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Development of Tolerant Crop Cultivars for Abiotic Stresses to Increase Food Security

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

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DEVELOPMENT OF TOLERANT CROP CULTIVARS FOR ABIOTIC STRESSES TO INCREASE FOOD SECURITY

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DEVELOPMENT OF TOLERANT CROP CULTIVARS FOR ABIOTIC STRESSES TO INCREASE FOOD SECURITY

PREPARED BY THE
JOINT FAO/IAEA CENTRE OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY
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FOREWORD

Salt stress (both salinity and sodicity) is the second most widespread soil problem after drought and is a serious constraint in rice production worldwide. Soil is considered saline if the electrical conductivity is above four units of its measurement scale. Rice is most sensitive to salt stress during the early seedling (1–3 weeks) and reproductive stages, when the threshold is as low as an electrical conductivity of three units of its measurement scale. Plant breeding for crop improvement can decrease the grain yield gap in salt-prone soils, thus contributing to food security and alleviating poverty.

This publication provides information on a system for phenotyping problem soils to identify tolerant lines for use in breeding programmes that target salt stress. The standard operating procedures presented here provide comprehensive information on (a) developing a suitable phenotyping system for the two main stages of crop sensitivity to salt stress, including prescreening of large sets of test lines in a controlled or semi-controlled environment and evaluation under field conditions, and on (b) reducing experimental error and improving repeatability of the experiment through proper stress management. The present publication is expected to be a valuable resource for plant breeders and field technicians involved in phenotyping of soils.

The IAEA wishes to thank N.K.A. Amoah (Côte d'Ivoire) and B. Manneh (Senegal) for their contributions to the preparation of this publication. The IAEA officer responsible for this publication was I.K. Bimpong of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture.

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CONTENTS

1. INTRODUCTION

The rapidly changing climate is causing different abiotic stresses, including salt stress, frequent floods, periods of drought and other stresses that reduce the yield potential of current rice varieties [1]. Among abiotic threats, salt stress is the second most devastating constraint in rice production after drought, affecting about a billion ha of land globally [2] and nearly 20% of the globally irrigated area [3]. Salt stress in arable land is mainly caused by the excessive use of irrigation water with improper drainage, poor quality of irrigation water containing an excess level of salts, and flooding from seawater [4]. Salt stress soils are usually in waterlogged conditions where other crops cannot grow and survive except rice. Rice is sensitive to salt stress during the early seedling and reproductive stages [5]. Sensitivity of the rice crop to salt stress at the early seedling stage (1–3 weeks) of crop development has a threshold as low as an EC of 3 dSm^{-1} [6, 7, 8].

Excess salt in soil adversely affects plant growth, development, and productivity when osmotic stress reduces water uptake by roots [9]. Direct accumulation of salts disturbs metabolic processes and all major morphophysiological and yield-related traits including tiller number, panicle length, spikelet number per panicle [7], grain filling [10], plant biomass [11] and photosynthesis[4, 12], leading to a significantly decreased yield.

The problem of salinization is expected to increase due to poor agricultural management practices and climate change. The decrease in rice productivity in salt affected areas can be addressed by plant breeding as its contribution to food security and poverty alleviation has been well documented [13, 14, 15]. Furthermore, other integrated approaches combining land reclamation and crop management can be used. Management practices are, however, not always feasible in the long term, as in coastal areas where salt stress is seasonal or in inlands where the cost of reclamation is high [16].

A screening method for salt stress tolerance could be readily acceptable when based on a simple criterion for selection and provides rapid screening of large numbers of materials and reproducible results.

This TECDOC is designed to provide two phenotyping methods to screen for tolerance to high salt at both the seedling and reproductive stages of a crop.

1.1. BACKGROUND

These guidelines were developed for Breeders and Field Technicians who manage phenotyping for tolerance to problem soils with the aim of identifying improved lines to use in breeding programmes targeting salt stress. The guidelines will enable them to:

(a) Develop a suitable phenotyping system for the two main stages of crop sensitivity to salt stress, including pre-screening of large sets of test lines in a controlled or semi-controlled environment and evaluation under field conditions;
(b) Phenotyping for salt stress tolerance using key effective secondary traits as a selection criterion. For a secondary trait to be effective as a selection, it needs to possess enough genetic variability, with high heritability, and it requires to be easier and less expensive to measure compared to grain yield itself and have positive genetic correlation with yield [17];

(c) Reduce experimental error and improve heritability of the experiment through proper stress management.

1.2. OBJECTIVE

This TECDOC aims to equip field crop breeders, agronomists, field technicians and students with detailed guidelines on how to manage phenotyping for problem soils with the aim of identifying tolerant lines in field crops to use in breeding programmes targeting salt and drought stresses.

1.3. SCOPE

The scope of this TECDOC covers how to manage crop phenotyping under controlled/semi-controlled environments and natural field conditions.

1.4. STRUCTURE

Two standard operation procedures (SOPs) guide the reader. These SOPs are: (1) A hydroponic system of screening under controlled conditions for the early seedling stage (1–3 weeks), and (2) natural field conditions for reproductive stages. However, a modification of the two SOPs will also be discussed under Artificial field environment.

2. HYDROPONIC SYSTEM OF PHENOTYPING IN CONTROLLED ENVIRONMENTS FOR EARLY SEEDLING STAGE TOLERANCE

The primary aim of this approach is its high-throughput nature which enables phenotyping large samples in a relatively short period (often under one month) to identify and select putative lines of interest for further advancement in the breeding cycle.

2.1. SCOPE AND FIELD OF APPLICATION

This SOP covers the pre-screening phase under controlled environments where basic items and chemicals are needed to establish and conduct the experiment.

2.2. BACKGROUND INFORMATION AND CONSIDERATIONS

This approach uses only nutrient solutions supplemented with excess salt (usual NaCl) without soil as the screening material with the assumption that differences in high salt tolerance expressed in these systems will result in improved performance in the field [18]. Furthermore, studies under controlled conditions generally involve imposing salinization on seedlings over a relatively short period and data generated does not generally include yield and its component traits [18].

This approach has proven to save time and resources compared to conventional phenotyping strategies [19].

2.3. SOME BASIC ITEMS NEEDED TO ESTABLISH THE HYDROPONIC EXPERIMENT

The key aspects for conducting a managed salt stress experiment under hydroponic conditions are described as per [20] and [21]. Most laboratories usually have most of the equipment mentioned (e.g. pH meter, balances, convection oven, magnetic stirrers, and others). Only very few items may be needed to complete the setup. This includes:

- pH meter;
- EC meter;
- Weighing balance (1000 g capacity and 0.0001 g readability);
- Magnetic stirrer;
- NaOH and HCI;
- Reagents (analytical grade) for nutrient solution [22] (Table 1);
- Glass bottles, 6 pieces of 1 L capacity, 12 pieces of 2.5 L capacity (dark glass);
- Volumetric flasks: 100, and 200 mL capacity;
- Graduated cylinders: 25, 50, and 100 mL;
- Plastic trays: 12 L capacity rectangular trays of size 14 × 30 × 35 cm (dark color trays are preferred);
- Beaker: 1000 mL;
- Styrofoam sheets (4.25 and 2.5 cm thick for making seedling floats);
- Nylon net (insect proofing type);
- Mixing containers: Cylindrical plastic containers, 50 L and 100 L capacity;
- Petri dish.

TABLE 1. PREPARATION OF NUTRIENT SOLUTION FOR HYDROPONIC SCREENING IN RICE

No.	Element	Reagent	Stock solution	Working solution
			Quantity to be used (g/L)	Quantity of stock solution/1 L nutrient solution (ml)
Macronutrient				
1	N	Ammonium nitrate (NH_4N0_3)	91.4	1.25
2	P	Potassium dihydrogen phosphate (KH_2PO_4)	29	1.25
		Potassium hydrogen phosphate (K_2HPO_4)	8	1.25
3	K	Potassium sulfate (K_2SO_4)	97.8	1.25
4	Ca	Calcium chloride, diydrate ($CaC1_2.2H_2O$)	175	1.25
5	Mg	Magnesium sulfate, 7-hydrate ($MgS0_4.7H_2O$)	324	1.25
Micronutrient				
6	Mn	Manganous chloride, 4-hydrate ($MnC1_3.4H_20$)	1.5	1.25
7	Mo	Ammonium molybdate, 4-hydrate [$(NH4)_6Mo_7O_{24}$-$4H_2O$]	0.074	
8	Zn	Zinc sulfate, 7-hydrate ($ZnS0_4.7H_20$)	0.035	
9	B	Boric acid (H_3BO_3)	0.93	
10	Cu	Cupric sulfate, 5-hydrate ($CuS0_4.5H_20$)	0.03	
11	Fe	Ferrous sulfate, 7-Hydrate ($FeSO_4.7H_20$)	2.5	
Supplement				
12		Agar (boil to dissolve and mix with nutrient solution at 60°C)	24	

2.4. CONSTRUCTION OF SEEDLING FLOATS

A float, as used here, refers to rectangular Styrofoam of size 28 × 32 × 1.25 cm having 100 holes (10 × 10) with a nylon net bottom and 2.5 cm thick frame pasted on top (Fig. 1). The frame helps fit the float to a rectangular plastic tray with 12-L capacity and 4 × 30 × 35 cm size (for detailed fabrication, see [20]).

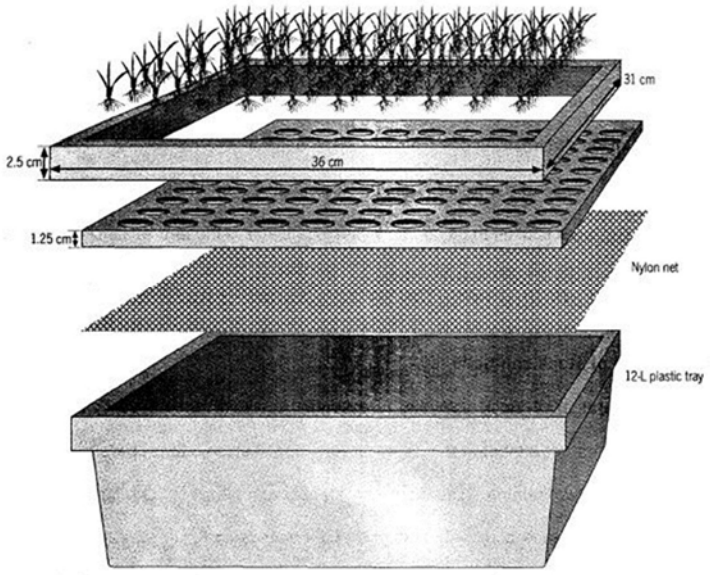


FIG. 1. Seedling float for high salt screening at the early seedling stage.

2.5. PREPARATION OF STOCK SOLUTIONS

Proper preparation of stock solutions is essential to avoid nutrient deficiencies and mineral toxicities not attributed to salt stress. For routine and continuing phenotyping, it is recommended to prepare fresh stock solutions every two months. The amounts prepared need to be dependent on the number of test entries to be screened during a two-month period. The pH of the nutrient solution requires to be maintained at 5.5 using NaOH or HCl as required.

2.6. PHENOTYPING FOR TOLERANCE TO SALT STRESS UNDER HYDROPONIC SYSTEMS

The hydroponic phenotyping method is adopted from Salinized Yoshida's solution [22], with slight modifications as per the protocol by Singh and Flower [23] whereby distilled water is replaced after three days in the experiment under salt stress.

Two sets of experiments need to be set up with one set representing the control (without treated salt) and the other with salt stress (treated with salt). If seed is not enough to replicate the experiment under control conditions, it is recommended to use two suggested checks (one tolerant and the other sensitive) to set up the control experiment. Besides, this check also need to be used in the salt treated set as a comparison to determine the performance of the tested lines.

Modifications related to the concentration of salt and the duration of the evaluation are as described below:

(a) Sterilize seeds' surface using 3% (v/v) sodium hypochlorite for 15 min and thoroughly rinse with tap water;
(b) Place the seeds on wet paper towel in clean Petri dishes and pre-germinate for three days under room temperature;
(c) Transfer individual germinated seeds into a perforated polystyrene sheet maintained floating in a plastic tank containing distilled water for three days (Fig. 2 and Fig. 3). This is followed by one week's growth in Yoshida culture solution [22] before the salt stress treatment is applied;
(d) Salt stress application consists of supplementing Yoshida nutrient solution with 5.13 mM of NaCl (approximately 3.0 gL^{-1}) to bring the electrical conductivity (EC) of the solution to 6.0 dSm^{-1} (Fig. 3 and Fig. 4);
(e) Increase salt concentrations to 12 dSm^{-1} after three days of being under the 6 dSm^{-1} treatment to reduce the immediate shock;
(f) Increase salt concentrations to 18 dSm^{-1} three days of being under the 12 dSm^{-1} treatment to reduce the immediate shock;
(g) In all, ensure that the daily pH is maintained at approximately 5.0 for the entire duration of the phenotyping (Fig. 5);
(h) For the non-stress setup, allow the seedling to grow without supplementation of NaCl to the nutrient culture;
(i) Maintain pH at approximately 5.0 as in the case of the stress setup;
(j) Renew the Yoshida nutrient solution every week and maintain pH at approximately 5.0 as reported in the protocol adopted from [20].

FIG. 2. Stage of transferring seedlings to perforated styrofoam to set up screening for early seedling stage salt stress tolerance.

FIG. 3. Transferring seedling to perforated Styrofoam to set up screening for early seedling stage salt stress tolerance.

FIG. 4. Chemical preparation of Yoshida nutrient solution and measurement of pH and EC of the experimental set up.

FIG. 5. Chemical preparation of Yoshida nutrient solution and measurement of pH and EC of the experimental set up.

2.6.1. Possible modification for high throughput optimization

Higher throughput can be achieved using about 10 floats per tank and a direct supply of nutrient solution through an easy drainage and filling of tanks. This has the chance of minimizing the variability between the floats (Fig. 6).

FIG. 6. New approach being optimized.

2.7. TEST ENTRIES AND CHECKS

Each Styrofoam float has 10 rows with 10 holes per row and it needs to contain the test entries and the two standard check varieties in every styrofoam float to guide in rating the visual symptoms of salt stress. At the AfricaRice Research Station in Ndiaye, Senegal, two check varieties are used; lR29 which is sensitive, (improved varieties from IRRI), and Hasawi, a new tolerant donor from Saudi Arabia [24] (Fig. 7 and Fig. 8).

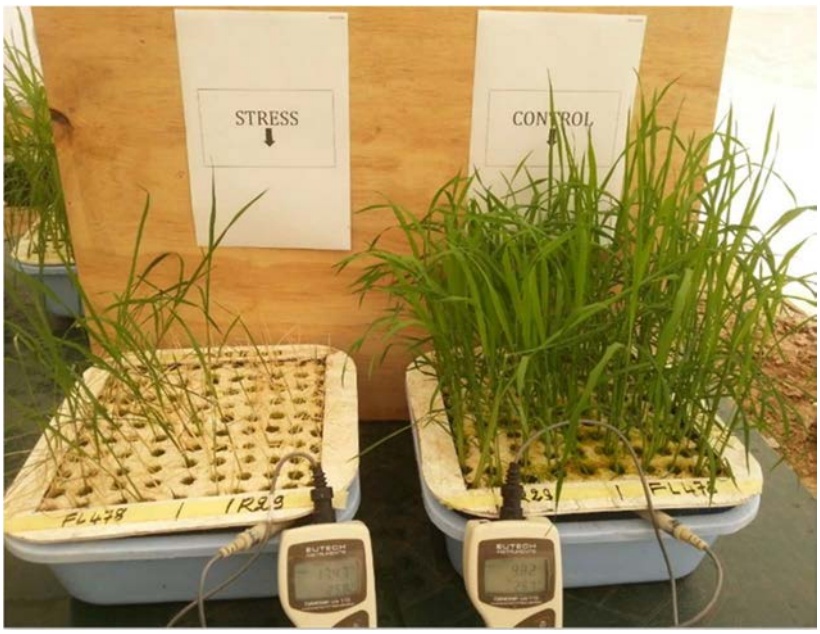


FIG. 7. Phenotyping at EC of 18 dSm^{-1} and non-stress (EC under 1 dSm^{-1}) between two checks (FL478: tolerant and IR29: susceptible).

FIG. 8. Phenotyping at EC of 18 dSm^{-1} and non-stress (EC under 1 dSm^{-1}) between two checks (Hasawi: tolerant and IR29: susceptible).

2.8. CALIBRATION OF PH AND ELECTRICAL CONDUCTIVITY (EC) METERS

The pH of the culture solution needs to be regularly maintained at approximately 5.0 as any error in the pH may cause some toxicity and/or deficiency of nutrients not related to salt stress. This is done using two buffers in calibrating at pH 4 and pH 7 (Figs 9a and b). The EC meter determines the number of electrolytes present in the solution, which is associated with the degree of salt stress. Errors in EC readings from the meter could easily be detected because a pre-measured amount of NaCl ought to register the expected EC level (6 g NaCl gives an EC of 12 dSm^{-1}).

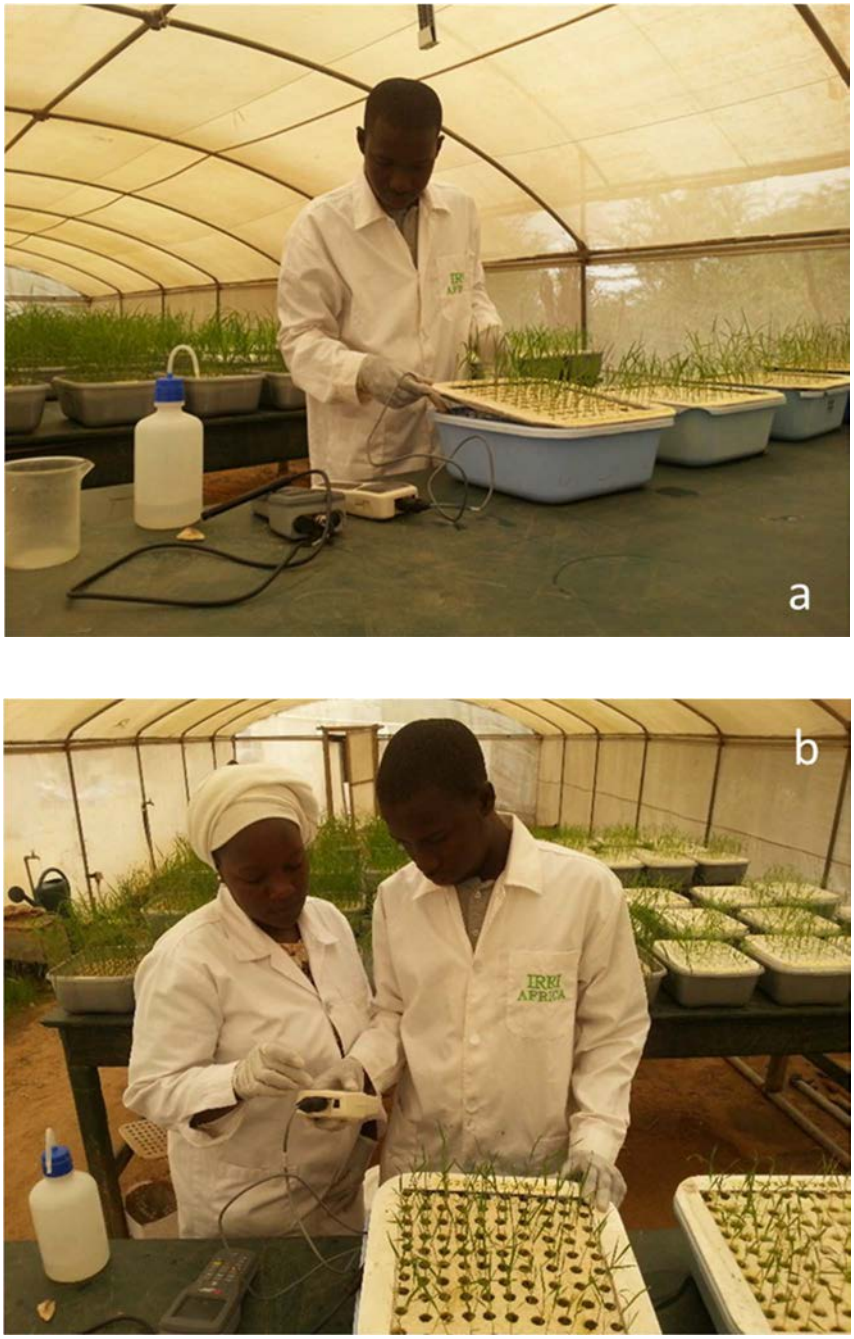


FIG. 9. (a) and (b) Monitoring of pH and EC of the experimental set up.

2.9. EVALUATION OF SALT STRESS SYMPTOMS

Salt injury symptoms ought to be visually assessed using a scale of one (no symptom on the plant) to nine (dead plant) following the Standard Evaluation System for rice [25] (Table 2). The initial, second and third data on evaluation of salt injury score (SIS), plant height [PH (cm)], shoot and root length [RL (cm)] needs to be recorded on each test entry.

TABLE 2. STANDARD EVALUATION SYSTEM FOR RICE

Score	Symptom/Observation	Degree of tolerance
1	Normal growth, only the old leaves show white tips while there are no symptoms on young leaves	Highly tolerant
3	Near normal growth, but only leaf tips burn, few older leaves become whitish partially and rolled	Tolerant
5	Growth severely retarded; most leaves severely injured, few young leaves elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dried; only a few young leaves still green	Sensitive
9	Almost all plants dead or dying	Highly sensitive

This scoring differentiates between the susceptible, tolerant and the moderately tolerant test entries.

Scoring may start at 10 days after salinization and final scoring at 16 days after salinization. At 10 days after salinization, the tolerant check, Hasawi needs to score one and the susceptible check IR29 ought to score seven. During this time, susceptible test entries could be distinguished from the rest of the test entries. However, tolerant types cannot be readily identified from the moderate ones. At 16 days after salinization, Hasawi needs to have a score of three, IR29 ought to be dead with a score of nine. At this stage, a clear distinction among the test entries can be observed between the tolerant, moderate and the susceptible test entries (Fig. 10 and Fig. 11). At the end of the experiment, surviving plants needs to be transferred into pots for seed production.

FIG. 10. Effect of salt stress on shoot biomass at the end of the phenotyping.

FIG. 11. Effect of salt stress on root biomass at the end of the phenotyping.

2.9.1. Some results of evaluating rice lines under salt stress condition

This method has been successfully used for screening germplasm and breeding populations at the screenhouse of the AfricaRice Sahel Regional Station in Senegal. We observed that this method is easier to differentiate between susceptible and tolerant types. In one such evaluation, 2380 segregated populations (BC_3F_2) were phenotyped under hydroponic systems of which 500 had a SES score of one and were advanced for screening in the naturally saline fields at Ndiol, Senegal to confirm the reliability of this screening technique (Fig. 12) [26]. This method of visual symptoms of salt stress after 16 days of salinization as the selection criterion for rapid screening of large populations of breeding materials is reliable.

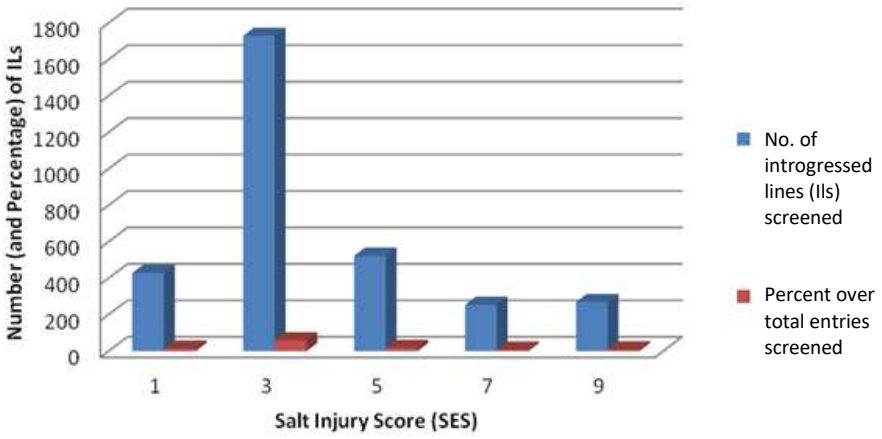


FIG. 12. Scoring of introgression lines after surviving at an EC of 12 dsm^{-1} under hydroponic conditions.

3. NATURAL FIELD CONDITIONS FOR PHENOTYPING AT THE REPRODUCTIVE STAGE

Screening of test entries under natural field conditions where there is no tampering of variables and the environment is not altered to select the best entries is important as the resultant variety will be grown under such very similar conditions for productivity.

3.1. SCOPE AND FIELD OF APPLICATION

This SOP covers (i) field preparation and experimental design(s), (ii) understanding the target population of environments, (iii) selection of the precision phenotyping site, (iv) establishing the salt stress phenotyping site, (v) managing variation in phenology, (vi) crop management, (vii) soil data, and (viii) management of salt stress.

3.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Very few studies have been reported on phenotyping for salt tolerance on yield, and yield components at the reproductive stage of rice, as most research has been limited to the early vegetative stages [27, 26]. Managing the field phenotyping of large populations in high salt soils with high precision and repeatability would be the ideal situation since varieties would be tested in the target environments or in very similar conditions.

"Hotspot" phenotyping for high salt stress (an area with a relatively high level of salt which can seriously affect crop production) is needed to design proper plant breeding programmes focused on developing varieties with tolerance to salt stress for the low-income farmers. At the AfricaRice Research farms at Institut Sénégalais de Recherches Agricoles (ISRA) research field in Ndiol, Senegal (16_32.141 N; 15_11.545 W) located in the Senegal River Valley and close to the coast (about 40 km inland), soil salt stress in the river delta is generally high due to the occurrence of marine salt deposits in the subsoil. EC of the saline field ranges between 6.5 and 9.5 dSm^{-1} [24]. This serves as a very good hotspot for phenotyping.

3.3. FIELD PREPARATION AND EXPERIMENTAL DESIGN(S)

In addition to controlling weeds and provision of soft soil mass for transplanting and a suitable soil surface for direct seeding, a well-prepared field also improves aeration and drainage, and provides easy space for growth and development of roots. Using experimental design enables researchers to control each variable on its own or in different combinations to study what possible outcomes are available for the experiments. This enables the researcher to obtain accurate results.

3.4. UNDERSTANDING THE TARGET POPULATION OF ENVIRONMENTS

Understanding the target population environment (TPE) is crucial in identifying and selecting the best suitable environment where the phenotyping site required to be established. Analysis of this information will help in describing the exact type of salt stress and understanding the necessary requirements for establishing a phenotyping site that is significantly related to the TPE.

3.4.1. Scope and field of application

This SOP covers information about the target population of environments to help analyse and define the type of salt stress (salinity or sodicity) and to understand all the requirements for

establishing a phenotyping site that is significantly related to the target population of environments.

3.4.2. Background information and considerations

The success of any phenotyping for stress depends on a clear understanding of the target population of environments. It is important to plan and select the best representative selection environment where the phenotyping site ought to be established. The phenotyping site needs to have a good representation of the target population of environments. Some basic information needed on the target population of environments to establish a good phenotyping site are:

- Weather information on Rainfall, Relative Humidity, Temperature;
- Daily data for soil PH and EC readings at least for the past three to five years;
- History of the field including soil profile, cropping season especially for the targeted crop (rice) and cropping system;
- Causes of salt stress at the site; is the cause due to marine salt deposits in the subsoil or from poor crop and water management through inefficient irrigation schemes, poor infrastructure, poor hydrological controls and/or poor use of fertilizer;
- Information on other major abiotic and biotic stresses and socio-economic constraints in that environment.

This information will help in defining the most relevant type of salt stress (sodicity or salinity) and also in understanding the requirements for establishing a phenotyping site that is representative of the target population of environments.

3.5. SELECTION OF PRECISION PHENOTYPING SITE

Based on the analysis of the past daily data for the soil pH and EC reading for all potential locations, a suitable site can be identified. Besides, other weather conditions ought to be suitable for growing the crop at the selected location in the planting window identified for the salt stress phenotyping trial. For example, in rice breeding for salt stress tolerance the targeted crop stage is flowering and early grain-filling stage.

3.5.1. Background information and considerations

It is important to conduct a managed salt stress screening during a rain-free period, where stress is imposed by broadcast application of NaCl (which is the common cause of salt stress) and managing the irrigation schedule in such a way that all the tested entries are exposed to a desired level of salt stress at the targeted stage.

Based on the analysis of the past daily data for the soil pH and EC reading for all potential locations, a suitable site can be identified. Besides, other weather conditions require to be suitable for growing the crop at the selected location in the planting window identified for the salt stress phenotyping trial. For example, in rice breeding for salt stress tolerance the targeted crop stage is flowering and early grain-filling stage.

At the AfricaRice Research farms at Institut Senegalais de Recherches Agricoles (ISRA) research field in Ndiol, Senegal the soil at the site is a typical Orthithionic gleysol and contains at least 10 mg C kg^{-1} of soil and 5 mg P kg^{-1} (P-Bray1). The climate at this site is characterized by a wet season with approximately 200 mm rainfall per year from July to October, which is suitable for growing the rice crop. Therefore, this site is identified as a suitable location for salt stress screening, where transplanting can take place in mid-July so that a trial with about 125 day maturity group of entries under irrigated conditions can reach the peak flowering stage

around mid-October, and most critical stages of reproductive phase complete within the month of October. At this site salt stress could be imposed at a desired intensity (and duration) and timing (targeted crop stage) with good uniformity by measurements and regular monitoring of the salt concentration in the fields for adjustment as necessary.

3.6. ESTABLISHING THE SALT STRESS PHENOTYPING SITE

Based on the analysis of the target population of environments and the identification of a representative testing site, a field screening can be established for salt stress.

3.6.1. Background information and considerations

It is important to ensure that the selected field satisfies some basic requirements such as:

- Being well characterized/mapped to determine the stress heterogeneity and the presence of other soil-related stresses. This can be achieved by growing a buffer crop of a single variety (preferably the same crop) in order to identify the heterogeneity in the field. A soil conductivity meter can also be used to access spatial field variations;
- A good irrigation (and drainage) facility to avoid random salt stress or false positive results during the experiment;
- A well-levelled field to facilitate smooth waterflow during irrigation to avoid water run-off and stagnation in patches.

3.7. MANAGING VARIATION IN PHENOLOGY

Managing variation in phenology is critical for establishing testing groups of similar maturity. If the experiments were not established with strict maturity blocks, salt stress would occur at different growth stages across the tested lines, preventing the breeders from interpreting the yield data properly. The goal is to impose the drought stress treatment at the same stage of development (flowering, for example) for all experimental lines within each testing.

3.7.1. Background information and considerations

The phenotyping experiment needs to be carried out with test entries grouped based on duration and height to ensure uniformity within the trial. This is important to avoid different levels of stress within the experiment. Staggered planting (where long duration entries are planted early, and early duration entries are planted late) can be considered in the situation where it is impossible to have a uniform group of test entries.

3.8. CROP MANAGEMENT

It requires proper crop management practices such as timely application of recommended inputs and agronomic operations to obtain high quality output from phenotyping.

3.8.1. Background information and considerations

- It is suggested to follow all recommended crop management practices such as timely application of recommended inputs and agronomic operations during the trials;
- Avoid missing hills as the number of plants per unit area is one of the components of final grain yield; it is recommended to plant extra seeds per hill and thin-out extra seedlings after plant establishment;

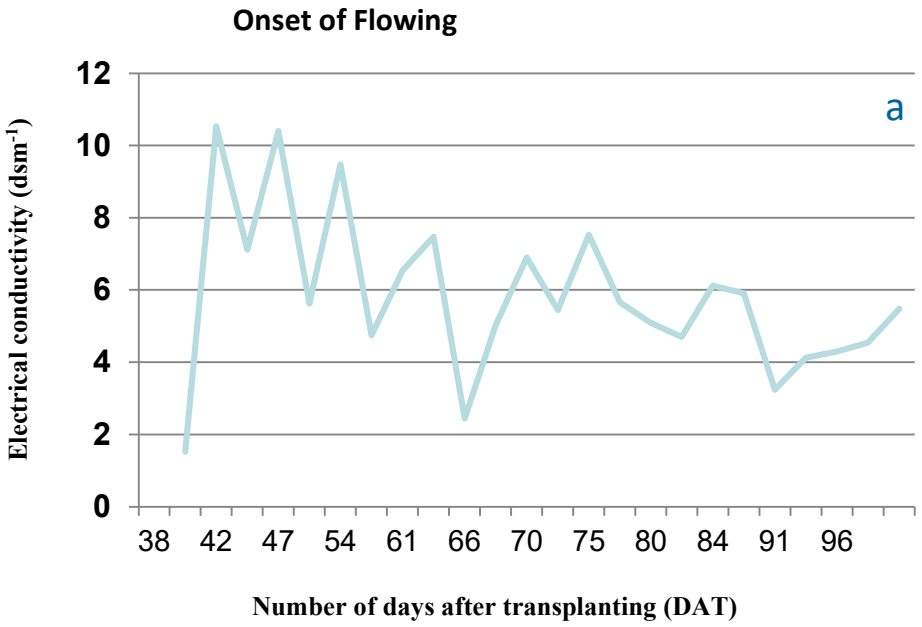
— Border rows ought to be planted around the trials to prevent border effects on test-entries and any physical damage;
— Applications of inputs such as fertilizer (time of application and doses), weed, insect-pest and disease control measures are usually location-specific and depend on soil physical and chemical properties and common biotic pressures[1]. It is essential therefore to have updated information on the recommended practices for the phenotyping site and ensure that they are implemented on time, in order to keep the crop free from nutrient stress and any biotic stresses[2];
— Presence of other stress agents that influence plant growth and functions and can limit the accuracy of phenotyping ought to be prevented so that it does not interfere in the screening process.

3.9. SOIL PROFILING

In field-based salt stress screening, it is extremely important to document soil pH and EC patterns that could significantly change the overall effects of salt stress experienced by the crop.

3.9.1. Scope and field of application

This SOP covers the need for soil profiling to determine the soil pH and EC, as well as how to install soil pH and EC probes and data recordings (Figs 13a and b).



[1] www.ncbi.nlm.nih.gov
[2] www.ncbi.nlm.nih.gov

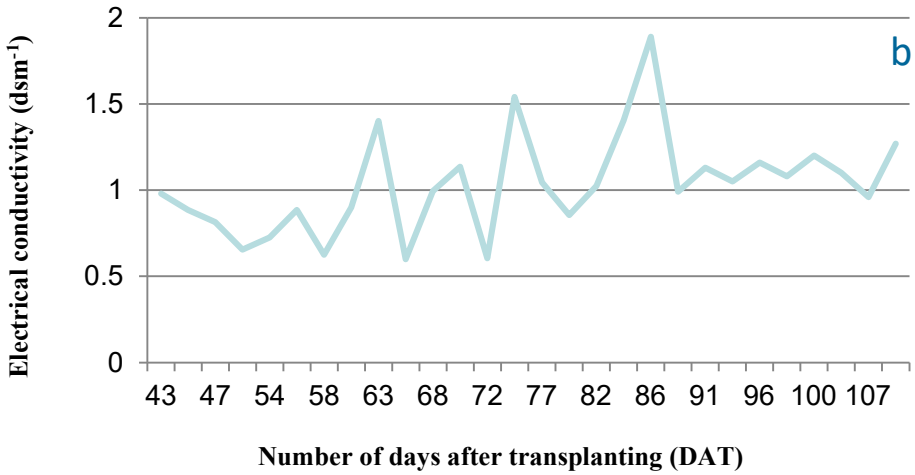


FIG. 13. Salt stress level monitored under (a) hotspot and (b) control or non-stress fields.

3.9.2. Background information and considerations

Regular recording of soil EC and pH data after imposing salt stress aids in regular monitoring of stress development in the field and achieving the desired level of stress, which will lead to the attainment of high-quality phenotyping data. The setup needs to be completed with the installation of piezometers after all mechanical field operations are completed. These piezometers are specially constructed and are supplied along with given guidelines for proper installation and use. The number of piezometers to be installed in a field depends on the spatial variability in the field. At least one piezometer in each block of experimental design is recommended.

3.9.3. Recording soil pH and EC

Soil pH and EC data need to be recorded at a regular interval, at least two times a week at the onset of the application of the salt, until the stress is relieved, or the field is drained and re-irrigated. Measuring soil pH and EC allows the repetition of such an experiment under similar conditions as well as more rigorous assessment and interpretation of the results. It also enables the quantification and documentation of the level of stress applied.

The most reliable criteria to make any informed decision about when to stop the stress (wash and re-irrigate) needs to be based on the monitoring of the soil pH and EC after imposition of the salt stress.

The three key steps for a successful salt stress phenotyping at the reproductive stage of the crop are (i) time of imposing salt stress (ii) monitoring soil pH and EC and (iii) terminating the stress at an appropriate time. Besides, high-quality data related to various agronomic and yield traits needs to be also collected.

3.9.4. Weather data

In hotspots of phenotyping trials, it is essential to generate weather data (including relative humidity and rainfall) that could have a significant impact on the overall effects of salt stress experienced by the crop, hence the need to install a portable weather data recorder within the phenotyping field for regular data recording.

Note: It is recommended that each activity carried out under the stress screening requires to be conducted for the non-stress trial.

3.10. MANAGEMENT OF SALT TOLERANCE TRIALS

The key factors for successful implementation of most abiotic stress phenotyping under field conditions are timing, intensity, and uniformity of the stress.

3.10.1. Scope and field of application

This SOP covers the three main determinants in managing a successful high salt phenotyping trial. This includes the application of salt stress at the desired intensity and precise uniformity.

3.10.2. Background information and considerations

The major aspects in conducting salt stress phenotyping under field conditions are described as follows:

- Timing of the stress needs to be planned such that the targeted growth stages are exposed to the desired level of salt stress;
- Intensity of the stress needs to be severe enough to identify the differences between important yield and related traits under stress when compared to non-stress fields;
- The uniformity of the stress is very crucial for the tested entries to express the variability within a trial that could be clearly observed and recorded.

3.10.3. Evaluation of tested lines for tolerance to salt at hotspot

For the present discussion, we will assume the use of the single tiller method in conducting the hotspot screening. The principle behind the single tiller approach is that annual cereal plants evolved from perennial wild species in which a single or a few tillers removed from the mother plant and planted could grow and produced grains. The performance/characters of replanted tillers are then compared with those of corresponding undisturbed plants. Enough seeds can be produced for generation advancement and grain yield [24, 28, 29].

3.10.3.1. Field layout

A proper layout of the field is important, as it offers a way to test hypothesis with higher external validity, because they simulate actual occurrences. It also serves as a better guard against potential biasness in the research output. The main guidelines for a proper field layout are as follows:

(a) Establish a seedling bed by growing >1000 seeds of tested lines, and the two checks (one tolerant and another sensitive);
(b) Prepare the field at the site where the experiment will be conducted by removing weeds, ploughing, proper levelling (with a laser leveller if available), raising of bunds (to contain standing water);
(c) Transplant approximately 500 seedlings from the tested lines 25 days after seeding under controlled conditions at a spacing of 20 × 20 cm;
(d) Transplant approximately 500 seedlings to each of the two checks 25 days after seeding, side by side of each tested line at a spacing of 20 × 20 cm;

(e) Four weeks after transplanting, remove a single tiller from each of the 500 mother plants (tested lines and each of the two checks) and transplant into the hotspot site to be used for the stress phenotyping;
(f) Follow the same design as used in points 3–5 for the management of transplanting and establishment of the stress phenotyping;
(g) After transplanting, maintain approximately 5 cm of standing water in the field until drainage before harvesting the control trial (no stress trial);
(h) Follow all recommended crop management practices such as timely application of recommended inputs and agronomic operations during the trials as per the local recommendations;
(i) Measurements of soil pH and EC ought to be monitored regularly to determine the salt concentration and adjust accordingly (Fig. 14 and Fig. 15).

FIG. 14. Monitoring of Soil pH and EC at a phenotyping hotspot.

FIG. 15. Monitoring of Soil pH and EC at a phenotyping hotspot.

3.10.3.2. Application of salt stress

The timely application and the uniformity of the stress is very crucial for the tested entries to express the variability within a trial that could be clearly observed and recorded. The following are the steps in the application of salt stress:

(a) Maintain approximately 5 cm of standing water in the field until initiation of the stress then drain the field;
(b) Induce salt stress at the booting stage of the crops by broadcasting the measured quantity of granular salt representing the type of salt stress in your location into the standing water in the field. At AfricaRice Sahel Regional research farms, granular NaCl, representing the type of salt stress found in most parts of Senegal River valleys, is used;
(c) Monitor the two-standard check varieties to guide in rating the visual symptoms of salt stress;
(d) Measure soil pH and EC regularly to determine the salt concentration and adjust accordingly. At AfricaRice Sahel Regional research farms, salt concentration is retained at EC of >6 dsm^{-1} and at 1 dsm^{-1} in the control plots;
(e) Start irrigation with water when you observe that all the leaves are rolling in the tolerant check. Then no more stress until maturity and harvest.

3.10.3.3. Scoring for tolerance

With the application of the stress at the appropriate time and intensity, the expression of the tested entries within a trial could be clearly observed and scored. Listed below are the steps to follow in scoring for tolerance to salt stress:

- The susceptible check will express stress symptoms (leaf rolling and whitening) about three weeks after initiation of salt stress;
- Salt tolerance score (SES score) at maximum tillering/panicle initiation stage, and two weeks after flowering (sterility) using the standard evaluation system (SES) of the International Rice Research Institute, in which a score of one was for a tolerant plant, three for moderately tolerant, and a score above five was for a susceptible plant (Table 2).

3.11. KEY SECONDARY TRAITS TO RECORD

Record the following data on each of the 500 tested lines, for each of the two check varieties:

- Days to flowering/heading (days), i.e. the average number of days from seeding until the panicles has flowered;
- Plant height (centimetre), measure from the soil surface to the tip of the tallest panicle (awns excluded); this needs to be measured 'prior to booting and just before harvesting';
- Number of tillers per plant; each tiller per plant require to be counted. This need to be measured 'prior to booting and just before harvesting';
- Panicle Sterility, i.e. number of unfilled spikelets deducted from the total number of spikelets per plant and expressed as percentage (%);
- Grain yield (g m^{-2}), number of grains per unit area;
- 1000-grain weight (g), i.e. the average weight of 1000 seeds from the sample harvested grains from the plants;
- Yield per plant (g m^{-2}), for each hill is harvested to estimate the grain yield from each plot. Seeds needs to be dried (50ºC), weighed, and adjusted to a moisture content of 14%;
- Yield-component data for each plot, based on the number of tillers, panicle counts, filled and unfilled grain;
- The percent reduction for each trait in stressed plants is calculated relative to the non-stressed control.

3.11.1. Some results of screening rice lines under hotspots and control conditions

To confirm the reliability of this screening technique, the 500 plants in the segregated population with visual salt-injury symptoms score of one from the hydroponic conditions were further screened for four seasons under hotspots and control conditions. Subsequently the results of the two screening conditions were compared for their actual grain yield and other parameters. Sixteen of them had less yield loss (3–26%) relative to the non- stress conditions, of which eight showed high yield potential under stress and non -stress conditions. Table 3 shows these comparisons. The score based on visual symptoms relates well to grain yield under salt stress field conditions and yield reduction due to salt stress [26].

TABLE 3. PROMISING BREEDING LINES IDENTIFIED THROUGH SCREENING UNDER SALINE STRESS IN 2013–2014 DS AND WS AT THE AFRICA RICE SAHEL STATION FARMS, NDIAYE AND NDIOL, SENEGAL

	Plant height (cm)		Tiller number / plant		Days to 50% flowering (days)		100-seed weight (g)		Percent panicle sterility (%)	Grain yield (gm^{-2})		Percent yield loss (%)
Designation	Stress	No stress	Stress	No stress	Stress	No stress	Stress	No stress	Stress	Stress	No stress	
IL-003	79.58	98.72	18.25	19.86	95	96	28.5	35.14	20.59	343.87	477.35	27.96
IL-009	79.08	97.21	14.67	15.06	96	95	26	30.72	22.82	359.36	421.62	14.77
IL-019	86.33	105.6	18	18.79	95	95	27	31.4	30.99	322.45	454.44	29.04
IL-022	74.96	107.6	17.29	18.25	95	94	27.34	29.15	17.35	240.54	319.82	24.79
IL-041*	81.75	98.26	14.63	16.3	95	94	13.67	29.69	48.17	474.41	676.26	29.85
IL-058*	77.75	100.01	23.5	19.9	92	95	28.17	38.39	18.4	406.39	647.66	37.25
IL-063*	82.92	100.74	15.08	23.7	95	95	23.67	32.33	45.77	431.38	665.31	35.16
IL-065*	88.63	111.37	18.88	22.94	94	93	28.25	38.75	13.04	314.21	399.38	21.33
IL-068*	86.17	116.28	13	22.14	95	94	16.67	22.43	43.03	289.6	293.7	1.4
IL-070	86.5	109.71	21.08	22.31	94	96	27	27.49	24	249.54	309.05	19.26
IL-071*	76.08	107.7	19	24.08	96	94	27.67	36.49	35.65	357.19	379.38	5.85
IL-072	82.08	106.24	18.17	23.08	95	96	26.67	35.33	29.77	359.69	538.73	33.23
IL-073*	80.17	116.04	23.92	20.14	93	95	29.17	36.35	25.59	429.29	527.09	18.55
IL-092	83	97.63	16.33	22.24	92	94	22.67	41.28	39.92	402.91	649.59	37.97
IL-106*	84.25	96.95	20	20.68	95	95	23.17	30.61	47.19	339.69	530.22	35.93
IL-112	77	99.93	16.25	17.78	94	94	23.33	26.6	39.28	377.03	507.78	25.75
Rassi (RP)	70.41	93.31	14.4	17.36	97	93	11.08		87.08	115.34	499.98	81.68
FL478 (donor)	77.44	90.67	15.2	24.34	83	86	27.03	27.39	63.1	255.16	411.2	37.95
Mean	80.78	103	17.65	20.5	93.94	94.11	24.28	32.33	36.21	337.11	483.81	
$LSD_{.05}$	3.68	2.35	1.53	1.39	1.5	1.1	2.63	2.6	9.11	42.52	60.77	

** top 8 ILs that are stable across the two environments (saline and non-saline)*

4. POSSIBLE MODIFICATIONS FOR FIELD PHENOTYPING

Natural field screening is challenging due to the heterogeneous nature of high salt occurrence even in hotspots. Artificial fields can be created through the erection of concrete tanks at the depth that does not hamper free growth of the roots of the tested lines.

4.1. PRE-SCREENING UNDER SEMI-CONTROLLED ENVIRONMENTS AT THE REPRODUCTIVE STAGE

In this system, the selection criteria for improving high salt stress tolerance is done using sand-based systems under semi-controlled environments, with the assumption that differences in high salt tolerance expressed in these systems will result in improved performance in natural fields [3].

4.2. SCOPE AND FIELD OF APPLICATION

Salt stress tolerance in crops during the flowering stage is important to warrant high yield under high salt environments. To achieve this, requires placing greater emphasis on developing an improved cultivar with reproductive stage salt tolerance, so as to minimize the yield penalty under salt stress. This SOP covers phenotyping for the reproductive stage of the crop.

4.3. BACKGROUND INFORMATION AND CONSIDERATIONS

Artificial fields can be created through the erection of concrete tanks at the depth that does not hamper free growth of the roots of the tested lines. This is preferred to pot experiments where there is a complete loss of plant-plant interaction when pots are used. However, the main drawback of this approach is the capital intensity of it to develop and maintain. Filling the tank may be challenging due to regulations on digging up and transporting target soils.

4.4. MATERIALS AND INSTRUMENTS NEEDED

The technique can be modified to suit any specific requirements. There are no restrictions on the type of soil, fertilizer use, salt stress level and compounds/chemicals used to obtain salt stress. The effects of salt stress on growth and reproduction is estimated by growing test entries in both salt stress and non-salt stress tanks.

4.5.CONSTRUCTION OF A CONCRETE TANK

- Concrete tank (75 m length, 2.5 m width, 1 m depth; subject to user preference), having a width of 2.5 m will allow 10 hills in a row, enabling the researcher to move freely in order to attend to the tested entries and also record data freely in any part of the tank without an obstruction to other tested plants (Fig. 16);
- Equipment for rain-out shelter;
- Soil; loamy and sandy -preferably fill the base of the concrete tank up to 20 cm with sand and 50 cm with loamy. The remaining 20 cm will be for irrigation water. Take precautions to ensure a level surface to avoid localization of nutrients at one side of the tank.

FIG. 16. Concrete tanks mimicking natural field growth conditions but with the advantage of regulating soil conditions (picture by Nana Kofi Abaka Amoah).

The setup needs to be completed with the installation of at least one piezometer per tank to monitor soil pH and EC.

4.6. TEST ENTRIES AND CHECKS

The test entries require to be grouped based on duration and height in order to have a uniform condition from flowering to maturity. Tolerant and sensitive checks need to be used for comparison with the test entries.

4.7. EVALUATION OF TESTED LINES FOR TOLERANCE TO SALT STRESS

- Pre-germinate the seeds of test entries in a petri-dish. Place the seeds in a petri dish and incubate in a cool, dry place at room temperature for two days; seeds ought to begin sprout during this period;
- By the time the seeds start sprouting, nursery beds ought to be already in place for the installation of a seed nursery;
- Make the soil ready by fertilizing (using the local recommendation for the basal application). At the AfricaRice Sahel regional station in Ndiaye, Senegal, a recommended rate of 12:22:22 NPK (200 kg/ha is used as the basal application);
- Transplant 21-day old seedlings using a spacing of 20 cm × 20 cm into the concrete tank following an appropriate field design/technique, one seedling per hill.

4.8. APPLICATION OF SALT STRESS

- To initiate salt stress, water is completely siphoned out of the concrete tank at the booting stage of the crops;
- Prepare a saltwater solution up to the desired EC level by dissolving table salt (NaCI) in water by stirring. With the table salt used at the AfricaRice Sahel regional station, 4.5 g in 1 L gives an EC of 8 dSm^{-1};

- Fill up the concrete tanks with saltwater solution. The water level needs to be maintained daily (1 cm above soil surface) by adding ordinary water and not saltwater;
- The salt level used at the AfricaRice Sahel Regional screenhouse is EC of 8.0 dSm^{-1};
- Salt stress in the concrete tank will gradually decrease and stabilize at around 2–3 EC units less than the original. The soil salt level is more than the water bath and climatic conditions seem to influence the degree of increase. The EC stabilizes at around 11 dSm^{-1};
- If the salt stress level needs to be adjusted in the course of the experiment, this is done by draining all the water and replacing with new saltwater to the desired EC level;
- Measurements of soil pH and EC needs to be monitored regularly to determine the salt concentration and adjusted accordingly;
- All recommended crop management practices such as timely application of recommended inputs and agronomic operations during the trials as per your local recommendations ought to be followed;
- Monitor the two standard checks varieties to guide in rating the visual symptoms of salt stress;
- The susceptible check will express stress symptoms (leaf rolling and whitening) about 21 days after application of the salt stress.

4.9. SCORING FOR TOLERANCE

A few days after application of the stress, the tested entries will express different stress symptoms, which will be clearly observed and scored. Below are the guidelines to follow in scoring for tolerance to salt stress:

- Monitor the two standard checks varieties to guide in rating the visual symptoms of salt stress. The susceptible check will express stress symptoms (leaf rolling and whitening) about three weeks after initiation of salt stress;
- Data recording on test entries can be done from then onwards based on visual symptoms as described by the IRRI SES for salt stress (Table 2);
- It is suggested that data on the test entries needs to be recorded every two weeks;
- Grain yield per plant and other yield parameters can be collected and used as an indicator for advancement of putative test entries.

4.10. PRIORITY TRAITS TO RECORD

In addition to the SES score data recorded, the following needs to be recorded:

(a) Days to flowering/heading (days), i.e. the average number of days from seeding until the panicles have flowered;
(b) Plant height (centimetre), measure from the soil surface to the tip of the tallest panicle (awns excluded); this needs to be measured ‘prior to booting’ and ‘just before harvesting’;
(c) Number of tillers per plant; each tiller per plant requires to be counted. This need to be measured ‘prior to booting’ and ‘just before harvesting’;
(d) Panicle Sterility, i.e. number of unfilled spikelets deducted from the total number of spikelets per plant and expressed as a percentage (%);
(e) Grain yield (g m^{-2}), number of grains per unit area;
(f) 1000-grain weight (g), i.e. the average weight of 1000 seeds from the sample harvested grains from the plants;
(g) Yield per plant (g m^{-2}), each hill is harvested to estimate the grain yield from each plot. Seeds needs to be dried (50ºC), weighed, and adjusted to a moisture content of 14%;

(h) Yield-component data for each plot, based on the number of tillers, panicle counts, filled and unfilled grain.

5. EXAMPLES OF SOME RELEVANT FIELD TECHNIQUES

5.1. SINGLE TILLER METHOD FOR GENERATION ADVANCE IN PLANT BREEDING

One of the foremost challenges to breeders is to identify trait(s) that would confer grain yield advantage in crops challenged by some abiotic stresses (low moisture, high salt stress, extremes of temperature and drought, etc.), and devise suitable selection strategies, as phenotyping some abiotic stress tolerance components involves destructive sampling.

The single tiller approach can be used as a method to select putative breeding lines for generation advance for many important traits [24, 28]. This is because the annual cereal plant evolved from perennial wild species in which a single or a few tillers removed from the mother plant and planted could grow and produce grains. The performance/characters of replanted tillers are then compared with those of corresponding undisturbed plants. Sufficient seeds can be produced for generation advancement and grain yield. It ought to be stated that grain yield for single-tiller crops does not represent the true potential of it but progeny testing on bulk advanced offspring could be used instead.

5.2. PROCEDURE

Drought is a recurrent phenomenon and an important constraint to rainfed rice production. It is the most significant environmental constraint for rice production in sub-Saharan Africa (SSA) [30]. Progress in genetic improvement of rice for drought environments has been slow and limited [31] due to understanding of the inheritance of drought tolerance in rice and lack of efficient techniques for screening breeding materials [32]. Grain yield under drought stress is a complex quantitative trait, and its heritability is thought to be low relative to yield under control [33]. Hence, much of the work on improving drought tolerance in rice has been done targeting secondary traits under water stress [34].

In several studies, broad-sense heritability of grain yield under reproductive-stage drought stress has been observed to be comparable to that of grain yield estimated under nonstress conditions [35, 36, 37, 38, 39, 40], indicating direct selection for yield under moisture stress is likely to be effective.

In this layout, our emphasis is on phenotyping for drought tolerance in cereals using rice as a case example.

(a) Establish a seedling bed by growing >1000 seeds of the tested lines, and the two checks (one for tolerant and another sensitive);
(b) Prepare the field at the site where the experiment will be conducted by removing weeds, ploughing, proper levelling (with a laser leveller if available), raising of bunds (to contain standing water);
(c) Transplant approximately 500 seedlings from the tested lines 25 days after seeding under controlled conditions at a spacing of 20 × 20 cm;
(d) Transplant approximately 500 seedlings from each of the two checks, 25 days after seeding side by side of each tested line at a spacing of 20 × 20 cm;
(e) Four weeks after transplanting, remove a single tiller from each of the 500 mother plants (tested lines and each of the two checks) and transplant/use to re-constitute in the site

earmarked for the drought phenotyping while conserving the same layout as used in the control stress setup. Thus, tested progeny number one under stress set-up is a tiller from progeny number one under control, hence the same genotype;

(f) Care needs to be taken so that tillers are not harvested too old, in which case, it will be induced to flower early and will not fully express its vegetative growth;

(g) Follow the same design as used in points 3–4 for the management of transplanting and establishment of the stress phenotyping;

(h) After transplanting maintain approximately 5 cm of standing water in the field until drainage before harvest for the control trial (no stress trial);

(i) For the drought stress trial, maintain approximately 5 cm of standing water in the field until initiation of drought stress then drain the field (Fig. 17).

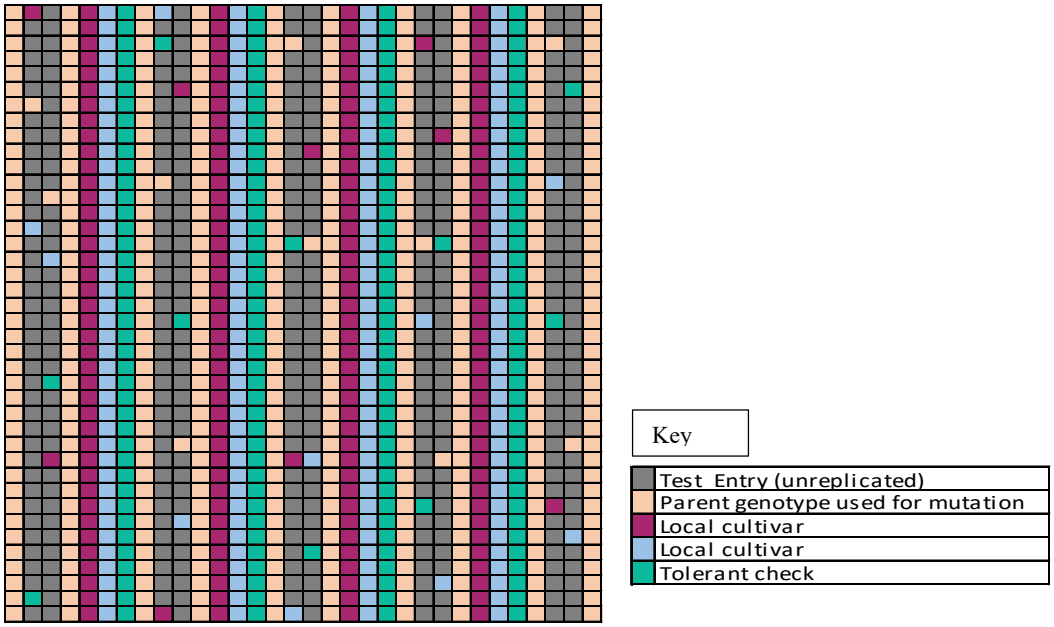


FIG. 17. Illustration of a single tiller approach embedded in a classical augmented design.

5.3. APPLICATION OF DROUGHT STRESS

Timely initiation of drought and the uniformity of its application is important for the tested entries to express the variability within the trial, which could be clearly distinguished and recorded. The following are the steps in the application of drought stress:

(a) Induce drought in the trial by restricting/preventing water from getting into the field for a period of 15–18 days, beginning at the flowering stage;

(b) Monitor only the drought tolerant check entries to see if all the leaves have started rolling and are burnt at the tip of the leaves;

(c) Start irrigation with water when you observe that all the leaves are rolling in the drought tolerant check;

(d) Repeat the stress cycle again by restricting or preventing water from entering the field for a period of 15–18 days. This regime will result in leaf-rolling and tip-burning at the end of each drying cycle;

(e) After this 15–18-day period, the treated plants need to be re-watered at 10-day intervals until maturity (No more stress until maturity and harvest);

(f) Leaf-rolling (LR) and leaf-drying (LD), needs to be recorded at weekly intervals after the second stress cycle. Visual scoring (0 to 9) need to be used according to the "Standard evaluation system for rice" [25] (in which a score of one is for tolerant line, three for moderately tolerant, and a score above five is for a susceptible line);
(g) In addition, all other secondary and yield-components traits listed elsewhere in this TECDOC needs to be recorded;
(h) All recommended crop management practices such as timely application of recommended inputs and agronomic operations during the trials as per your local recommendations ought to be followed.

Conclusion

The present publication is expected to be a valuable resource for field crop breeders, agronomists, field technicians and students who manage phenotyping for problem soils with the aim of identifying tolerant lines in field crops to use in breeding programmes targeting salt and drought stress.

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ABBREVIATIONS

EC	Electrical conductivity
FAO	Food and Agriculture Organization of the United Nations, Rome
dSm^{-1}	DeciSiemens per metre
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
HCI	Hydrochloric acid
L	Litre
mL	Millilitre
g/L	Gram per litre
^{o}C	Degree Celsius
cm	Centimetre
IRRI	International Rice Research Institute
SES	Standard evaluation system
Gm^{-2}	Gram per metre square
N	Nitrogen
P	Phosphorus
K	Potassium

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