the last century, and the concentration of CO_2 had increased during the last 250 years from 270 to 370 ppm [8]. When temperature increases, the evaporation of water increases, and the water used by plants decreases [9].

Mediterranean climates like the Syrian Arab Republic [10] receive rainfall between 200 and 800 mm a^{-1}, with <100 rainy days during the winter months [11]. In addition, unpredictably high temperatures combined with drought restrict the growth and yield of crops [12]. Drought is the most critical factor causing yield loss worldwide. According to FAO, only 20% of the total agricultural areas can be irrigated, and therefore most research is concentrated in the rain fed areas. The main challenge is to obtain higher production per unit area, since the potential for horizontal expansion is finite [13, 14]. Crop simulation models could be useful tools to predict scenarios during the weather cycle, including crop managements (e.g. planting date), phenology, morphological and physiological traits such as transpiration rate, water use efficiency (WUE) or carbon isotope discrimination (CID, $\Delta^{13}C$, Δ).

CID has been shown to have potential application as a screening tool in breeding programmes to increase transpiration efficiency (TE) and therefore yield, especially during extreme events such as drought [15, 16]. The isotopic ratio of ^{13}C to ^{12}C in plant tissue is less than the ratio in atmospheric CO_2, indicating that plants discriminate against ^{13}C during photosynthesis, particularly C_3 plants such as wheat, peanuts and cotton. Plants with high transpiration rates consume more water, and have high Δ values under limited rainfall [17]. Positive correlations between Δ and yield have been reported in a range of crops including wheat [18], barley [19] and cotton [20].

The objective of the present investigation was to validate the SIMWASER simulation model [21] to predict yield and WUE of wheat cultivars varying in TE and Δ grown under rain fed conditions in the Syrian Arab Republic.

2. MATERIALS AND METHODS

2.1. Field experiments

Field experiments were conducted during the 2004–2005, 2005–2006 and 2006–2007 seasons at Tel Hadya, the main field research station of the International Centre for Agricultural Research in the Dry Areas (ICARDA), 30 km south of Aleppo in northern Syria. The site is located at 36.01 ºN, 36.54 ºE, 284 m above sea level with an annual rainfall (28-a average) of 348 mm, falling mainly in the cold winter months, with a high year-to-year variation.

The experiment consisted of six durum wheat genotypes (durum wheat programme–ICARDA), which had a similar phenology to ensure minimum genotype x environment (G*E) interactions, but with contrasting grain Δ (range 14.78 to 15.88‰). Optimum agronomic practices were used including cultivation, fertilization and weed control. Seed was planted at a rate of 120 kg ha^{-1} using an Oyjord planter in a Randomized Complete Block Design (RCBD) with three replicates. Planting dates were 25th December 2004, 28th November 2005 and 18th January 2007 for the 2004–2005, 2005–2006 and 2006–2007 seasons, respectively, and the corresponding harvest dates were 28th May 2005, 1st June 2006 and 5th June 2007, i.e. 155, 191 and 139 d from sowing to maturity.

In 2004–2005 plant samples were taken only at physiological maturity (0.5 m^2) from every plot. In 2005–2006 plant samples were taken at the 3–4 leaf stage (every plot, 0.8 m^2), at tillering (0.6 m^2), at stem elongation and anthesis (0.2 m^2) and at physiological maturity (1 m^2). In 2006–2007 plant samples were taken at the 3–4 leaf stage (0.8 m^2), at tillering and anthesis (0.2 m^2) and at physiological maturity (1 m^2). Plant samples were cut at ground level,

counted, oven dried at 65 ^{o}C for 48 h and then weighed. At physiological maturity, samples were treated in the same manner to determine biomass yield and were then hand- or machine-threshed (depending on sample size) to determine grain yield. Leaf and grain samples were were finely ground for $\delta^{13}C$ analysis at the IAEA Laboratories, Seibersdorf, Austria, and $\Delta^{13}C$ was calculated from the $\delta^{13}C$ data [22].

Soil water content was monitored in every plot in 2005–2006 and 2006–2007 using the Diviner 2000. PVC access tubes were manually installed to a depth of about 130 cm. Measurements were made in 10 cm intervals throughout the season, and the probe was calibrated at the end of the season by artificially wetting the dry soil.

2.2. Weather data

Air temperature (minimum and maximum), relative humidity (minimum and maximum), wind speed, rainfall and solar radiation were recorded at the site on a daily basis. Also, the daily weather data from 1980 to 2007 were used as long term data in the simulation model.

2.3. Soil sampling

Samples were taken at depth intervals of 0–20, 20–60, 60–100, 100–120 and 120–170 cm. Some physical and chemical properties were determined including texture, bulk density, pH, electrical conductivity, total N, organic matter and $CaCO_3$. Olsen P was determined only on 0–20 and 20–40 cm samples.

2.4. Simulation modeling

2.4.1. Characteristics of SIMWASSER

The deterministic simulation model SIMWASER describes the one dimensional vertical water flux within a soil profile, neglecting inter-flow and preferential flow [23, 24]. The water balance and the growth of plants are interrelated by the physiological interaction between transpiration and assimilation. The accumulation of plant material depends on the amount of CO_2 incorporated via the stomata, through which water vapor is simultaneously lost from the saturated vacuole into the unsaturated ambient air. Potential assimilation and therefore potential growth is only possible as long as the water supply towards the stomata can meet the potential transpiration loss. If this is not the case, stomata will close and the formation of plant biomass will be restricted. All of these processes depend on the respective plant development stage that determines the partition of the daily assimilates between leaves, stems and roots. SIMWASER calculates the actual development stage by dividing the accumulated growing degree days by the sum of growing degree days necessary for ripeness of the crop. A growing degree day corresponds to the mean daily temperature minus a base temperature which is specific to the crop.

Actual plant growth is calculated from the potential plant growth rate as the proportion of the actual to the potential transpiration (Eq. 1):

$$P_{act} = P_{pot} \times T_{act}/T_{pot} \quad (1)$$

where P_{act} and P_{pot} = actual and potential plant growth (kg CH_2O m^{-2} d^{-1}), respectively, and T_{act} and T_{pot} = actual and potential transpiration (mm d^{-1}), respectively.

Potential evapo-transpiration (PET) is calculated according to Penman-Monteith [25] (Eq. 2):

$$PET = (f_t \times Q + 0.864 \times H_{0/}r_{air})/(f_t + r_{crop/}r_{air}) \quad (2)$$

where PET = potential evapo-transpiration (mm d^{-1})
f_t = weighting factor depending on temperature
Q = evaporation equivalent of available energy (mm d^{-1})
0.864 = factor converting (g H_2O m^{-3})/(s cm^{-1}) to (mm d^{-1})
H_0 = saturation deficit of air (g H_2O m^{-3} air)
r_{air} = aerodynamic resistance against water vapour exchange (s cm^{-1})
r_{crop} = crop resistance (s cm^{-1})

Both r_{air} and r_{crop} are variables, depending on weather as well as on the actual plant development stage. Potential transpiration (T_{pot}) is deduced from the proportionality of PET to the global energy absorbed by the leaves of the crop stand (Eq. 3):

$$T_{pot} = PET\ [1.0 - \exp(-0.6 \times totlai)] \quad (3)$$

where totlai = total leaf area per unit soil surface (m^2 m^{-2})
exp = exponential function

Actual transpiration T_{act} is determined by comparison of potential transpiration T_{pot} with the amount of water which can be withdrawn by the roots from the soil SUMROOT. If SUMROOT is larger than T_{pot} then $T_{act} = T_{pot}$ otherwise T_{act} = SUMROOT, which will increase with rooting depth. Actual rooting depth depends on the respective crop as well as on the penetration resistance of the soil and is calculated each day.

When calculating the soil water flux within the soil profile (Fig. 1) one must determine if it may be influenced by the ground water level. If not, it may be assumed that there is no capillary rise from the coarse aquifer, whereas in the other case the variable ground water level will form the lower boundary of the profile.

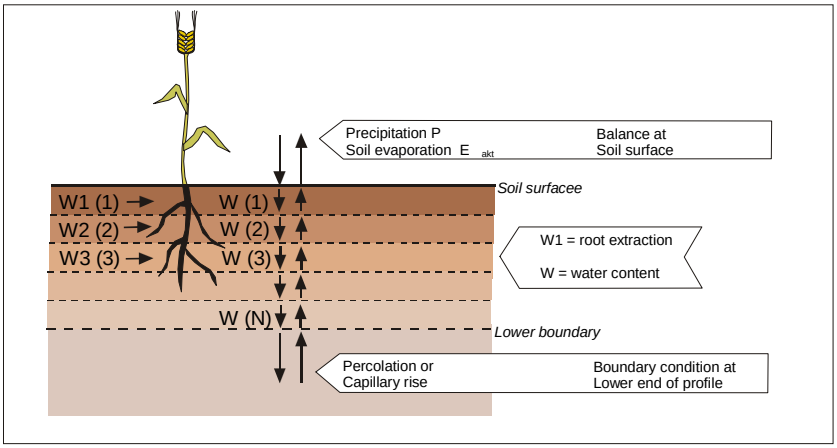

FIG. 1. Schematic illustration of the soil water flux taken into account by SIMWASER [26].

Water flux between the soil layers is calculated according to Darcy's law as a function of the capillary conductivity and the gradient of the matric potential using small but variable time steps which restrict changes of water content to 0.1 % v. Filter velocity at the lower end of a soil layer V_i is calculated according to Eq. 4:

$$V_i = \frac{(K_i + K_{i+1})}{2} \cdot \left(\frac{\Psi_{i+1} - \Psi_i}{Z_i} + 1\right) \quad (4)$$

where V_i = filter velocity
K_i, K_{i+1} = capillary conductivity of layers i, i+1
ψ_i, ψ_{i+1} = matric potential of layers i, i+1
Z_i = distance from centre of layer i to centre of layer i+1

The filter velocity V_i of the water flowing out of the bottom of layer i is at the same time the filter velocity of the water flowing into the top of the next layer i+1. The soil profile model may be divided into a maximum of 50 layers, each 5–10 cm thick, down to a depth which is outside the range of plant roots. A soil profile influenced by ground water must be deeper than the deepest ground water level at that site.

2.4.2. Input data

There are three groups of input data:

The daily weather data are: minimum and maximum air temperature (ºC); rainfall (mm); minimum and maximum relative humidity (%); wind speed (2 m height); solar radiation (MJ m^{-2}); rain duration and day length.

Soil data (for every depth interval) are: texture (particle size percentage); structure; organic matter (%); bulk density (g cm^{-3}); pF curve; hydraulic conductivity; salinity (dS m^{-1}); gravel (%); compaction (loose, normal, dense, hard and severe) and pore volume (%).

Plant parameter data are: extinction coefficient (ext); leaf area weight (lawgt); plant height (hgt); stomatal resistance (rs); assimilation (ass); temperature class (ft); root length (rlg); root density (rd); root strength (ss); leaf width (lfw); ripening (ripe); leaf area index at emergence (lai0); minimum air temperature (amin) and critical day length (cdayl).

2.4.3. Output data

The output data are: date (day); sum water content (SUMWG, mm); sum rain (SRAIN, mm); sum evapotranspiration (SETA, mm); sum deep percolation (SPER, mm); sum transpiration (STR, mm); sum run off (SROFF, mm); evapotranspiration of current day (ETA, mm d^{-1}); daily rain (RAIN, mm); daily snow (SNOW, mm); accumulated flux during current day (FLXG, mm d^{-1}); accumulated capillary rise (GWRIS, mm d^{-1}); green leaf area index (GRNLAI, m^2 m^{-2}); root length (CROOTL, dm); daily dry matter accumulation (DRYMAT. kg ha^{-1}) and daily grain yield (GRAIN, kg ha^{-1}).

2.5. Calibration and validation of the model

Calibration of the SIMWASER model is normally restricted to the soil and plant parameters. The plant parameters to be calibrated are mostly the stomatal resistance and the sum of growing degree days necessary for ripeness as well as the potential root length. Essential files which contain the following input data need to be prepared:

2.5.1. Information on the soil profile: (File 'soilprofile')

Name of the site and its elevation (m above sea level); name of the relevant weather station and number of the layer (layer of balance) at which the soil water balance will be calculated; number of the last soil layer of the profile; depth and thickness of the soil layers; soil type in each of the layers; pore volume and soil water content at the beginning of the simulation.

2.5.2. Information on soil

The hydraulic parameters that must be given in tabulated form: the name of the file; the number of curves; pore volume of the curve; the next three lines contain the headings of the four columns of the table: soil water tension (Psi, kPa), soil water content (W, % v), capillary conductivity (Ku, mm d^{-1}), penetration resistance (PE, MPa)

2.5.3. Plant parameters

For each of the cropping elements to be simulated (actual.sim) the typical physiological plant parameters must be given in tabulated format; as bare soil is a cropping element too, its soil resistance is also given formally, while all other 'physiological' parameters are set to zero.

2.5.4. Weather data

Daily weather data are given as a binary file stored in a master file. The data must be converted from ASCII or EXCEL tables, and the data are: day length (daylgt, h × 10); maximum temperature (Tmx, °C × 10); minimum temperature (Tmn, °C × 10); maximum relative humidity (Rhmx, %); minimum relative humidity (Rhmn, %); wind velocity at 2 m height (Wind, m s^{-1} × 10); precipitation (Prec, mm d^{-1} × 10); duration of rain (Durn, min) and solar radiation (Glob, MJ m^{-2}).

2.6. Simulation scenario

Genetics scenarios were initiated by using different Δ values as parameters in the SIMWASER model, and these were used as 'assimilation'. The model was then run for the Tel Hadya weather data from 1980 to 2007.

3. RESULTS AND DISCUSSION

3.1. Calibration

Each depth interval was prepared to include data on pF curve [the relation between soil water content (% v) and soil water suction (kPa)], Ku curve [the relation between capillary conductivity (mm d^{-1}) and soil water suction (kPa)], PE curve [the relation between penetration resistance (MPa) and soil water suction (kPa)]. An example for the 0–20 cm depth interval is given in Table 1. The model used soil files of 26 layers, 5 cm for each layer, to cover the measured soil depth of 130 cm. The plant file was constructed using default values, except for the assimilation values, where grain Δ values averaged over 3 years were used for the six genotypes.

Leaf-Δ (4th fully expanded leaf) was consistently higher than grain Δ (Table 2), due to the greater availability of water early in the season compared with the end of the season. A similar trend of grain Δ between genotypes was evident for the different seasons, despite the fact that inter-seasonal rainfall was very different. The data used for assimilation were grain Δ values averaged over three seasons; 14.90, 15.30, 15.40, 15.50, 15.60 and 15.70‰ for Omruf-2, Brachoua, Korifla, Waha, Massara-1 and Omrabi-14, respectively.

TABLE 1. THE SOIL FILE+ USED FOR SIMULATION OF THE 0–20 CM DEPTH INTERVAL AT TEL HADYA

Psi (kPa)	W (% v)	Ku (mm d^{-1})	PE (MPa)
1.0E – 2	58.0	7.6E + 03	2.4E + 00
1.0E – 1	57.9	6.2E + 03	2.4E + 00
2.0E – 1	57.8	5.6E + 03	2.4E + 00
4.0E – 1	57.7	4.7E + 03	2.3E + 00
1.0E + 0	57.2	2.8E + 03	2.3E + 00
2.0E + 0	56.3	1.2E + 03	2.4E + 00
4.0E + 0	54.1	5.4E + 02	2.4E + 00
1.0E + 1	49.8	6.3E + 01	2.6E + 00
2.0E + 1	44.9	1.6E + 01	2.8E + 00
4.0E + 1	41.0	3.7E + 00	3.0E + 00
1.0E + 2	37.7	4.7E – 01	3.2E + 00
2.0E + 2	34.6	1.0E – 01	3.5E + 00
4.0E + 2	32.0	2.3E – 02	3.7E + 00
1.0E + 3	29.1	3.2E – 03	4.0E + 00
2.0E + 3	26.8	8.3E – 04	4.3E + 00
4.0E + 3	24.3	1.2E – 04	4.6E + 00
1.0E + 4	20.9	1.8E – 05	5.3E + 00
4.0E + 4	14.8	5.4E – 07	7.0E + 00
1.0E + 5	10.8	5.6E – 08	8.9E + 00
1.0E + 6	0.10	1.2E – 12	5.1E + 01

+Psi, soil water tension; W, soil water content; Ku, capillary conductivity; PE, penetration resistance.

TABLE 2. THE 4TH FULLY EXPANDED LEAF AND GRAIN Δ (‰) OF SIX DURUM WHEAT GENOTYPES AT TEL HADYA IN 2004–2005, 2005–2006 AND 2006–2007

Genotypes	Leaf-Δ		Grain-Δ		
	2005–2006	2006–2007	2004–2005	2005–2006	2006–2007
Omruf-2	21.95	21.45	15.36	15.35	14.08
Brachoua	22.36	21.12	15.34	15.70	14.78
Korifla	22.52	21.71	15.72	15.68	14.83
Waha	22.51	22.08	15.80	15.81	14.88
Massara-1	22.39	21.96	16.13	15.62	15.21
Omrabi-14	22.76	22.20	16.26	15.71	15.17
F test	***	*	***	***	**

*, **, ***, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

3.2. Validation

The assimilation value used in the simulation for the wheat plot was 15.0, which is the average value of the six genotypes (Tables 2 and 3).

Good agreement was found between measured and simulated values of soil water storage (Fig. 2), confirming the prediction [26] that SIMWASER could be successfully used to simulate the influence of water availability on crop yield. This agreement was valid throughout the two seasons for both fallow and wheat plots, suggesting that SIMWASER is able to reproduce the water balance (Fig. 2) of the investigated site in a realistic manner. In addition, the measured dry matter accumulation as well as grain yield was very close to that simulated by SIMWASER for the two seasons (Fig. 3), despite the very different growing conditions, reflected by a two-fold difference in yield.

TABLE 3. THE PLANT PARAMETERS+ OF THE SIX DURUM WHEAT GENOTYPES USED FOR SIMULATION

Crop	Code	ext	lawgt	Hgt	rs	ass	ft	rlg	rdf	rr	lfw	ripe	lai0	amin	cdayl
Fallow	0	0.0	.000	0	5	00.0	0	00	0	0	0.0	0000	0.00	0	00.0
Wheat	1	0.4	.003	1	1	15.0	1	10	9	1	0.5	1600	0.05	5	10.5
Omruf-2	1	0.4	.003	1	1	14.9	1	10	9	1	0.5	1600	0.05	5	10.5
Brachoua	1	0.4	.003	1	1	15.3	1	10	9	1	0.5	1600	0.05	5	10.5
Korifla	1	0.4	.003	1	1	15.4	1	10	9	1	0.5	1600	0.05	5	10.5
Waha	1	0.4	.003	1	1	15.5	1	10	9	1	0.5	1600	0.05	5	10.5
Massara-1	1	0.4	.003	1	1	15.6	1	10	9	1	0.5	1600	0.05	5	10.5
Omrabi-14	1	0.4	.003	1	1	15.7	1	10	9	1	0.5	1600	0.05	5	10.5

+ext, extinction coefficient; lawgt, leaf area weight; hgt, plant height; rs, stomatal resistance; ass, assimilation; ft, temperature class; rlg, root length; rdf, root density; rr, root strength; lfw, leaf width; ripe, ripening; lai0, leaf area index at emergence; amin, minimum air temperature and cdayl, critical day length.

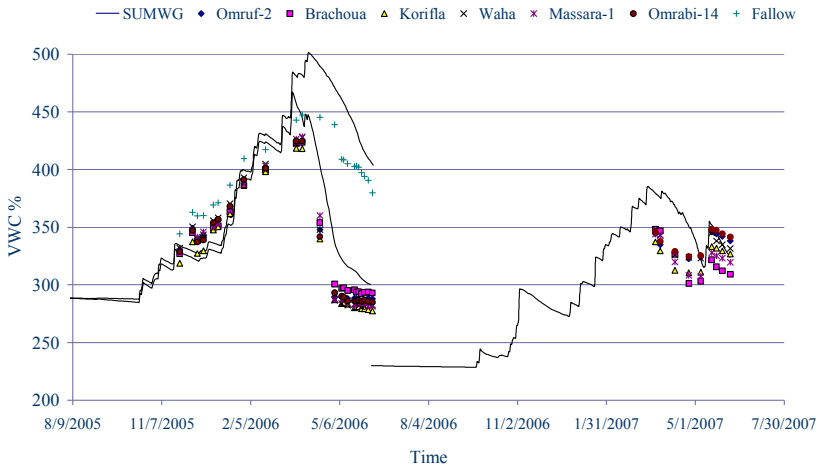

FIG. 2. Simulated volumetric water content (SUMWG, continuous line) and measured volumetric water content (VWC, %) of the six wheat genotypes (symbols) and the fallow plot during the 2005–2006 and 2006–2007 seasons at Tel Hadya.

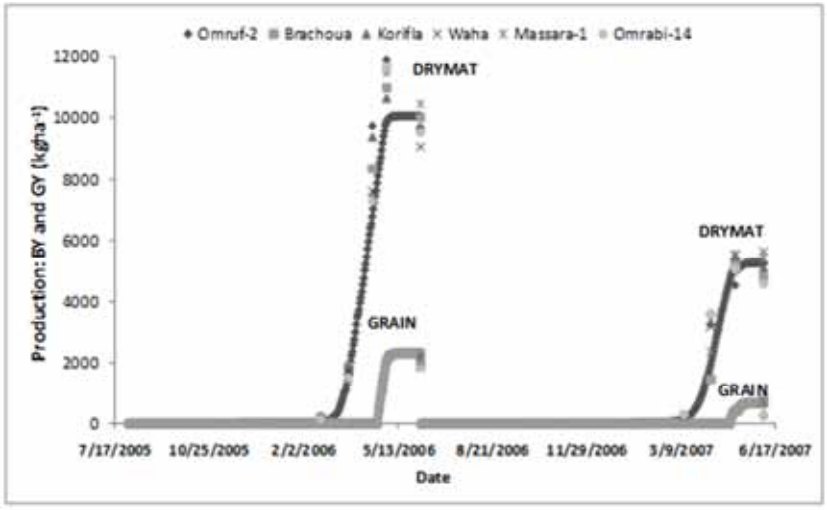

FIG. 3. Measured (symbols) and simulated (continuous line) dry matter accumulation and grain yield (DRYMAT and GRAIN) during the 2005–06 and 2006–07 seasons at Tel Hadya.

3.3. Simulation using long term weather data and genetics scenarios

The simulation of volumetric soil water content (SUMWG), transpiration (STR), evapo-transpiration (SETA) and measured rainfall (SRAIN) for the 1980–2007 period at Tel Hadya is illustrated in Fig. 4. The simulated volumetric water content generally followed the measured rainfall, with some exceptions, which could be due to erratic and ineffective rainfall causing run off, as shown by the high frequency of daily rainfall in excess of 40 mm (Fig. 5).

The fluctuation of volumetric soil water contents and evapo-transpiration were similar to that of the simulated dry matter production and grain yield (Fig. 6). Yield (either total or grain) followed the rainfall pattern but the agreement was not close, possibly due to the occasionally ineffective intense rainfall events (Fig. 5).

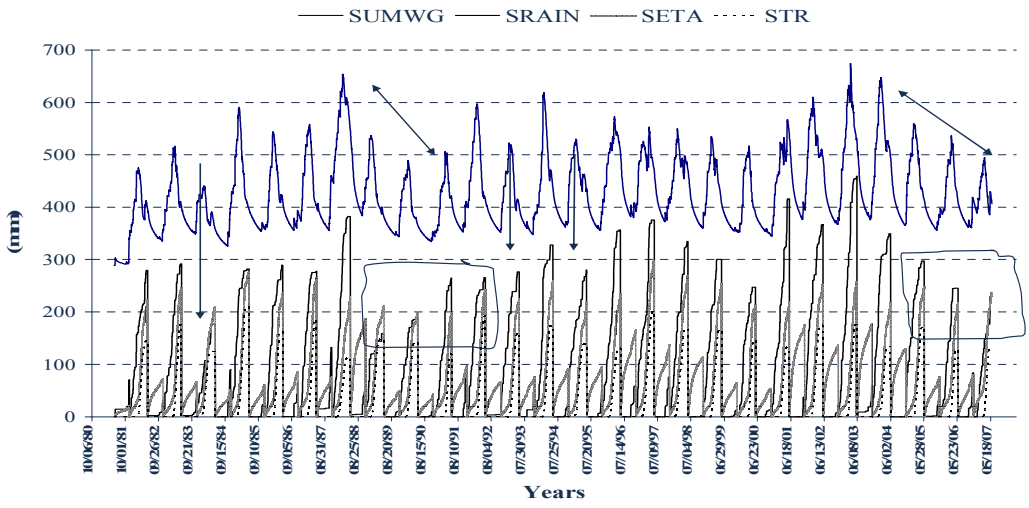

FIG. 4. Simulated volumetric water content (VWC, %), evapo-transpiration and transpiration (mm) and measured rainfall during 1980–2007at Tel Hadya using SIMWASER.

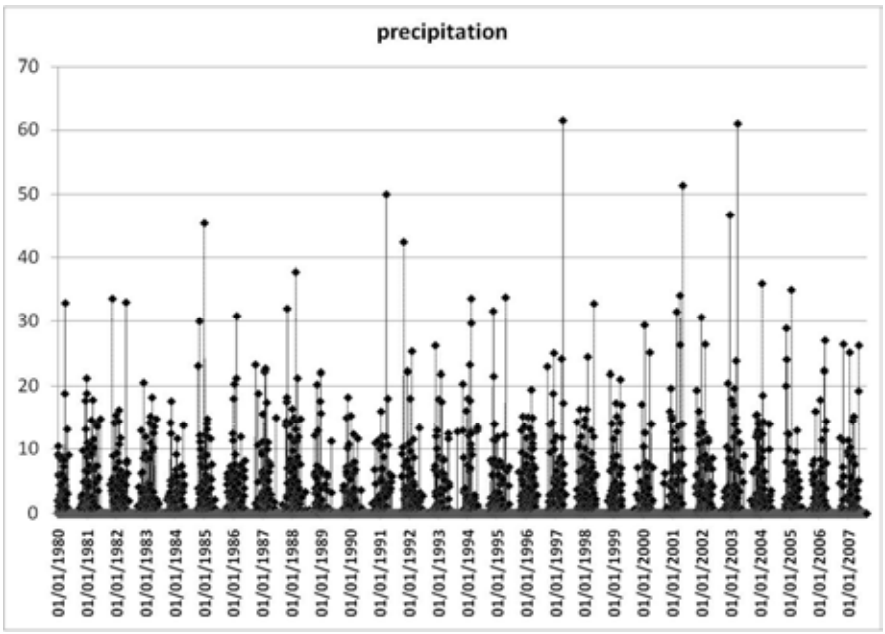

FIG. 5. Measured daily rainfall at Tel Hadya during 1980–2007.

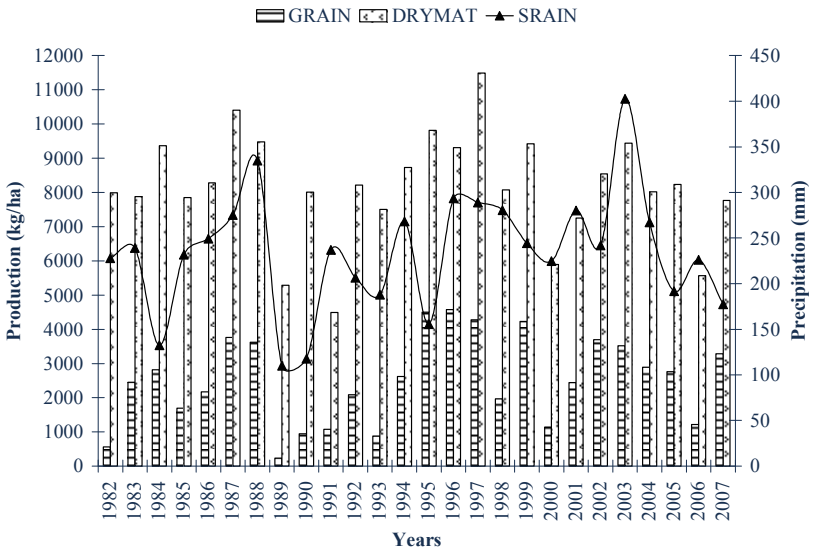

FIG. 6. Simulated dry matter production and grain yields using SIMWASER with measured precipitation at Tel Hadya during 1980–2007.

The relationships between yield (total or grain) and precipitation were linear, but with low values of R^2 (Fig. 7). However, when the data were divided into low (<175 mm) and high (>175 mm) rainfall categories significant relationships were obtained (Fig. 8). The slope of line was much higher in the low rainfall years (90 kg ha^{-1} mm^{-1}), while in the wetter years it was about 13 kg ha^{-1} mm^{-1}, a 7-fold difference. This suggests that a unit of water produced higher dry matter production at lower rainfall, and the low yield elasticity of genetic variation positively responds to high rainfall. Stronger relationships were found between yield and plant transpiration (Fig. 9), especially for total biomass, since grain yield could be adversely affected by temperature and rainfall during grain filling.

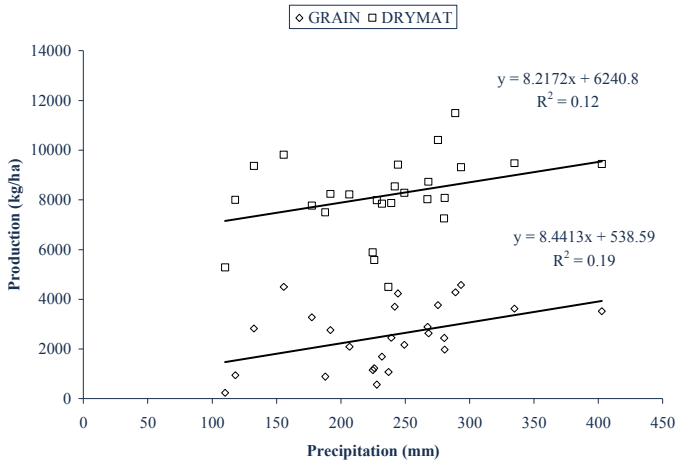

FIG. 7. Relationships between simulated dry matter production or grain yields using SIMWASER and measured precipitation at Tel Hadya during 1980–2007.

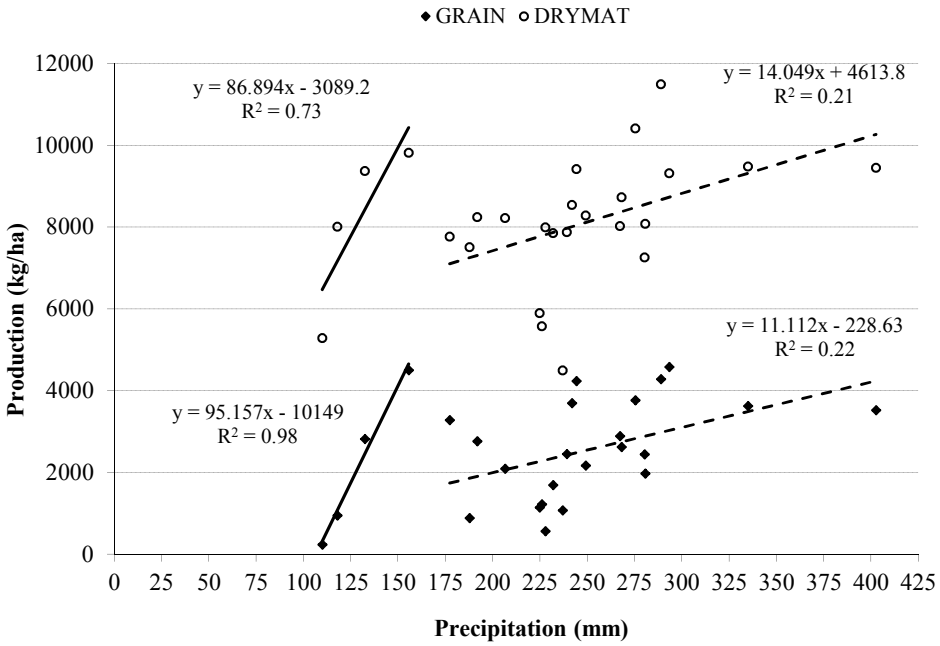

FIG. 8. Relationships between simulated dry matter production or grain yield using SIMWASER and measured precipitation at Tel Hadya during 1980–2007divided into low (continuous line) and high (dashed line) rainfall groups.

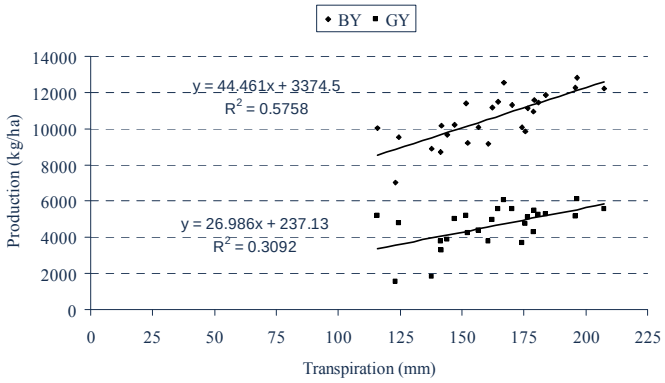

FIG. 9. Relationships between simulated dry matter production (BY) or grain yield (GY) using SIMWASER and transpiration at Tel Hadya during 1980–2007.

The simulation of the different scenarios of assimilation represented by Δ values for the period between 1980 and 2007 at Tel Hadya showed that highly significant differences (P<0.001) occurred between the six genotypes in grain and total yield (GRAIN and DRYMAT), transpiration (STR), evapo-transpiration (SETA), water use efficiency for dry matter (WUE-DRYMAT) and total volumetric soil water content (SUMWG) (Table 4).

TABLE 4. SIMULATION FOR $\Delta^{13}C$ OF SIX DURUM WHEAT GENOTYPES AT TEL HADYA (1980–2007)

Parameter†	GRAIN	DRYMAT	STR	SETA	WUE-DRYMAT	WUE-GRAIN	SUMWG
Omruf-2	2437	7853	136	186	42.5	13.22	458
Brachoua	2507	8107	140	190	42.9	13.29	454
Korifla	2522	8172	141	191	43.1	13.31	453
Waha	2534	8232	142	191	43.2	13.30	453
Massara-1	2549	8287	143	192	43.3	13.30	452
Omrabi-14	2552	8347	144	193	43.5	13.27	451
F test	<0.001	<0.001	<0.001	<0.001	<0.001	0.884	<0.001
SE (±)	17.9	16.7	0.69	0.68	0.17	0.08	0.66
$LSD_{0.05}$	35.43	33.13	1.37	1.35	0.33	0.16	1.31
CV (%)	2.6	0.7	1.8	1.3	1.4	2.1	0.5

†SE, standard error; $LSD_{0.05}$, least significant difference (P<0.05); CV, coefficient of variation.

A systematic increase in yield (grain or total), STR, SETA, and WUE-DRYMAT occurred while the total volumetric soil water content (SUMWGT) decreased. i.e. the high Δ genotypes used more water as transpiration and evapo-transpiration increased and therefore produced higher yields compared with the low Δ genotypes. This result is consistent with the findings of another study [27]. Genotypes differed in physiological behaviour where genetic variation in TE was strongly influenced by environment [28]. CID in shoot tissue is a good indicator of cumulative stress of the crop as Δ decreased with increased salinity and reduced shoot biomass [29]. The differences in CID values were probably caused by stomatal limitation rather than by nutrient-related changes in photosynthetic efficiency [30]. The low values of the coefficients of variation between the different genotypes for the 26 years' data are very important for yield stability across different years. In general, the high Δ genotypes transpired more soil water, had higher WUE and therefore produced higher yield (grain or total biomass) compared with the low Δ genotypes.

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COMPARATIVE EVALUATION OF OXYGEN ISOTOPE COMPOSITION AND CARBON ISOTOPE DISCRIMINATION IN SELECTING FOR GREATER AGRONOMIC WATER USE EFFICIENCY IN WHEAT

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Abstract

The efficacy of using oxygen isotope composition ($\delta^{18}O$) and carbon isotope discrimination ($\Delta^{13}C$, CID or Δ) in selecting for greater agronomic water-use efficiency in wheat was studied. The objective was to determine whether natural-abundance measurements of $\Delta^{13}C$ or $\delta^{18}O$ of crop dry matter can be used to separate differences in access to soil water during grain filling from differences in assimilate re-translocation to the grain. The study consisted of carefully-controlled irrigation and rainfall-exclusion, which established two treatments with different soil-water availability at depth when they entered the post-anthesis (p-A) phase. Values of $\Delta^{13}C$ and $\delta^{18}O$ were measured on green leaves, senesced leaves, stem, non-grain ear parts (chaff) and grain from samples taken several times p-A from a field trial grown in SE Australia in 2004. Soil water content was measured from sowing to maturity using a neutron moisture meter, while roots were extracted from soil cores. Leaf porosity was also measured p-A using a Thermoline viscous-flow porometer and the values were related to stomatal conductance; in addition, canopy temperature was measured using a hand-held Mikron M-120 infrared thermometer. Substantial variation was observed in $\Delta^{13}C$ and $\delta^{18}O$ among plant parts, with the grain having the smallest values of $\Delta^{13}C$ and the largest values of $\delta^{18}O$. There were large changes in grain $\Delta^{13}C$ and grain $\delta^{18}O$ with time p-A. Grain-$\Delta^{13}C$ was smallest and grain $\delta^{18}O$ largest at 12 d p-A. At 27, 34 and 48 d p-A, the value of grain $\Delta^{13}C$ had increased by up to 1.5‰ and grain $\delta^{18}O$ had decreased by up to 2‰. The magnitudes of these changes with time p-A were consistent with observed contributions to grain dry weight from re-translocation of stored assimilates. No treatment differences were observed for grain $\delta^{18}O$. Small treatment differences found for grain $\Delta^{13}C$ late in grain filling may have been due to the small treatment difference in sub-soil water use. Additional data are needed from experiments in which differences in soil water use are greater than those found in this experiment to determine whether measurements of $\Delta^{13}C$ or $\delta^{18}O$ of crop dry matter could be used to separate the contributions to grain yield of assimilate re-translocation and use of extra soil water.

1. INTRODUCTION

Genotypic variation in $\Delta^{13}C$ measured in leaves of wheat plants has been shown to be negatively correlated with transpiration efficiency (TE) of leaf gas-exchange (i.e. the ratio of the instantaneous rates of photosynthesis and transpiration) and of plant growth (i.e. the amount of plant dry matter accumulated per unit water transpired by the plant over an extended period of days-months). These observations have prompted the hypothesis that selection for low $\Delta^{13}C$ in breeding populations may result in yield gains in dry environments. This hypothesis has been verified for wheat in some drought environments [1], but among cereal cultivars grown in Mediterranean-type climates, many studies have found that grain $\Delta^{13}C$ is actually positively correlated with grain yield [2]. Some plausible explanations proposed by Condon et al. [3] are: (i) that TE and yield are actually negatively correlated in these environments, due to pleiotropic effects on rate of growth associated with high TE, (ii) that high $\Delta^{13}C$ of grain reflects better access to subsoil water, which also contributes to higher yield, and (iii) that high $\Delta^{13}C$ reflects more effective re-translocation to the grain of stored assimilates acquired before grain filling, also contributing to higher yield. It is quite possible

that all three of these explanations are correct, and that all three may be operating at the same time.

In the absence of rainfall, wheats that are better able to access subsoil water may have more-open stomata than wheats that are less able to access such water. So, genotypic differences in stomatal conductance, and also canopy temperature, may reflect differences in access to water. But stomatal conductance is tedious to measure on large numbers of breeding lines, and measurements of stomatal conductance and canopy temperature are highly dependent on stable, sunny weather conditions. Measurement of stable isotopes laid down in plant dry matter may have more potential as screening tools for water extraction because they could provide a time integrated measure of differences in stomatal conductance. Variation in CID is somewhat related to variation in stomatal conductance, but variation in oxygen isotope composition ($\delta^{18}O$) may be more strongly related, and therefore may provide stronger relationships with water extraction. Genotypes that are better able to extract subsoil water during grain filling might be expected to have higher values of $\Delta^{13}C$ and/or lower values of $\delta^{18}O$ in the grain.

There may be another influence on the C and O isotopic composition of grain. Grain-filling in wheat relies to varying degrees on carbohydrates assimilated by photosynthesis during the grain filling period, and on carbohydrates acquired earlier in the season before grain filling, and stored mainly in the stems for later re-translocation to the grain. The C and O isotopic signatures of carbohydrates acquired by the crop during grain filling should reflect the prevailing conditions of evaporative demand and soil water availability during that period, whereas the C and O isotopic signatures of stored assimilates re-translocated to the grain are likely to be different, i.e. representative of conditions when those assimilates were first acquired. In Mediterranean-type environments, conditions are usually more favourable before grain filling than during grain filling, when soil water is being rapidly depleted and evaporative demand (vpd) is rapidly increasing. Given this scenario, genotypes that are more reliant on re-translocation of assimilates for grain filling might be expected to have higher values of $\Delta^{13}C$ and/or lower values of $\delta^{18}O$.

From plant breeding, molecular-marker development and gene-discovery perspectives, it would be very useful if the contribution to yield attributable to better access to subsoil water could be dissected from the contribution to yield attributable to re-translocation of assimilates. This may be possible by measuring the C and O isotopic composition of grain and other plant parts harvested before and during grain filling. Here we report on research aimed at evaluating whether measurements of $\Delta^{13}C$ or $\delta^{18}O$ could be used to distinguish effects of differences in access to soil water from effects of differences in assimilate re-translocation by wheat.

2. METHODS AND TECHNIQUES

Plant samples analysed for C and O isotope composition were taken from a field trial grown in 2004 for which detailed measurements of soil water extraction and assimilate re-translocation were obtained [4]. The experiment used carefully-controlled irrigation and rainfall-exclusion so that plots were manipulated to differ in soil-water availability at depth when they entered the post-anthesis phase.

2.1. Trial setup

Comprehensive details of the trial are presented in Kirkegaard et al. [4]. Briefly, a replicated field trial was established in the 2004 season near Junee, NSW, Australia (34° 43’

S; 147° 48' E). A single variety (cv. Janz) was sown in early June (early-winter) at a seeding-rate of 80 kg ha^{-1}. In July, the soil profile was filled to a depth of 1.35 m or to a depth of 1.85 m. There were four replicate plots (4 m × 2 m) for each of the two water-availability treatments. Up to ear-emergence (6 d before anthesis), rainfall and supplemental irrigation were applied to all plots so that the treatments were maintained with minimal stress. Then, moveable shelters were used to exclude all rainfall so that the subsoil water was depleted in a terminal stress during the post-anthesis (p-A) period.

Samples for total and component dry matter were harvested from each plot in the different treatments at regular intervals from anthesis (early October) through to crop maturity (early December). The plant sampling was complemented by measurements of leaf porosity and canopy temperature, soil water extraction using a neutron-moisture meter and of rooting depth at maturity (by soil coring and washing out the roots).

2.2. Measurements

Dry-matter samples for isotope analysis were dried (70 °C), finely ground, and analysed for carbon isotope composition ($\delta^{13}C$) using a Europa 'ANCA' sample preparation system and a Europa '20-20' isotope ratio mass spectrometer. Values of carbon isotope discrimination ($\Delta^{13}C$) were calculated assuming a $\delta^{13}C$ of air of –8‰. The same samples were analysed for oxygen isotope composition ($\delta^{18}O$) using the same make of mass spectrometer after pyrolysis of dry matter at very high temperature and separation of CO from N_2 using gas chromatography [5]. From each plot at each sampling time after anthesis, isotopic composition was measured on green leaves, senesced leaves, stem, non-grain ear parts (chaff) and grain. A sample of total above ground biomass taken in early September was also analysed. The post-anthesis samples were also analysed for water-soluble carbohydrate (WSC) concentration [6].

Soil water content was measured weekly from sowing to maturity using a neutron moisture meter calibrated at the site. Soil cores (3 $plot^{-1}$) were taken at maturity, roots were extracted by washing and root-length density determined to a depth of 1.9 m. Leaf porosity (POR) was measured using a Thermoline viscous-flow porometer. Raw data from the porometer (counts) was inverted (1/counts) to generate POR data linearly related to stomatal conductance over the range of counts measured on irrigated wheat plants [7]. POR data were collected on cloud-free days without high wind between 0900 h and 1500 h. Canopy temperature was measured under the same conditions using a hand-held Mikron M-120 infrared thermometer. Measurements were taken from the northern side (i.e. with the sun behind the user but without shading the plot) by directing the instrument diagonally downwards at the plot from a distance of 1–2 m.

2.3. Statistical analysis of data

Data transformation was not required. Statistical analysis (analysis of variance) was done using Genstat software.

3. RESULTS AND DISCUSSION

Pertinent data from Kirkegaard et al. [4] have been extracted and presented here in Tables 1 and 2, and in Fig. 1. Key points to note from the data are summarised below.

TABLE 1. BIOMASS ACCUMULATION, GRAIN YIELD AND POST-ANTHESIS WATER USE IN THE TWO TERMINAL STRESS TREATMENTS

Treatment	Biomass (g m^{-2}) at		Grain yield (g m^{-2})	Post-anthesis water use (mm)
	Anthesis	Final		
1.35m	1081	1505	602	75
1.85m	1083	1581	664	88
F-value	*ns*	*0.05*	*0.05*	*0.01*

TABLE 2. INDICATORS OF STRESS DEVELOPMENT DURING THE POST-ANTHESIS (P-A) PERIOD

Terminal stress treatment	Stomatal conductance, gL† (mmol m^{-2} s^{-1}) at			CTD‡ (°C)
	-2 d p-A, 1300 h	6 d p-A, 1000 h	12 d p-A, 0915 h	
1.35 m	540a	118a	8a	–1.1a
1.85 m	592a	104a	75b	–1.6b
Irrigated (adjacent)	-	748b	301c	–3.1c

† Within columns, numbers followed by the same letter are not significantly different.
‡Canopy minus air temperature.

3.1. Crop growth, water use and yield

- Anthesis biomass was high (~10.8 t ha^{-1}) and the same for both treatments (Table 1).
- The final biomass (≥15 t ha^{-1}) and grain yields (≥6 t ha^{-1}) obtained in the study (Table 1) were high for this region, reflecting the optimal irrigation and N management in the pre-anthesis period.
- Post-anthesis water use was greater for the 1.85 m treatment (Table 1). The 13 mm extra water extracted from this treatment was substantially less than the 35 mm of plant-available water between 1.35 and 1.85 m. The poor extraction presumably reflected the low root-length density observed below 1.35 m (data not shown here).
- The 13 mm extra water extracted from the 1.85 m treatment resulted in significantly greater final biomass and grain yield for this treatment, relative to the 1.35 m treatment (Table 1).
- Stomatal conductance (gL) at 2 d before anthesis (i.e. 4 d after the last water was applied to the terminal-stress treatments) was high and similar in both treatments (Table 2).
- Throughout the p-A period, the stomata in both treatments were substantially more-closed than in adjacent irrigated plots. The stomata in both terminal-stress treatments had begun to close by 6 d p-A, i.e., only 12 d after irrigation and rainfall were withdrawn from the terminal-stress treatments.
- The greater water extraction from depth for the 1.85 m treatment was reflected in higher gL at 12 d p-A and a more-negative CTD at 34 d p-A for this treatment, relative to the 1.35 m treatment (Table 2). By 12 d p-A, the stomata of the 1.35 m treatment were effectively shut. Those of the 1.85 m treatment were somewhat more-open, but not very open.

3.2. Contribution of re-translocation to grain yield

Grain yield achieved in both the terminal-stress treatments (602 and 664 g m^{-2}, see Table 1) was substantially greater than the calculated increase in standing biomass during the p-A period (424 and 498 g m^{-2}). Taken at face value, the yield and biomass data indicate that re-translocation of stored assimilates to the grain accounted for ~30% of the grain yield for the 1.35 m treatment and ~25% of the grain yield for the 1.85 m treatment. These estimates are supported by observations on time course changes in total biomass, stem biomass, stem WSC, grain yield and kernel growth (Fig. 1).

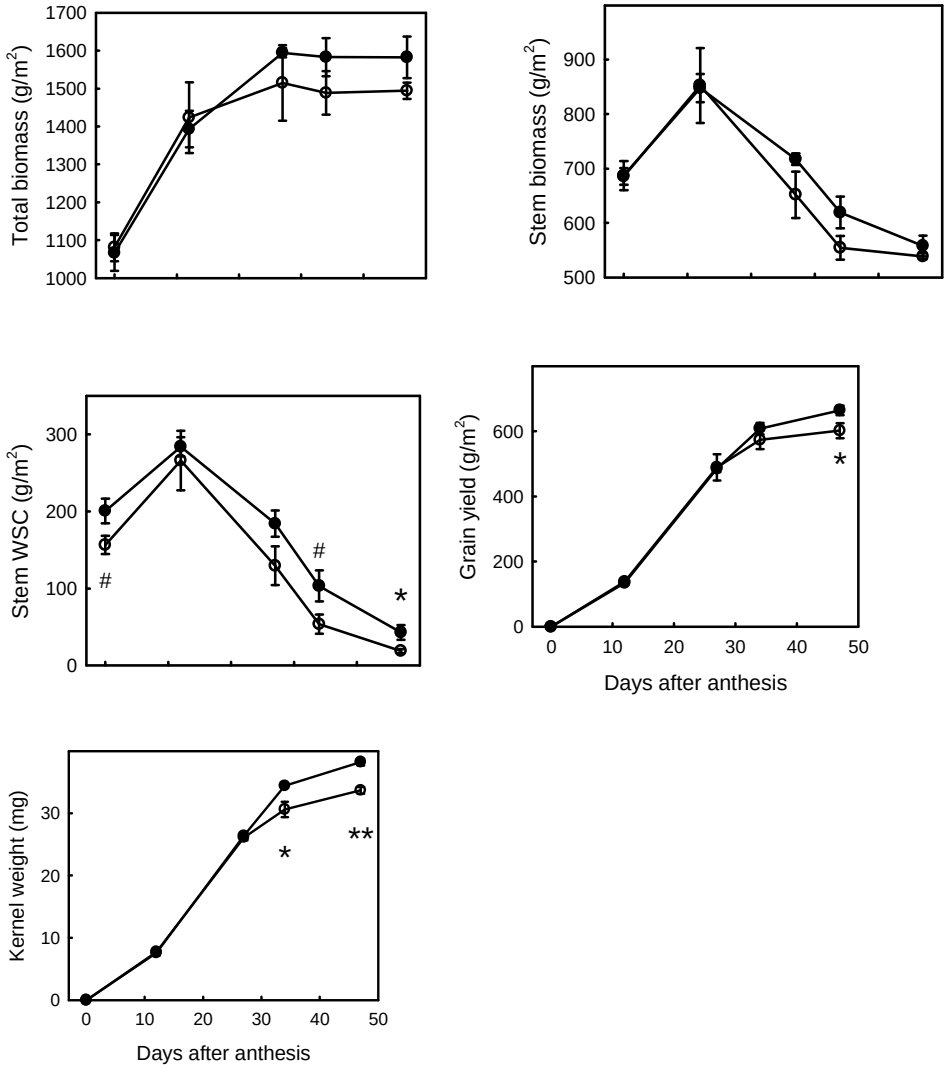

FIG. 1. Time courses of change in total biomass, stem biomass, stem WSC, grain yield and kernel weight during the post-anthesis (p-A) period with subsoil water to 1.85 m (●) and 1.35 m (o). The bars represent s.e.m, n = 4 at significance levels P<0.10 (#), P<0.05 (*) and P<0.01 (**).

Key points to note from Fig. 1 are:

- For the first 12 d p-A, increases in total biomass, stem biomass, stem WSC, grain yield and kernel weight were the same for both treatments.
- From 12 d to 27 d p-A, grain yield and kernel weight changes were the same for both treatments. Over the same period, the total biomass growth of the 1.35 m treatment slowed, relative to the 1.85 m treatment.

- From 12 d to 27 d p-A, stem biomass and stem WSC declined in both treatments. The declines seemed faster for the 1.35 m treatment, most clearly for stem biomass.
- Beyond 27 d p-A, there was no further increase in total biomass of either treatment, presumably reflecting the very low values of gL by this time (Table 2).
- Beyond 27 d p-A, grain yield and kernel size continued to increase. The rate of increase was slower for the 1.35 m treatment. Over this final phase, stem biomass for the 1.85 m treatment declined more than for the 1.35 m treatment.
- For both treatments, the declines in stem WSC beyond 12d p-A (~200 g m^{-2}) were in excess of that needed to account for the difference between final grain yield and the gains in total biomass during grain filling (~180 g m^{-2}). For stem biomass, the declines (~300 g m^{-2}) were well in excess of that required to account for the achieved grain yields.

For both treatments, re-translocation of assimilates from the stems could account for a substantial proportion of achieved grain yield (~25–30%). Total changes in stem biomass and stem WSC were very similar for the two treatments (i.e. the amounts of re-translocated assimilates were very similar), yet final biomass, grain yield and kernel weight were significantly greater for the 1.85 m treatment. The extra biomass growth and yield achieved for this treatment can be reasonably attributed to the extra water available at depth (i.e. the greater yield of this treatment was associated with greater water extraction). Time course changes in $\Delta^{13}C$ and $\delta^{18}O$ of dry matter components harvested from the two treatments were examined to determine whether measurements of $\Delta^{13}C$ or $\delta^{18}O$ of crop dry matter could be used to separate the contributions to grain yield of assimilate re-translocation and use of extra soil water.

3.2. Variation in carbon isotope discrimination

The mean $\Delta^{13}C$ of leaves (21.5‰), stems (20‰) and chaff (19‰) remained relatively constant during p-A (Fig. 2). Differences in $\Delta^{13}C$ between green and dead leaves and changes with time in $\Delta^{13}C$ of green and dead leaves are interpretable as a reflection of the changes in the relative amounts of these leaf components and the leaf positions contributing to them as the canopy senesced after flowering, from the base of the canopy (earliest-formed leaves) upwards. The trend to lower values of $\Delta^{13}C$ at anthesis, from leaf to stem to chaff, is similar to the trend observed in previous studies, in this case probably reflecting increasing stomatal closure in response to seasonally-increasing vpd and relatively minor depletion of soil water before anthesis. There were few significant difference between treatments for $\Delta^{13}C$ of leaf, stem and chaff dry matter (Fig. 2), apart from treatment differences in stem-$\Delta^{13}C$ observed at 2 d and 12 d p-A.

Grain sampled 12 d p-A had mean $\Delta^{13}C$ for both treatments of 15.5‰, substantially lower than for other plant parts. By 27 d p-A, mean grain $\Delta^{13}C$ had increased by 1‰ in both treatments, to 16.5‰. Over the following 7 d, grain $\Delta^{13}C$ remained at 16.5‰ for the 1.35 m water treatment but increased to 17‰ for the 1.85 m water treatment. This separation between treatments was maintained at maturity, 50 d p-A.

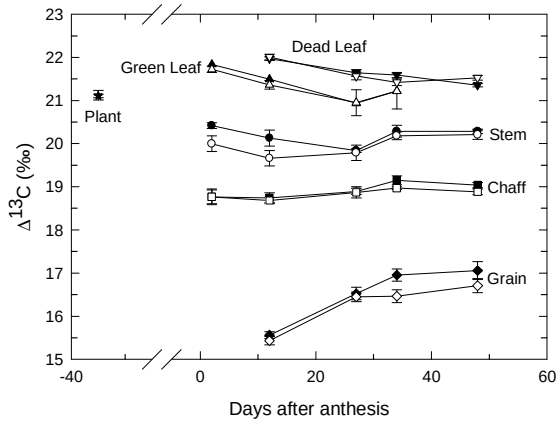

FIG. 2. Time course of variation in carbon isotope discrimination ($\Delta^{13}C$) measured in components of dry matter sampled during the post-anthesis (p-A) period with subsoil water to 1.85 m (closed symbols) and 1.35 m (open symbols). The bars represent s.e.m, n = 4.

3.3. Variation in oxygen isotope composition

There was a general trend to greater values of $\delta^{18}O$, from leaf to stem to chaff to grain (Fig. 3), the inverse of that observed for $\Delta^{13}C$. For $\delta^{18}O$, there was greater fluctuation with time of sampling than was observed for $\Delta^{13}C$. The $\delta^{18}O$ of leaves increased by ~3‰ from 2 d to 12 d p-A, and there was a similar change in $\delta^{18}O$ of chaff. In fact, at 2 d p-A, the $\delta^{18}O$ of chaff was ~1‰ lower than for the stem component. Over the course of sampling, the $\delta^{18}O$ of the green leaf component increased by ~4.5‰. As observed for $\Delta^{13}C$, there were few significant differences between treatments for $\delta^{18}O$ of leaf and chaff dry matter. Interestingly, and somewhat puzzlingly, the treatment difference of ~1‰ in stem-$\delta^{18}O$ observed throughout the p-A period was in the same direction as the differences in stem-$\Delta^{13}C$ observed at 2 d and 12 d p-A.

Grain sampled 12 d p-A had mean $\delta^{18}O$ for both treatments of 34‰, substantially greater than for other plant parts (Fig. 3). By 27 d p-A, mean grain $\delta^{18}O$ had decreased by 2.5‰, to about 31.5‰, in both treatments. Over the following period to maturity, grain $\delta^{18}O$ remained at 31.5‰, unchanged for both treatments, i.e. there was no separation between treatments as had been observed for grain Δ.

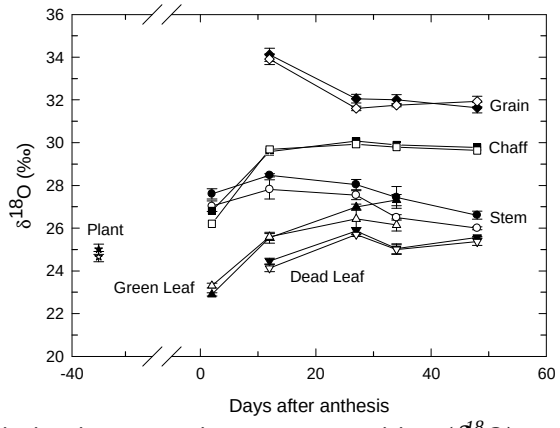

FIG. 3. Time course of variation in oxygen isotope composition ($\delta^{18}O$) measured in components of dry matter sampled during the post-anthesis (p-A) period with subsoil water to 1.85 m (closed symbols) and 1.35 m (open symbols). The bars represent s.e.m, n = 4.

3.4. Is it possible to interpret the measurements of $\Delta^{13}C$ or $\delta^{18}O$ to separate the contributions to grain yield of assimilate re-translocation and access to extra soil water?

The increases in grain $\Delta^{13}C$ (Fig. 2) and decreases in grain $\delta^{18}O$ (Fig. 3) with time between 12 d and 27 d p-A are consistent with the substantial declines in stem biomass and of stem WSC content observed over the same period (Fig. 1). There were substantial differences between values of stem/chaff $\Delta^{13}C$/ $\delta^{18}O$ and grain $\Delta^{13}C$/ $\delta^{18}O$ at 12 d p-A. Re-translocation of assimilates from the stem to the grain would be expected to change the values of grain $\Delta^{13}C$/ $\delta^{18}O$ in the directions observed. The sizes of the increases in grain $\Delta^{13}C$ (~1‰) and of the decreases in grain $\delta^{18}O$ (~2.5‰) are consistent with the calculated contribution from re-translocation of ~25–30% of final grain dry matter. If there was no such re-translocation, then the expectation would have been for a further small decline in grain $\Delta^{13}C$ and a further small increase of grain $\delta^{18}O$ with time as soil water was depleted and vpd of the air increased during p-A, noting that kernel weights were already ~70–80% of maximum by 27 d p-A (Fig. 1).

Re-translocation of assimilates, rather than effects of soil-water use *per se*, are the most likely explanation for changes in isotopic composition of grain beyond 27 d p-A. During the latter half of grain filling, the increase of grain yield and of kernel size in the 1.85 m treatment was about twice that in the 1.35 m treatment (Fig. 1). During the same period, the decline in stem biomass was also about two-times greater for the 1.85 m treatment (although less different for stem WSC). It was during this latter phase of grain growth that grain $\Delta^{13}C$ of the two treatments diverged. Puzzlingly, there was no divergence in grain $\delta^{18}O$ during this period. By 27 d p-A, the stomata of both terminal-stress treatments were almost closed (Table 2) and there was no further gain in biomass, in either treatment, after 27 d p-A (Fig. 1). Thus, any contributions to the values of grain $\Delta^{13}C$ or of grain $\delta^{18}O$ from assimilation during this period are likely to have been trivial and differences in isotopic signals due to differences in soil water use also trivial.

4. CONCLUSIONS

The observed changes in grain $\Delta^{13}C$ and grain $\delta^{18}O$, between treatments and with time p-A, are most easily explained on the basis of observed differences in assimilate re-translocation. The small differences between treatments in the use of sub-soil water would have contributed little to these changes. It would be useful to examine data from experiments in which differences in soil water use were greater than those found here. Despite rigorous and inventive generation of treatment differences in access to water available at depth, the actual amount of extra water used at depth was only a small component of total p-A water use, possibly due to low root density at depth. Stomata of both treatments closed quite quickly after anthesis, limiting the opportunity for generation of isotopic signals attributable to differences in soil water use.

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