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J. Neil Rutger in his "laboratory" in September 2002, at Stuttgart, Arkansas, observing an early flowering indica mutant line (left) and its late flowering parent (right) (see page 9)

To Our Readers

First of all, I would like to inform you that the Plant Mutation Reports (PMR) is now indexed in the CAB ABSTRACTS and GLOBAL HEALTH databases, run by the CABI, Wallingford, UK. This is not only an important recognition of the publication and, ultimately, the quality of your papers, but also provides a great opportunity for the broad dissemination of papers published in PMR.

Secondly, it is a great honour to include a review paper from Dr. J. Neil Rutger, former director of Dale Bumpers National Rice Research Center USDA-ARS-SPA, USA. Dr. Rutger's paper summarizes the extraordinary success of his 30 years of work on induced mutations in rice genetics and breeding, together with his contribution to the Joint FAO/IAEA Programme. In particular, you will learn how a single induced mutation can contribute to the substantial yield increase in rice. We are aware that great success has also been achieved in rice as well as in other crops by various groups around the world; we invite you to submit papers of this kind to highlight your accomplishments or summarize your professional careers in mutation induction, application or basic studies on mutagenesis.

Thirdly, I would like to share with you our plan for further improvement of the quality of the PMR: (1) We are planning to establish an editorial board next year (see be-

low); (2) We are planning to transform the cover appearance and paper format into the style of a scientific journal; (3) For expanding the manuscript's source and quality, we decided that all final technical papers from the Agency's coordinated research projects (CRPs) and regional and interregional technical cooperation projects (TCPs) in the field of plant breeding and genetics be published in the PMR. (4) We will also strive for a broader distribution of the PMR; free electronic subscription will be granted to all interested institutions and individuals.

However, subscription to the printed copy will still only be free for institutions, including research units, within a large organization (see page 20).

Last but not least, we encourage you to contribute your manuscript to the PMR. We assure you that once your paper is identified as being of scientific value, we will offer you our assistance with improving the presentation and the language of the manuscripts. For more information, see page 37 for Author's Guidelines.

Qingyao Shu

Plant Mutation Reports Editorial Board

We are planning to establish an editorial board for the Plant Mutation Reports. It will consist of about 15 editors / associate editors specialized in the following fields of plant research: (1) DNA damage, repair and mutagenesis; (2) Insertion mutagenesis; (3) Experimental mutagenesis with artificial mutagens; (3) Crop breeding and genetics with induced mutations; (4) Genomics and molecular genetics of induced mutations; (5) Mutational analysis and mutant germplasm.

Interested scientists, please submit a short C.V. (2-3 pages) to plant.mutation@iaea.org for consideration; final selection will be made on the basis of both the candidate's expertise and balance of each field.

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Review

Thirty Years of Induction, Evaluation, and Integration of Useful Mutants in Rice Genetics and Breeding

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Abstract

The author's 30 years of achievements with induced mutations in rice genetics and breeding are summarized, beginning with temperate japonica mutants in California, and then continuing to tropical japonica and indica mutants in Arkansas. Throughout these studies, emphasis has been on selecting agronomically useful mutants such as semidwarfism and early maturity. The author notes that to realize the full value of mutants, induction should be closely followed by evaluation of the mutants and then their integration into cross-breeding programs. Evaluation and integration should be done concurrently insofar as possible. The best example was induction and release of Calrose 76, the first semidwarf table rice cultivar in the USA. Subsequent evaluation and integration steps by rice breeders have resulted in 25 improved semidwarf cultivars that trace their ancestral source of semidwarfism to Calrose 76: 13 in California, 10 in Australia and 2 in Egypt. Since 1993 induced mutation has been used to develop and release numerous tropical japonica mutants at the Dale Bumpers National Rice Research Center in Stuttgart, Arkansas. In the last six years induced mutation has been used to develop indica rice germplasm adapted to the USA. Previous and current induced mutants were used to establish the Genetic Stocks-Oryza Collection (GSOR) in 2003. Over 50 rice mutants collected by the author and his associates have been released as germplasm or entered into GSOR. The author has had extensive involvement with IAEA in participation in international symposia, Research Coordination Meetings, and as a mutation breeding consultant in IAEA activities in Latin America and Asia.

Key words: Rice mutants, temperate japonica, tropical japonica, indica, cross-breeding, genetic stocks, improved germplasm

Introduction

My 30 years of experience with rice mutants started in 1971 in Davis, California so it might seem like that I cannot subtract very well and should say 35 years, but in 1989 I began an administrative assignment which took me away from rice research for nearly 5 years. I was fortunate to be able to return to full-time rice research in late 1993 at the Dale Bumpers National Rice Research Center (DB NRRC) in Stuttgart, Arkansas, with most of my emphasis again on rice mutants. Throughout my career I have concentrated on selecting agronomically useful mutants such as semidwarfism and early flowering, with occasional detours into mutants such as male steriles and marker genes as genetic tools. My "laboratory" for induced mutation studies has been the rice field, since that is where useful mutants eventually will have to pass muster. In the last decade I have ventured into a base-broadening program with indica rice that is, developing indica rice for adaptation to our japonica rice-growing nation. Once again, in the indicas, induced mutation is playing a useful role.

During most of my 30 years I literally walked over or ignored a lot of what I called "curio" mutants, such as extreme dwarfs which were only 20 cm tall-a real problem when the irrigation water is 25 cm deep!-albino mutants, etc. In the last 5 years I have come to realize that these "curio" mutants can have value as genetic stocks for basic research studies, so my colleagues and I began collecting them for our Genetic Stocks-*Oryza* (GSOR) Collection which was founded at Stuttgart in 2003.

California temperate japonicas

The author's introduction to useful applications of induced mutation in rice genetics and breeding was in the early 1970s in California, with the development and release of the first semidwarf table rice cultivar in the USA, Calrose 76 (Rutger et al., 1977). Prior to the author's arrival in 1970, Dr. Chao-hwa Hu of Taiwan, who had experience with induced mutation in his country, had received an IAEA research award to study in California. In advance of his arrival, Dr. Hu asked Dr. C.O. Qualset of the University of California Davis (UCD) to mutagneize seeds of leading California rice cultivars. Dr. Qualset arranged for the seeds to be mutagenized at UCD and planted by Dr. W.F. Lehman at the UC Imperial Valley Field Station in southern California. When Dr. Hu's California visit was delayed the M1 seeds were stored at the Imperial Valley Field Station until the author picked them up and planted them at UCD in 1971. The semidwarf plant which ultimately became Calrose 76 was selected in the M_2 generation by Dr. Hu in 1971 during his year as a Visiting Scientist at UCD. He also made other selections for short stature and early maturity. After Hu's return to Taiwan in 1972 Rutger pursued genetic and agronomic evaluation of the mutants (Rutger et al., 1976), resulting in Calrose 76 (Figure 1) (Rutger et al., 1977).

Another cultivar, M-101, was developed by integration of the mutant semidwarf gene into cross-breeding by combining it with an early flowering mutant gene, and the gene for glabrous leaves and hulls from the closely related cultivar CS-M3 (Rutger et al., 1979). Keys to the success of this work were 1) inducing mutants in a very good cultivar, Calrose, which needed only a couple of corrections, in this case, semidwarfism and earlier maturity, and 2) immediately evaluating the mutants agronomically and genetically, and 3) then integrating them into conventional crossbreeding efforts.

Figure 1. The induced mutant Calrose 76, released in 1976, was the first semidwarf table rice cultivar in the USA. It is about 25% shorter than its tall parent, Calrose, and the closely related tall cultivar CS-M3. Calrose 76 and its derivatives have been widely used as the ancestral source of semidwarfism in breeding programs in California, Australia, and Egypt. Calrose 76 carries the sd1 semidwarfing allele.

Concurrently with the cultivar releases Rutger and his students determined that the semi dwarfing gene in Calrose 76 was allelic to *sd1* from DGWG (Foster and Rutger, 1978a), and that it was independent of the widely used gene for glabrous leaves and hulls (Foster and Rutger, 1978b). Other studies included inheritance of an early maturity mutant (McKenzie et al., 1978), and inheritance of additional semidwarfing genes (Mackill and Rutger, 1979).

Rice breeding colleagues in California immediately pursued cross-breeding Calrose 76 with the tall cultivar CS-M3 to produce M7 (Carnahan et al., 1978). In cultivar x nitrogen fertilizer rate studies the two semidwarf cultivars Calrose 76 and M7 averaged 14% more grain and 13% less straw than the tall check cultivar CS-M3 (Figure 2) (Brandon et al., 1981). In farm practice yields of 20-25% were commonly observed since the higher nitrogen fertility levels resulted in lodging and consequent yield decreases in the tall cultivars. California growers very quickly began adopting semidwarf cultivars.

By 2005 the total number of California cultivars, including Calrose 76 itself, that traced their semidwarfism ancestry to Calrose 76 had grown to 13 (Table 1). The most recent of those cultivars, Calamylow-201, not only carries the semidwarf gene but also was itself an induced mutant for a second characteristic, a speciality low amylase (ca 6%) type which is expected to be useful for a new developing rice market (McKenzie et al., 2006). So Induced mutations are being pyramided! Calrose 76 ancestry appears in the pedigrees of 10 additional California cultivars resulting from crosses between the Calrose 76 source and other semidwarf sources, mostly IR8 or DGWG (K.S. McKenzie, Director of California Cooperative Rice Research Foundation, personal communication, August 22, 2005). Molecular technology now makes it possible to determine exactly which parent contributed the semidwarf allele gene in such semidwarf x semidwarf crosses. For example, the most successful cultivar in

California for the last two decades, M-202 (Johnson et al., 1986), derived from crossing the Calrose 76 source and the IR8 source, was recently shown to carry *sd1* from IR8, while S-101, another cultivar resulting from crossing the two sources, carries *sd1* from Calrose 76 (T.H. Tai, Rice Geneticist, Davis, California, personal communication, October 17, 2006).
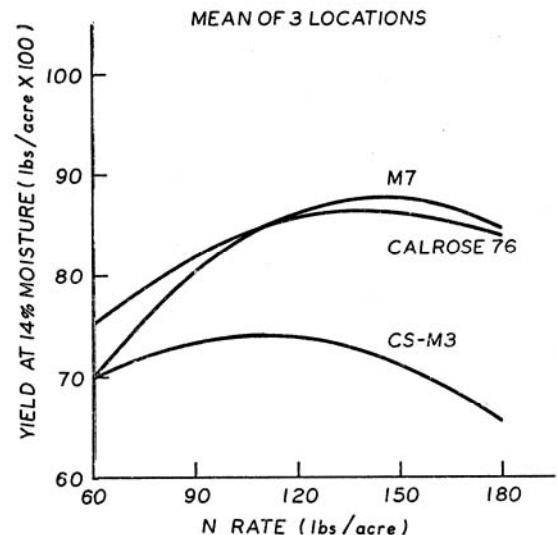
Figure 2. Averaged over nitrogen fertilizer rates from 60 to 180 lb/acres (67 to 202 kg/ha), the two semidwarf cultivars Calrose 76 and M7 yielded 14% more grain and 13% less straw than the tall cultivar CS-M3 (Brandon et al., 1981).

In Egypt 2 semidwarf cultivars were developed using the Calrose 76 source of semidwarfism (Table 2). In Australia an additional 10 semidwarf cultivars traced their ancestry to M7, the California glabrous leaf cultivar which received its semidwarfing gene from Calrose 76 (R. Reinke, Rice Breeder, Yanco Agricultural Institute, personal communication, December 21, 2005).

The experimental semidwarf Short Labelle was selected in California from an M_2 population grown from bulk M_1 seed of the cultivar Labelle supplied by C.N. Bollich, USDA-ARS, Texas. Short Labelle was determined to be nonallelic to *sd1* and was evaluated for productivity in the southern US, but was not released as yields were generally lower than the parent cultivar (McKenzie and Rutger, 1986). The semidwarf cultivar Mercury, released in Louisiana, was selected from the cross Short Mars/Nato (McKenzie et al., 1988). Short Mars, a semidwarf selection made at Davis, California, from a mutagenized population of the tall Arkansas cultivar Mars, showed some segregation for maturity and may have resulted from an outcross. Genetic studies indicated that Short Mars carried a semidwarfing gene allelic to *sd1* (McKenzie et al., 1988).

An interesting spontaneous mutant for elongated uppermost internode, *eui*, inherited as a recessive tall plant type, was postulated to have breeding value in hybrid rice seed production, that is, tall male plants would be desirable for pollen dispersal onto short female plants, and

being recessive, the tall plant type would not be expressed in the desired semidwarf F_1 crop (Rutger and Carnahan, 1981). Allelic *eui* mutants were later found to occur in japonica germplasm (Mackill et al., 1994), as well as in indica germplasm (Rutger, 2005).

Table 1. California cultivars for which Calrose 76 served as the ancestral source of semidwarfism

Cultivar	Year	Pedigree	Reference
Calrose 76	1976	Induced mutant of Calrose	Rutger et al., 1977
M7	1978	Calrose 76/CS-M3	Carnahan et. al., 1978
M-101	1979	CS-M3/Calrose 76//D31	Rutger et al., 1979
M-301	1980	Calrose 76/CS-M3//M5	Johnson et al., 1980
S-201	1980	Calrose 76/CS-M3//S6	Carnahan et al., 1980
M-302	1981	Calrose 76/CS-M3//M5	Johnson et al., 1981
Calmochi-101	1985	Tatsumimochi//M7/S6	Carnahan et al., 1986
S-101	1988	70-6526//R26/Toyohikari/3/M7/74-Y-89//SD7/73-221	Johnson et al., 1989
M-103	1989	78-D-18347/M-302	Johnson et al., 1990
S-301	1990	SD7/730221/M7P-1/3/M7P-5	Johnson et al., 1991
S-102	1996	Calpearl/Calmochi-101//Calpearl	McKenzie et al., 1997
Calhikari-201	1999	Koshihikari/(Koshihikari/S-101)*2	McKenzie, 2001
Calamylow-201	2006	Induced low amylose mutant of Calhikari-201	McKenzie et al., 2006

Table 2. Egyptian rice cultivars for which Calrose 76 served as the ancestral source of semidwarfism

Cultivar	Year	Pedigree	Reference
Giza 176	1989	Calrose 76/Giza172//GZ 242	Badawi, 1999
Sakha 101	1997	Giza 176/Milyang 79	Badawi, 1999

Other rice mutants produced in California included various short stature, early maturing marker gene stocks, and several genetic male steriles (Rutger et al., 1982, 1987; Mese et al., 1984). An attempt at inducing cytoplasmic male sterility (CMS) in rice with streptomycin was not successful but did result in another genetic male sterile (Hu and Rutger, 1991); a parallel streptomycin study in sunflower resulted in several CMS mutants (Jan and Rutger, 1988).

The genetic male steriles were used as a tool to search for apomixis in rice, by interplanting male steriles between rows of world collection rices, producing 3,178 F_1 plants, then looking for abnormal segregation in the resulting F_2 populations. The male steriles were homozygous for three recessive marker genes, semidwarfism, glabrous leaves, and male sterility. Abnormal segregation of the three genes, specifically for excess of maternal-type plants, would be evidence of apomixis. Although abnormal segregation ratios was observed in 14 out of 3,728 families, detailed analysis indicated these were due to sampling error, and thus not evidence of apomixis (Rutger, 1992a).

An environmentally sensitive genetic male sterile mutant recovered from anther culture of Calrose 76 appeared promising for using genetic male sterility in hybrid seed production (Rutger and Schaeffer, 1994). In the long-day environment at Davis, California, this mutant segregated as a recessive male sterile, in the short day winter nursery environment in Kapaa, Hawaii, no segregation occurred,

i.e., genetically sterile plants became fertile. This offered the potential of producing bulk quantities of seeds from "sterile" plants in the short-day environment, seeds which would produce all-sterile plants in a crossing block in the long-day environment. Although the trait was reproducible in selfed generations, it was not transmitted in controlled crosses, thus limiting its utility (Rutger and Schaeffer, 1994).

In the author's studies on induced mutants, emphasis has been on finding applications, primarily for breeding, so detailed cytogenetic and molecular studies have not been pursued. The rationale behind this decision has been to get the mutants documented and available for others to use, as in the molecular studies that have been reported on *sd1* (Monna et al., 2002), *eui* (Ma et al., 2006) and *lpa1* (Andaya and Tai, 2005). Such basic studies have been very helpful in understanding which alleles are being used in breeding (T.H. Tai, Rice Geneticist, Davis, California, personal communication, October 17, 2006). Meanwhile, the various mutants found over the last 30 years are listed in Table 3.

The California work up to the early 1990s was summarized in IAEA venues (Rutger and Peterson, 1981; Rutger, 1984, 1991, 1992b) and elsewhere (Rutger, 1983).

Arkansas tropical japonicas

After the five-year administrative assignment, the author returned to rice research in 1993 as the first Director of the Dale Bumpers National Rice Research Center in

Stuttgart. Arkansas, and began work on useful applications of induced mutation in southern US rice. Nearly half of the total US rice production is in Arkansas, all with tropical japonica cultivars, primarily long grain rices with intermediate amylose contents (21-23%). At that time no cultivars carrying the semidwarfing gene had been released in Arkansas, although the modern Arkansas cultivars were only 15 to 25 cm taller than semidwarfs from Texas and Louisiana. Therefore considerable effort was directed at inducing and evaluating semidwarf mutants in the tall Arkansas cultivars. Over 100 putative semidwarfs were selected in the next 5 years, but through agronomic evaluation these were reduced to just 12 single recessive gene mutants which equaled their tall parents in yield; those not equaling the tall parent were summarily discarded (Rutger et al., 2004b, 2004c, 2006). Each mutant was test crossed to the Calrose 76-derived induced *sd1* source from California. Surprising, all 12 proved to be nonallelic to *sd1* (Figure 3). The fact that none of these 12 mutants gave the 14% or higher yield increase that was customarily observed with *sd1* in California, gave further credence to the previously postulated "*sd1* mystique" (Rutger, 1992b).
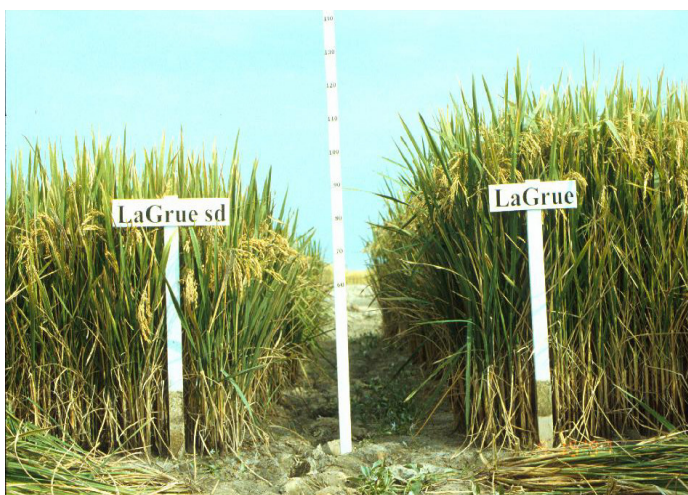

Figure 3. A semidwarf mutant of LaGrue and its tall parent, LaGrue. As with all 12 semidwarf germplasm mutants that Rutger induced in Arkansas cultivars, the semidwarfing gene in the LaGrue mutant was nonallelic to sd1, the worldwide semidwarfing gene.

In addition to the semidwarf mutants in the tropical japonica germplasm, a recessive early flowering mutant that was about 16 days earlier than its parent was induced in the cultivar LaGrue (Rutger et al., 2004e). Also induced was the low phytic acid mutant lpa1 in the cultivar Kaybonnet (Rutger et al., 2004a). This mutant reduces the phytic acid phosphorus content of rice about 45%, with a concomitant increase in free phosphorus (Larson et al., 2000). Phytic acid phosphorus is largely indigestible for monogastric animals, and phytic acid also interferes with iron and calcium uptake, while free phosphor-

rus is digestible. The phytic acid differences are concentrated in the bran portion of the rice grain (Bryant et al., 2005). Thus to get full benefit of the reduction one should eat brown (unmilled) rice, which to date has been a minor use of rice but may increase with current interest in consuming whole grain cereals. The low phytic acid mutant has about a 10% yield penalty (Rutger et al., 2004a). Otherwise the mutant is phenotypically identical to its parent, creating seed purity challenges. Therefore the low phytic acid mutant was crossed with an older goldhull-color cultivar, Bluebelle, and low phytic acid, goldhull color recombinants were obtained to form the germplasm designated GLPA, for goldhull low phytic acid (Rutger et al., 2004d). Currently there are no other goldhull cultivars in production in the USA so GLPA has identity preservation in the field, in the farm truck and in the grain elevator.

Two dominant genetic male sterile mutants were induced, one in the long grain cultivar Kaybonnet and one in the medium grain cultivar Orion (Zhu and Rutger, 1999). Genetic male sterility is usually advocated for population improvement schemes such as recurrent selection. The principal merit of dominant genetic male sterility is that the sterility recurs every generation, in contrast to recessive male sterility, where the male sterility recurs in every second generation.

Another example of integration of mutants was provided by development of *aromatic se* germplasm as a semidwarf (*s*), early maturing (*e*) recombinant from a cross between a late maturing semidwarf mutant, DM 107-4, and the early maturing tall cultivar Kashmir Basmati (Rutger and Bryant, 2004). Both of the parents were induced mutants of Basmati 370 developed in Pakistan (Awan, 1984). The *aromatic se* germplasm retains the aroma and cooking quality of the original basmati source. Yield of *aromatic se* has been low relative to its tall parent in Arkansas (Rutger and Bryant, 2004). Awan and Cheema (1999) reported that DM 107-4 has a semidwarfing gene nonallelic to *sd1*. Therefore attention in Arkansas has shifted to recombinants from crossing another late maturing semidwarf mutant, DM2 (Awan, 1984), with Kashmir Basmati, in hopes that DM2 may carry the "mystical" *sd1*. Evaluation of F_4 generation early maturing, semidwarf recombinants show this new germplasm, designated *aromatic se2*, indeed has higher yield potential than the first germplasm, *aromatic se* (Rutger, unpublished).

Table 3. List of rice mutants collected in California and Arkansas

California mutants

semidwarf Calrose	CI 9966	Calrose 76 *sd1*	Rutger et al., 1977
" "	CI 11033	D66 *sd2*	Foster and Rutger, 1978a; Rutger et al, 1979
" "	CI 11034	D24 *sd4*	Mackill and Rutger, 1979; Rutger et al., 1979
" "		D32 *sd1*, D23*sd4*, D25*sd4*	Mackill and Rutger, 1979
early flowering Calrose	CI 11037	D18	McKenzie et al., 1978; Rutger et al, 1979
" " "	CI 11038	D31	Rutger et al, 1979
doubledwarf Calrose	CI 11036	DD1 *sd1 +sd2*	"
semidwarf Colusa	CI 11035	*sd* unknown	"
short Labelle		*sd? ≠ sd1**	McKenzie et al., 1986
short stature M5	CI 11045	*sd? ≠ sd1*	Rutger et al., 1982
short stature M5	CI 11046	*sd? ≠ sd1*	"
short stature Maxwell	CI 11047	*sd? ≠ sd1*	"
short stature WC 1403	CI 11048	*sd* unknown	"
narrow leaf semidwarf	CI 11049	*sd? ≠ sd1*	"
short stature Tsuru Mai	CI 11050	*sd* unknown	"
early flowering Calrose 76	CI 11051	*sd1*	"
early flowering M5	CI 11052	*ef* unknown	"
early flowering S6	CI 11053	*ef* unknown	"
early flowering Terso	CI 11054	*ef* unknown	"
Calady, Earlirose, Caloro,CS-M3 male steriles		4 recessive male steriles	Trees and Rutger, 1978
elongated uppermost internode	CI 11055	*eui*	Rutger and Carnahan, 1981
early flowering S-201	PI 506219	ef unknown	Rutger et al., 1987
light green panicle M-101	PI 506221	*lgp*	"
yellow-green panicle ESD7-3	PI 506222	*yp*	"
waxy M-101	PI 506223	*wx*	"
goldhull M101	PI 506224	*gh*	"
streptomycin-induced male sterile	PI 543853	*m*s	Hu and Rutger, 1991
Arkansas mutants			
dominant male sterile KBNT 1789	GSOR 1	*Ms*	Zhu and Rutger, 1999
dominant male sterile Orion 1783	GSOR 2	*Ms*	" "
recessive male sterile Cypress 1819	GSOR 3	*ms*	" "
semidwarf KBNT 4	PI 632276	*sd? ≠ sd1*	Rutger et al., 2004b
semidwarf KBNT 5	PI 632277	*sd? ≠ sd1*	"
semidwarf LGRU 12	PI 632278	*sd? ≠ sd1*	"
semidwarf LGRU 13	PI 632279	*sd? ≠ sd1*	"
semidwarf ADAR 10	PI 632280	*sd? ≠ sd1*	"
semidwarf ORIN 172	PI 632281	*sd? ≠ sd1*	"
semidwarf ADAR 22	PI 632951	*sd? ≠ sd1*	Rutger et al., 2004c
semidwarf KATY 1	PI 632952	*sd? ≠ sd1*	"
semidwarf KBNT 11	PI 632953	*sd? ≠ sd1*	"
semidwarf LGRU 2	PI 632955	*sd? ≠ sd1*	"
semidwarf LGRU 14	PI 632956	*sd? ≠ sd1*	"
semidwarf DR1	PI 642749	*sd? ≠ sd1*	Rutger et al., 2006
low phytic acid KBNT lpa1	PI 632282	*lpa1-1*	Rutger et al., 2004a
goldhull low phytic acid	PI 632954	*lpa1-1 + gh*	Rutger et al., 2004d
Guichao 2 eui	GSOR 11, PI 634574	*eui*	Rutger, 2005
early flowering LaGrue	GSOR 7, PI 632957	*ef* unknown	Rutger et al., 2004e

Arkansas indicas

By the mid-1990s it had become evident that indica germplasm had greater yield potential in the southern US than tropical japonica germplasm (Eizenga et al., 2006), although indicas generally had higher amylose contents than the intermediate amylose (21-23%) level desired for US markets. Therefore the first approach was to cross a very early indica from China, Zhe 733, which had high amylose, with intermediate amylose indicas from IRRI that were about a month too late in maturity for Arkansas (Figure 4). The IRRI lines, which very closely approach US long grain quality standards, were graciously supplied by G. S. Khush of IRRI (G. S. Khush, personal communication, December 20, 1995). Nine early maturing, intermediate amylose recombinants, *indica-1* to *indica-9*,

from this program were released (Rutger et al., 2005), but these still suffered from lower whole grain milling yield (white rice) than US tropical japonicas. Meanwhile it had become apparent that the IRRI germplasm lines used in these crosses had both the appropriate intermediate amylose levels and high whole-grain milling yields. It was then hypothesized that induction of earlier maturity in the IRRI materials could enhance their value for the US. This indeed proved to be the case, resulting in induction and evaluation of four early flowering mutants, *indica-10* to *indica-13*, which were 18 to 28 days earlier than their parent (Figure 5). The mutants were nearly as early as local tropical japonica cultivars, and most importantly, the mutants retained not only the desired intermediate amylose levels of the parents but also high whole-grain milling yields, giving indica germplasm that is competitive in quality with US long grains (Rutger et al., 2007) (Figure 6).

Figure 5. The induced early flowering mutant *indica-12* (right) is 28 days earlier than its IRRI parent IR53936-60-3-2-3-1 (left), making it useful for the US since the IRRI parent is about a month too late when grown in the US.

Figure 4. J. Neil Rutger in his "laboratory" in September 2002, at Stuttgart, Arkansas, observing an early flowering indica germplasm line (left) and its late flowering parent (right).

Meanwhile, the induced low phytic mutant, KBNT *lpa1-1*, was crossed with Zhe733 to determine the chromosome location of the *lpa1-1* gene (Larson et al., 2000). Subsequently this japonica/indica population was advanced to the F_{10} generation in order to create a mapping population (Rutger and Tai, 2005). This mapping population has been distributed to interested colleagues for study of blast disease and insect resistance.

A series of 21 early flowering mutants was induced in the famous blast resistant cultivar from Colombia, Oryzica llanos 5 (Roca et al., 1996), another indica cultivar that is a month too late for the US. All 21 mutants, which were from 24 to 40 days earlier than the parent cultivar, retained the parental resistance to six blast isolates; the group has been narrowed down to two for germplasm release (Rutger and Lee, 2007).

Figure 6. The four induced early flowering indica mutants, *indica-10, indica-11, indica-12* and *indica-13* have head rice (whole kernel) yields similar to the japonica check cultivar Francis. This is the first time that indicas with high head rice yield.

Genetic stocks – *Oryza* collection (GSOR)

Development of the various mutants in the US led to the 2003 establishment of the Genetic Stocks-*Oryza* Collection (GSOR) at the DB NRRC in Stuttgart, Arkansas, USA. The GSOR is a much-needed effort since introductions of rice germplasm from overseas is hindered by strict quarantine introduction procedures within our country, which are directed at keeping unwanted diseases and pests out. Therefore we set out to make our own collection of genetic stocks. The first rice genetic stocks contributed to the collection were GSOR 1, 2, and 3, which were two dominant and one recessive genetic male sterile mutants induced at the DB NRRC (Zhu and Rutger, 1999). Sixteen more previously developed entries from California and Arkansas, including induced mutants for genetic male sterility, early flowering, semidwarfism, and elongated uppermost internode were included in the ini-

tial contributions to the GSOR (Rutger and Carnahan, 1981; Rutger et al., 1982, 1987; McKenzie and Rutger, 1986; Rutger, 2005). Currently the GSOR has 902 entries including: a lesion mimic mutant, GSOR 20 (Jia, 2005), the Stuttgart-developed japonica/indica mapping population with 355 lines (Rutger and Tai, 2005), a second mapping population with 325 doubled haploid lines from Louisiana State University (Chu et al., 2006), and the former "Jodon collection" (Jodon, 1977). A set of 191 Hokkaido University mutants, donated by Japan's Dr. Toshiro Kinoshita, via Dr. Susan McCouch, Cornell University (T. Kinoshita, personal communication, November 22, 2005), has been entered and is part of the 902 total. Four indica genetic stocks, apoptosis, chives, extreme dwarf, and gold leaf, designated as GSOR entries 21, 22, 23, and 24, respectively, were added in 2006 (Rutger and Bernhardt, 2006), and more DB NRRC-developed genetic stocks are in the pipeline. A very recent example mutant is a giant embryo mutant, in the long grain cultivar Drew (Figure 7). This recessive mutant increases oil content from the 2.7% level in the parent cultivar, to 3.7% in the mutant. The mutant, preserved as GSOR 25, may have potential for brown rice consumption, assuming that the increased oil content will have a favorable effect on taste (GSOR, 2006). Two indica doubledwarf mutants also are recent GSOR additions. These are of interest because in the work with indicas, all of which apparently carry the DGWG-TN1-IR8 source of *sd1*, it was usually observed these semidwarfs were 10 cm or so taller than tall Arkansas check cultivars (Rutger et al., 2005, 2007). Since the indicas generally are more susceptibleto lodging than local checks, short plants were sought in mutagenized populations of earlier releases.

Figure 7. The giant embryo mutant (left) has an embryo 44 % larger than the long grain parent cultivar Drew (right), and has whole kernel oil content of 3.7% compared to 2.7% for the parent. This mutant, which has been placed in the Genetic Stocks—*Oryza* Collection as GSOR 25, may have value for consumption as whole grain brown rice, which is a growing market in the US.

Thus one was found in a sister line of *indica-9*, and another one in *indica-12*. These "doubledwarf" mutants, which are 15 to 20 cm shorter than their respective sin-

gle-dwarf parents, were placed in the GSOR collection, as GSOR 27 and 28, respectively (GSOR, 2006). Finally, an indica mutant population, GSOR 26, which segregates for albinos, was preserved to serve as an elementary school teaching project, that is, 3 normal: 1 albino segregation can be observed by germinating the seeds for 5 to 7 days (GSOR, 2006). Entries in the GSOR may be viewed at the GSOR homepage http://ars.usda.gov/Main/docs.htm?docid=8318.

Cooperation with IAEA

The author has been fortunate to have numerous opportunities to cooperate with IAEA over the past 30 years, through participation in international meetings and /or workshops, beginning in 1977:

1. October 7-9, 1977. Presented invitational paper on "Utilization of induced mutants for short stature and early maturity in japonica rice" at the FAO/IAEA Regional Seminar on Improvement of Rice Production Through Research Using Nuclear Techniques, Jakarta, Indonesia.

2. March 1-14, 1981. Participated in FAO/ the IAEA RCM on Evaluation of Mutant Stocks for Semidwarf Plant Type as Cross-Breeding Materials in Cereals, and in the FAO/IAEA International Symposium on Induced Mutations as a Tool for Crop Improvement. Vienna, Austria.

3. September 15, 1983. Hosted FAO/ IAEA RCM on Evaluation of Mutant Stocks for Semidwarf Plant Type as Cross-Breeding Materials in Cereals. Davis, California.

4. December 13-21, 1985. Participated in Fourth RCM on Evaluation of Semidwarf Mutants for Cross-Breeding. Rome, Italy.

5. June 16-23, 1990. Presented invited paper on "Mutation breeding of rice in California and the USA, at the FAO/IAEA International Symposium on the Contribution of Plant Mutation Breeding to Crop Improvement. Vienna, Austria.

6. May 6-14, 1994. Participated in the First FAO/IAEA RCM on Radiation-induced Mutations and other Advanced Technology for the Production of Seed Crop Mutants Suitable for Environmentally Sustainable Agriculture. Vienna, Austria.

7. June 19-23, 1995. Participated as invited *advocatus diaboli* at the FAO/IAEA Symposium on the Use of Induced Mutations and Molecular Techniques for Crop Improvement. Vienna, Austria.

8. November 6-10, 1995. Participated in the Second FAO/IAEA RCM on Radiation-induced Mutations and other Advanced Technology for the Production of Seed Crop Mutants Suitable for Environmentally Sustainable Agriculture. San Jose, Costa Rica.

A major effort in the author's induced mutation career was as a consultant in the IAEA program for Evaluation of Cereal Crop Mutants (ARCAL

XXIA) from 1995 to 2001 in six Latin American countries, organized by M. Maluszynski, former Head of the Plant Breeding Section at IAEA. Twenty three rice mutants, including one from the US, were evaluated in multi-location yield trials by cooperators in Brazil, Colombia, Costa Rica, Cuba, Guatemala, and Uruguay (Blanco et al., 2000). In general, the 22 indica mutants performed better in these tropical and subtropical environments than did the one US mutant, OR172, which was in a japonica background. The Latin American model subsequently was adopted for similar mutant evaluations organized by Maluszynski in 11 Asian countries (Eizenga et al., 2004). Both the Latin American and Asian evaluation trials showed the usefulness of mutant germplasm in contributing to food security in participating countries (Blanco et al., 2000; Eizenga et al., 2004). Individual country meetings are summarized in items 9 to 14, below.

9. December 2-9, 1995. Began service as a coordinator for evaluation of 23 rice mutants, 10 parent lines, and a local check, by scientists in Brazil, Colombia, Costa Rica, Cuba, Guatemala, and Uruguay. I contributed one of my tropical japonica semidwarfs to this otherwise all-indica collection of mutants. Campinas, Brazil.

10. October 19-26. 1996. Continued as rice expert/coordinator. Buenos Aires, Argentina.

11. July 5-12, 1997. Continued as rice expert/coordinator. San Jose, Costa Rica.

12. March 22-29, 1998. Continued as rice expert/coordinator. Pointe del Este, Uruguay.

13. November 7-12, 1999. Continued as rice expert/coordinator. Guatemala City, Guatemala.

14. December 11-15, 2000. Continued as rice expert/coordinator. Guatemala City, Guatemala.

15. August 24-28, 1998. Hosted FAO/IAEA RCM on Radiation-induced Mutations and other Advanced Technology for the Production of Seed Crop Mutants Suitable for Environmentally Sustainable Agriculture. Stuttgart, Arkansas, USA,

16. October 4-8, 1999. Participated in First FAO/IAEA RCM on "Molecular characterization of mutated genes controlling important traits for seed crop improvement." Vienna, Austria.

17. June 3-10, 2000. Invited participant in IAEA Regional Training Course on New Frontiers of Developing and Handling mutants. Hangzhou, China.

18. June 10-14, 2002. Participated in FAO/IAEA RCM on "Molecular characterization of mutated genes controlling important traits for seed crop improvement." Krakow, Poland.

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Research Article

Development of Three Groundnut Varieties with Improved Quantitative and Qualitative Traits through Induced Mutation

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Abstract

With a view to develop high yielding, bold seeded and disease resistant varieties of groundnut, 200 dry seeds of an established mutant, Mut-6, was irradiated with 200 Gy gamma rays. All the M_1 plants were harvested and kept separately. In M_2-M_4 generations, selection and evaluation were made following high mean and high/low variances compared to the check cultivar Dhaka-1 (the parent of Mut-6). In M_4 generation, 16 true breeding lines were obtained. Through preliminary, advance, zonal and farmers' field trials during 1997-1999, three mutant lines M6/20/42-M(2), M6/20/44-3 and M6/20/62-4, were identified to be dwarf, high yielding, bold podded and seeded and moderately resistant to cercospora leafspot, rust and collar rot diseases, compared to the check variety, Dhaka-1. Moreover, these mutants have higher oil contents and higher/similar protein contents. The National Seed Board has registered these mutant families in 2000 as BINAchinabadam-1, BINAchinabadam-2 and BINAchinabadam-3, respectively, for commercial cultivation by the farmers.

Key words: groundnut; induced mutations; new cultivar

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil and food crop, currently being grown on approximately 17 million hectares of land worldwide with the production of 23.2 million metric tons [1]. Globally, it is the third major oil seed crop next to soybean and cotton. India, China and the United States of America have been the leading producers for over 25 years and produce about 70% of the total production. In Bangladesh, it ranks third after rapeseed-mustard and sesame based on both acreage and production despite per hectare yield is the highest in groundnut (1150 kg) [2]. It is a multipurpose crop and can help reduce edible oil, food and fodder shortages of the country. Apart from its rich oil content (45 to 50%), groundnut seeds are good source of proteins (25 to 30%), carbohydrates (20%) and vitamins E and B. A pound of peanuts provides food energy that is equivalent to 2 pounds of beef, 9 pints of milk, or 36 medium sized eggs [3]. For its high digestibility it is an excellent component of children's food. Being a legume it fixes atmospheric nitrogen to soil through its nodule bacteria and thus keeps environment most friendly [4]. Groundnut yield is one of the lowest in Bangladesh compared to the very high yields in the developed countries, particularly in the USA (2400-3200 Kgha^{-1}). The low genetic potential, smaller pod size and high susceptibility to disease and insect-pests of the widely grown land race, Dhaka-1, mostly attribute this low yield. Mutation breeding technique is one of the important accessories of the main stream plant breeding. Compared to conventional methods, it saves nearly half the time to create a new cultivar. Pleiotropic effects are very common and help fix true breeding lines even in M_3/M_4 generations. Genetic improvement of any yield attributes, both quantitative and qualitative in nature, has been successful through this technique [5-9]. Keeping these in mind this study was initiated to develop high yielding, bold seeded and disease resistant varieties using mutation breeding technique.

Materials and methods

Two hundred dry seeds of an established mutant, Mut-6, were irradiated with 200 Gy dose of gamma rays. The mutant, Mut-6, was originally developed during 1980-1986 by treating seeds of Dhaka-1, a widely cultivated cultivar, with gamma rays at a dose of 400 Gy. Treated seeds of Mut-6 were immediately sown for M_1 population development and at maturity plants were harvested separately and dry pods were kept for growing M_2 population. The following year, M_2-plant-progeny-rows were grown with check-rows of Dhaka-1 in every 15-row interval. In this generation, the most competitive ten plants from each and every M_2-plant-progenies including Dhaka-1 check were recorded. Approximately 200 families that showed high mean yields with either high or low variances compared to Dhaka-1 were selected. Exactly similar procedure of selection was practiced in M_3 generation and 98 out of 200 families were selected on the basis of high means and low variance compared to the mean yield and variance of Dhaka-1. An observational yield trial was then conducted in M_4 generation during the winter season of 1997 with these 98 mutant families. This trial identified 16 true breeding lines with higher mean yields.

Preliminary yield trial (M_6 generation)

These 16 true breeding families, along with their grand parent, Dhaka-1, were put into preliminary yield trial in Kharif-II season of 1997 at Mymensingh. The experiment followed a randomized complete block design with three replications having plot sizes of 4.5m x 1.0m. Seeds were sown at 15cm distances within rows of 45 cm apart. All plots were fertilized with 40kg N, 120 kg P_2O_5 and 120 kg K_2O ha^{-1} during final land preparation. Recommended cultural practices were followed as and when necessitated. Data on different yield attributes were recorded only from 10 randomly competitive selected plants. Moreover, yield from 1.4 m^2 area was recorded. Disease reaction data on cercospora leaf spot (*Cercospora arachidicola*), rust (*Puccinia arachidis*) and collar rot (*Aspergillus niger*) were also recorded from this experiment. Fifteen randomly selected plants from each plot were scored before 15-20 days of harvest for cercospora leaf

spot and rust as per standard 9 point rating scale developed by Subrahmanyan et al. [9]. For collar rot, data on germination, pre-and post emergence seedling deaths were recorded and finally, graded following standard

scale (1-9) developed by Nene et al. [10] and presented in Table 1.

Table 1. Means pod yield over three locations and different yield attributes over two locations in some elite groundnut mutants

Mutant	PH (cm)	PBPP (no.)	NPP (no.)	HPW (g)	HKW (g)	Shelling (%)	Pod yield (kg ha^{-1})			
							Mean	Mymensingh	Ishurdi	Natore
M6/20/42-M(2)	31.8	5.0	24.2	85.7	47.6	73.4	2594	3734	2070	1976
M6/20/44-3	27.5	5.0	25.1	83.4	40.1	68.6	2486	3168	2423	1866
M6/20/62-4	27.6	5.2	22.3	86.0	42.3	68.9	2371	2991	2184	1939
Dhaka-1(c)	37.8	4.8	26.4	67.6	34.1	70.0	1922	2610	1949	1207
Zhingabadam (c)	49.5	3.7	21.9	75.3	29.0	65.6	1734	2448	1383	1372
LSD(0.05)	**04.6**	**0.5**	**5.0**	**9.6**	**2.8**	**3.9**	**197**	**478**	**373**	**179**

PH=plant height; PBPP=number of primary branches per plant; HPW/HKW=100 pod/kernel weight

Advanced yield trial (M_6 generation)

An Advanced Yield Trial was conducted with 7 M_6 mutant families along with two check cultivars, Dhaka-1 and Zhangabadam, during the winter season of 1998 at BINA farm, Mymensingh. The experiment followed a randomized complete block design with three replications. Seeds were sown in unit plot sizes of 4.5 m x 1.35m at 15 cm distances within rows of 45 cm apart. All plots were fertilized following BARC Fertilizer Recommendation Guide (1995) during final land preparation. The experiment followed rainfed condition and recommended cultural practices. Pod yield data was taken from an area of 1.42 m^2/plot having uniform population size of 21 plants.

Farmers' field trial

Farmer's field trials were conducted with 3 elite M_7 mutant families together with two check varieties, during winter season of 1998-99, at Mymensingh, Ishurdi and Natore. The experiment followed randomized complete block designs with three replications, in each location. Seeds were sown in unit plot sizes of 4.05m x 2.25m at 15 cm plant distances within rows of 45 cm apart.

Fertilizers were applied following BARC Fertilizer Recommendation Guide (1995) during final land preparation. The crop was raised in rainfed condition where recommended cultural practices were followed. Pod yields were gathered from an area of 3.375 m^2/plot. Reaction to cercospora leaf spot, rust and collar rot diseases were recorded following the procedure as described in preliminary yield trial. Oil and protein contents were also determined [11, 12]. The yield in various tests was transformed into Kg ha^{-1} and was subjected to proper statistical analyses by following the given design.

Results and discussion

Performance in preliminary yield trial

The check cultivar, Dhaka-1, had the tallest height and was significantly different from some of the mutant lines,

e.g., M6/20/60-1, which was only less than half of Dhaka-1 (Table 1). Interestingly, we found most of the mutant lines were higher than Mut-6, although not always significant (Table 2). Variations of other agronomic traits and yield attributes and disease resistance were also observed and seven lines were selected for advanced yield trial.

Performance in advanced yield trial

Zhingabadam had the largest pod size while Dhaka-1 had the least sized pod. The mutant family M6/20/62-4 had shown significantly higher pod size than Dhaka-1 control. Shelling percentage had not differed significantly among the mutant families and check cultivars. Mutant genotype M_6/20/62-4 had produced the highest yield and did not differ from the 5 other mutants and two check cultivars. Results of this advance trial were further assessed through pool analyses with yield and yield attribute records of the previous preliminary trial for proper evaluation of the mutant families for future farmer's field trial.

It could be seen clearly that not all mutant lines had consistently high yield in preliminary and advanced yield trial (Table 1, 2). Based on comprehensive consideration of earliness, mature pod numbers, dwarf types, shelling percentages and yields, three lines M_6/20/42-M(2), M_6/20/62-4, M_6/20/44-3 were selected.

Performance in farmer's field trials

The yields of the three mutants, M_6/20/42-M(2), M_6/20/44-3 and M_6/20/62-4, were significantly higher than the two check varieties, Dhaka-1 and Zhingabadam, both on average and in each location (Table 5). M6/20/42-M(2), for example, out-yielded on average the two check cultivars Dhaka-1 and Zhingabadam by 35.0% and 49.6%, respectively. All mutants and check cultivars showed resistance (R) to moderately resistance (MR) to all the three most prevailing diseases, i.e. collar rot, CLS and rust.

Table 2. Means of yield and yield attributes of 16 M_5 mutant families evaluated at BINA farm, 1997 Kharif-II season

Mutants/ Varieties	Plant height (cm)	Primary branch/ plant (no.)	Days to 50% flowering	No. of pods/ plant	100 pod weight (g)	Shelling (%)	Yield kg /ha	Resistance		
								CR	CLS	Rust
M_6/20/42-M(1)	17.5	5.0	31.0	14.0	75.2	79.3	1562	MR	MR	MR
M_6/20/105-2(1)	19.5	5.5	29.5	12.0	75.8	80.5	1118	MR	S	MR
M_6/20/42-M(2)	21.5	5.0	31.0	20.0	82.0	80.2	2365	MR	MR	MR
M_6/20/187-4	24.0	4.5	29.0	15.5	85.2	77.6	1604	MR	MR	MR
M_6/20/105-4	22.0	4.0	30.0	14.0	82.9	73.2	1432	MR	S	MR
M_6/20/62-4	16.0	5.5	30.5	17.5	80.0	78.1	1556	MR	MR	MR
M_6/20/44-3	17.0	5.5	30.0	15.5	86.3	73.6	1660	MR	MR	MR
M_6/20/105-3	18.0	5.0	32.0	12.0	77.4	81.6	1093	MR	S	MR
M_6/20/44-1(3)	19.5	4.0	28.0	16.0	79.8	76.9	1578	MR	MR	MR
M_6/20/60-1	11.0	4.0	32.0	12.5	73.9	76.8	1027	MR	MR	MR
M_6/20/41-2	13.0	5.0	30.5	15.5	64.9	75.3	1335	MR	S	MR
M_6/20/105-3(1)	24.5	5.5	29.5	14.0	78.0	80.1	1279	MR	MR	MR
M_6/20/40-2	13.5	4.0	32.0	16.5	60.4	85.2	1164	MR	MR	MR
M_6/20/133-1(1)	17.0	4.0	29.5	14.0	87.9	68.2	1466	MR	S	MR
M_6/20/44-1(2)	17.5	4.5	29.0	17.0	67.1	77.9	1314	R	S	MR
M_6/20/105-2	26.0	5.0	30.5	14.0	88.5	77.9	1555	R	MR	MR
Mut-6(parent)	15.5	5.0	32.0	13.0	79.9	75.8	1304			
Dhaka-1(C)	27.5	4.0	28.0	16.0	74.4	79.6	1317	MR	S	MR
LSD(0.05)	**6.9**	**1.2**	**1.7**	**3.2**	**16.4**	**3.2**	**186**			

C=control; R=resistant; MR=moderately resistant; S=susceptible; CR=collar rot; CLS=cercospora leaf spot

Registration of new variety

The National Seed Board has registered the mutant lines M_6/20/42-M(2), M_6/20/44-3 and M_6/20/62-4 as BINAchinabadam-1, BINAchinabadam-2 and BINAchinabadam-3, respectively, for commercial cultivation in 2000.

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Research Article

Induced Pusa Dwarfing Genes in *T. turgidum* var. *dicoccum* and their Inheritance

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Abstract

Two dicoccum varieties NP 200 and HW 5011 were subjected to 0, 10, 20, 30 and 40 kR doses of gamma ray irradiation. A desirable dwarf mutant was selected in the mutant population of NP 200 treated 20 kR at M_2 stage. This plant is conferring resistance to yellow rust and powdery mildew under natural epiphytotic conditions at Wellington and also having high tillering with dark green foliage. The mean plant height of the mutant is 71cm as compared to NP 200 with 110cm. The non-segregant semi-dwarf line was constituted at M_4 generation and named as HW 1095. The F_1 plants between NP 200 and HW 1095 showed the dominant character for tallness as that of NP 200, with the dark green foliage as that of the mutant. The other traits of HW 1095 remain similar as compared to NP 200. The F_2 segregation revealed that the semi-dwarfism in *dicoccum* is controlled by two independent complementary genes from AB genomes, which are designated as 'Pusa Dwarfing Genes of Dicoccum'. The HW 1095 plants exhibited 00-10 lodging score as compared to its parents with the score of 20-90 per cent in special dicoccum trials conducted by the All India Wheat Coordinated Improvement Programme (AICWIP). In the winter 2004-05 trials of AICWIP (HW 1095) was ranked 2nd only after bread wheat MACS 2496 and gave 40 qtls. per hectare across all locations. This mutant line has given 43.3 and 49.1 q/ha. grain yield in Karnataka and Maharashtra, respectively.

Key words: Dicoccum wheat, Mutation, Complementary, Semi-dwarfism, Pusa Dwarfing gene

Five decades ago several dwarf bread wheat varieties (*T. aestivum)* were isolated in Japan from the Cross Turkey Red Fulz- Daruma, resulting in Norin10, Norin14 and other dwarf varieties [1]. The present day wheat production of 72 million tones with average productivity of 27.5 q/ha, is made possible only due to the incorporation of dwarfing genes Rht_1 and Rht_2 (Norin10) into tall wheat varieties [2]. However, no such plant type is available even today in commercial cultivars of *T.turgidum* subsp. *dicoccum,* with better *dicoccum* grain quality.

In India common wheat (*T. aestivum* L.) has been grown since ancient times. The other two cultivated tetraploid species of wheat in India viz., macaroni wheat (*T. durum*) and Emmer/Khapli wheat (*T. dicoccum),* which might have gotten introduced from Middle East and are now grown generally in Central and Southern States [3]. The first systematic selection and introduction of *T. dicoccum* was carried out by the scientists at IARI, Wellington, in the 1950's. Since 1959, three *T. dicoccum* wheat varieties at national level were released as (New Pusa) NP-200, NP-201 and NP-202. They were local selections from the Rishi Valley of Andhra Pardesh. NP 200 is tall, high yielding, however non-responsive to irrigation and fertilizers [4]. Under increased levels of irrigation and fertilizer, NP 200 lodged heavily in several places of its cultivation. Till recently, NP 200 is being used as the national check which has best dicoccum quality.

Materials and methods

Dry and healthy seeds of *T. dicoccum* var. NP 200 New Pusa along with HW 5011 were irradiated with 0, 10, 20, 30 and 40kR doses at BARC, Mumbai. Treated seeds of NP 200 and HW 5011 were space planted in M_1 generation along with untreated checks during Rabi 02-03 at Regional Station, Wellington. The seeds of M_1 plants were harvested separately and sown to obtain M_2 generation during Kharif 2003. Interestingly one semi-dwarf plant with vigorous growth and high tillering habit was picked in M_2 population of 20kR treated NP 200. The seed of this plant was harvested separately and grown in Rabi (Kharif) 2003-04. One single row progeny No. 21 exhibited uniform plant height ranging from 63-79cm as compared to NP 200 (100-110cms) tall. The semi-dwarf *T. dicoccum* plant was crossed with tall NP 200 to study the inheritance pattern based on the data of its F_1, F_2 and F_3 families.

Results and discussion

1. Selection of mutants

Several dwarf and semidwarf mutant plants were selected in M_2 of both NP 200 and HW 5011 (Table 1).

Table 1. Some agronomic characters of selected mutant at M_2 and M_3 generations[1]

Material	Plant height (cm)			No. of ears/plant			No. of spikelets/ear		
	Mean.	Min	Max	Mean	Min.	Max.	Mean	Min.	Max
NP-200	110.0	97	120	15.6	18	22	19.0	21.0	24
20kR M_2 (NP 200)	71.0	Nil	Nil	11.0	Nil	Nil	19.5	Nil	Nil
30 kR M_2 (NP 200)	55.2	-	-	14.0	Nil	Nil	18.0	Nil	Nil
20kR M_3 (NP 200)	**70.7**	**63**	**79**	**19.2**	**5**	**25**	**20.5**	**18**	**22**
HW 5011	74.80	72	80	12.4	6	19	21.4	17	23
20kR M_2 (HW 5011)	68.3	Nil	Nil	16.0	Nil	Nil	22.6	Nil	Nil
20kR M_3 (HW 5011)	69.5	67	75	15.8	7	21	18.5	20	22

[1] Measurement of M_3 lines and parents was made on 15 plants

The dwarf plant in M_2 of 30 kR (NP 200) although showed dwarfness, but was rejected due to its lateness in maturity. However, the semi-dwarf plant of M_2 20 kR (NP 200) was selected because of its suitable non-lodging height, thick stem and maturity at par with parent NP 200. This semi-dwarf mutant showed uniformity in plant height, ear number etc. in M_4 generation and was designated as 'Semi-Dwarf Dicoccum' and named as HW 1095 (Figure 1).

NP 200 Semi-Dwarfs *Triticum dicoccum* HW 1095

HW 1095

Figure 1. Plants and grains of NP 200 and its semi-dwarf mutant HW 1095

2. Inheritance of semi-dwarf stature

The F_1 plants of HW1095 x NP 200 were all as tall as NP 200. Visual analysis of 243 F_2 plants showed segregation in a 9:7 model (137:106 X^2=1.850), which suggested that two independent semi dominant genes are responsible for this semi-dwarf mutant phenotype. All semi-dwarfs became bred true in the later generations. This suggested that the semi-dwarfism in *T. dicoccum* was due to two major complementary genes A and B. This is the first report of its kind in *T. dicoccum*. The term for this type of semi-dwarfism is "Pusa Dwarfing Genes of *dicoccum*".

3. Quality characteristics of HW 1095

The new plant type HW 1095 exhibited lowest lodging resistance score i.e. 00-10 as against its parents which showed 00-90 (Figure 1) (Table 2). Hence it is grouped as non-lodging. The quality traits viz., thousand grain weight, protein content, sedimentation value and beta carotene, indicated that these quality traits are at par with parent NP-200.

Table 2. Quality traits of *T. dicoccum* and lodging resistance *T. aestivum / durum*

Variety	Triticum Species	Lodging Resistance	Thousand Grain Wt (gm)	Protein Content (%)	Sedimentation Value (ml)	Beta Carotene (ppm)
DDK 1025	*T. dicoccum*	35-90	41.7	13.56	31	4.08
HW 1095	*T. dicoccum*	00-10	41.4	13.42	25	3.21
NP-200	*T. dicoccum*	00-90	41.3	13.40	23	3.28
DDK 1009	*T. dicoccum*	00-60	35.7	14.19	23	4.97
MACS 2846	*T. durum*	00-35	52.4	13.28	24	5.45
MACS 2496	*T. aestivum*	00-00	37.6	13.85	43	4.10

Data average of CZ, PZ and SHZ from DWR Progress Reports 2004-05

4. Performance in regional trials

A special trial on dicoccum under irrigated condition was conducted in Gujarat, Karnataka, Maharashtra and Tamil Nadu during Rabi 04-05 over 12 locations through All India Co-ordinated Wheat Improvement (Anon 2005). The data for grain yield was pooled and discussed. HW 1095 (Filler) was tested with other 7 new *T. dicoccum* varieties along with two check varieties (NP 200 and DDK 1009) as well as one *T. durum* variety MACS 2846 and one *T. aestivum* variety MACS 2496. The results are presented in the Table 3. The variety HW 1095 (Filler) had the highest grain yield and stood first in Karnataka and Maharashtra with 43.3 and 49.1 q/ha respectively, while it ranked 4th in Gujarat with 29.0q/ha as against dicoccum checks DDK 1009 23.5q/ha, while in Tamil Nadu it gave 21.6q/ha. The over all average of 3 zones i.e. Central, Peninsular and South, the variety HW 1095 ranked 2nd (40.0q/ha) and surpassed all dicoccum, durum checks and interestingly at par with *T. aestivum* check MACS 2496 (40.4 q/ha).

Emmer wheat types tend to have lower stomatal conductance, transpiration rate and leaf temperature than other form of wheat, which confirmed the heat tolerance to the plant [5]. This new genotype with Pusa dwarfing gene in the background of *T. dicoccum (tetraploid)* can be transferred to *T. durum* background to evolve the high yielding durum varieties with desirable quality traits to prepare the therapeutic food; as the *dicoccum* are suitable for diabetes and cardiovascular diseases; due to it's capacity to lower blood glucose and lipid levels [6]. The fast growing milling and baking industries have been demanding segregation of wheat spp. for specific end use [7]. Hence, there is a need for quality therapeutic emmer wheat end products. This new type will help to maintain and upgrade the quality of end product.

The results clearly indicated it's high yielding ability with resistant to black and brown rusts (5MS, tMS).

Table 3. State and zonal means (Q/Ha), Rabi: 04 – 05

Variety	GUJ Yld	Rk	KAR Yld	Rk	MAH Yld	Rk	T.N Yld	Rk	Peninsular zone Yld	Rk	Yld	Rk	All zones Yld	Rk
1. DDK – 1025*	21.7	12	42.5	3	39.4	11	31.8	2	44.1	6			36.3	7
2. DDK – 1028	22.7	10	37.8	11	37.9	12	22.1	6	37.6	12			33.2	11
3. DDK – 1029	31.5	3	41.4	4	46.5	3	10.9	11	43.5	3			37.9	4
4. DDK – 1030	23.5	8	39.7	7	41.2	7	7.2	12	40.3	7			33.6	10
5. MACS – 2956	25.4	7	40.0	5	39.6	10	35.9	1	39.7	9			36.6	6
6. MACS – 2947	26.8	5	39.5	8	40.9	8	27.2	4	40.0	8			36.2	8
7. MACS – 2961	26.8	5	42.7	2	46.0	5	23.4	5	44.1	2			38.6	3
8. FILLER (HW 1095)	29.0	4	43.3	1	49.1	1	21.6	7	45.8	1			40.0	2
9. NP - 200#	22.6	11	34.4	12	42.7	6	20.6	8	37.9	11			33.2	11
10. DDK – 1009#	23.5	8	38.5	10	40.2	9	29.4	3	39.2	10			35.1	9
11. MACS – 2846#	34.7	2	38.6	9	46.4	4	12.4	10	41.8	5			37.5	5
12. MACS – 2496#	45.3	1	40.0	5	47.0	2	12.5	9	43.2	4			40.4	1
S.E(M) =	.637		.655		1.084		.436						.439	
C.D=	1.8		1.8		3.0		1.3						1.2	

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Short Communication

Seedless and Citrus Cranker Tolerant Mutant Clones in Sweet Orange Induced by Gamma Rays

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Abstract

Seven mutant clones of sweet orange, induced by gamma rays, were field tested together with their parent variety, 'Pêra', from 1997-2001. Six lines were seedless mutants while the other was a less seed mutant. Two lines were identifies to be more tolerant to the citrus cranker disease. Although all lines had lower fruit yield per plant, it may not influence the yield per area.

Key words: Sweet orange, citrus cranker tolerance, seedless fruit, induced mutations

Introduction

Sweet oranges present great economical and social importance in Brazil, the main world producer of orange fruits and frozen concentrated juice [1]. 'Pêra' is the most important orange cultivar in this country being superior in fruit quality both for fresh fruit and processed juice [2], which has been grown in approximately 38% of the total area of orange groves. This cultivar presents superior fruit quality which determines its use either as fresh fruit or processed juice [2]. However, Pêra is very susceptible to citrus canker, one of the most severe citrus diseases caused by *Xanthomonas axonopodis* pv. *citri*. Citrus canker affects all citrus species and can cause fruit and leaf drop; infected plants must be eradicated from citrus groves since there are no control measures to eliminate this disease. [3]

Spontaneous or induced mutants have been contributed to the production of new citrus varieties [4, 5]. In a prior work, 127 putative mutant clones of 'Pêra' sweet orange were selected, in a population of 7,579 plants produced from irradiated buds with a dose of 40 Gy of gamma-rays [6]. In the present work several mutant clones were field evaluated for disease tolerance, fruit yield and quality, under natural infestation conditions in a five-year period.

Materials and methods

The experiment was a randomized complete block design with one plant of each mutant per plot replicated five times. Non-irradiated trees of 'Pêra' were used as a control. The experiment was planted in 1993 in Presidente Prudente, São Paulo State, Brazil, and was evaluated from 1997 to 2001.

Fruit length (L) and width (W), L/W ratio, number of seeds per fruit, fruit mass (g), peel thickness and juice content (%) of 10 samples (one fruit each sample) were evaluated. Fruit yield per plant was evaluated annually.

Citrus canker incidence was evaluated in four plant quadrants (north, south, east and west) based on the number and size of the canker pustules on leaves and fruits observed in five plants. It is recorded in a 0 to 5 scale, with 0 for no symptoms, 1 for 1-10%, 2 for 10-20%, 3 for 20-30%, 4 for 30-40% and 5 for above 40% of disease incidence.

All variables were averaged for a period of five years and were statistically compared using Dunnett´s test.

Results

Mutant clones 27, 28, 42, 58, 59 and 101 had average seed number per fruit equal or less than one, significantly lower than the control that had six seeds per fruit, therefore, these clones were considered seedless mutants. Because they presented average seed number per fruit equal or less than one, while control showed six seeds (Table 1). On the other hand, clone 9 was considered a low seed number mutant, with average of 1.5 seed per fruit. For all clones, a high stability for this characteristic was observed in all years of the experiment evaluation (Figure 1).

Table 1. Fruit characteristics of new mutant clones sweet orange c.v. 'Pêra' [1]

Clone No.	Fruit length (cm)	Fruit width (cm)	L/W Ratio	No. of seeds per fruit	Peel thickness (cm)	Fruit mass (g)	Juice content (%)
9	7.7	7.2	1.08 **	1.5 **	0.45 **	208	40.99
27	7.3	7.0	1.04	0.4 **	0.33	196	41.95
28	7.6	7.1	1.06	0.2 **	0.36	207	39.23 **
42	7.6	7.1	1.07	1.0 **	0.37	206	42.30
58	7.3	7.0	1.05	0.4 **	0.37	195	42.77
59	7.3	7.0	1.05	0.7 **	0.38	195	41.63
101	7.4	7.1	1.04	0.3 **	0.38	206	41.36
Pêra	7.5	7.2	1.05	6.0	0.37	207	43.82

[1]Average values (*n* = 10) were compared with the control Pêra using Dunnett´s test; values followed by ** are significantly different from Pêra (P=0.01)
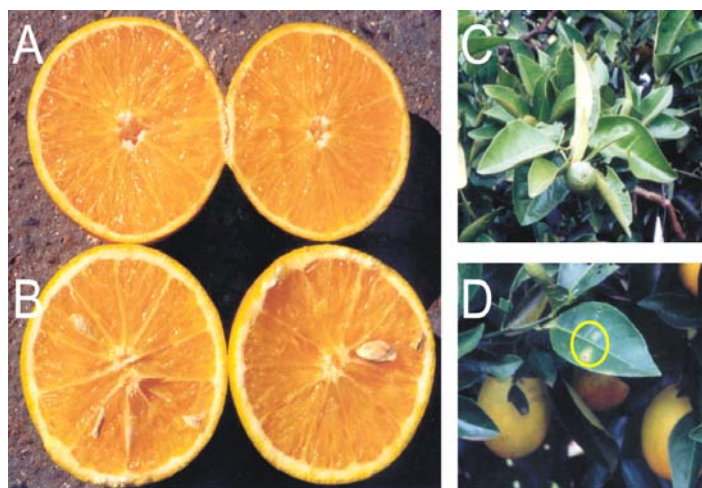
Figure 1. Fruits and leaves of sweet orange cv. 'Pêra' and it's mutant clones. A and B – Fruits of 'Pêra' and clone 28 (seedless mutant); C and D – Leaves of 'Pêra' and clone 9 (mutant tolerant to citrus canker), the symptoms of citrus canker infection is highlighted in the circle.

Clones 9 and 42 significantly presented higher tolerance to citrus canker in leaves and fruits compared to the control, during all five years of experiment, under natural infestation conditions (Table 2; Figure 1).

All clones showed lower fruit yield than the control, five of them at significant levels. However, it is commonly observed in citrus and other species with seedless fruits. Moreover, the fruit yield of young bearing trees (four to nine years old) might only be considered preliminary, this characteristic and the lower yield per plant does not impair their commercial use, especially if its groves are established with high tree density.

The seven mutants have been tested within cultivar competition in multi-trial local experiments with greater number of plants per clone, in order to evaluate yield potential of trees for a longer period. Two clones that showed improved citrus canker tolerance are under evaluation in other experiments involving artificial inoculation of the pathogen.

Table 2. Citrus canker incidence on leaves and fruits and fruit yield of new mutant clones of sweet orange c.v. 'Pêra'

Clone No.	Leaf canker incidence	Fruit canker incidence[1]	Fruit yield [2] (kg/plant)
9	1.42 **	1.33 **	23.52 **
27	2.24	2.59	25.55 **
28	2.35	2.47	19.16 **
42	1.62 *	1.83 *	33.10
58	2.17	2.38	25.22 **
59	1.99	2.28	31.31
101	2.09	2.05	26.84 *
Pêra	2.14	2.30	42.78

[1]Data were present as the average ($n = 10$) and were compared with the control using Dunnett´s test.
[2]Average for five harvest seasons.
Values followed by ** and * are significantly different from the control P=0.01 and P=0.05, respectively.

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Mutant Variety

Mutant Durum Wheat Varieties Devleoped in Bulgaria

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Abstract

Six durum mutant varieties were developed from 1984-2000 in Bulgaria. The yield of these varieties increased steadily in regional trials and all possessed good quality characters. These mutant varieties have been the leading durum variety in Bulgaria during the past 20 years.

Key words: Durum, mutant variety, gamma rays

Introduction

Mutation techniques have been successfully deployed in durum wheat improvement in Bulgaria since early 1980's. A total of 5 mutant varieties were developed, which have substantially and continuously increased the yield level of durum wheat.

Variety "Gergana"

Variety Gergana is the first Bulgarian mutant – hybrid durum wheat variety, originated from the crossing between durum wheat variety No. 788 and mutant line M-5574/109. The F_2 hybrid seeds from the above cross were irradiated with gamma rays – 10 krad again. It was recognized as an original cultivar in 1984. Cultivar Gergana covered 40-45% from the durum wheat cultivated area in the country.

The stem height of Gergana is 90-100 cm with good lodging resistance. The grains are very large, with a 1000 grain weight of 55-58 g. The vitreousness of the grains is 90-95%. Gergana possesses very good winter hardiness. It has 30-33% higher productive tillering than the old standard Zagorka and 15-20% higher productive tillering than the current standard Progress. Gergana possesses higher productivity than Zagorka, but lower than Progress. The content of protein in grains reaches to 14-16%, wet gluten-30-34%. Productivity – 6000-7000 kg/ha.

Variety "Progress"

Variety Progress is an essential variety in the country and national standard of the Variety State Commission since 1992, originated from the crossing between two mutant lines – M-1193/258 and M-5574/109. Currently Progress covers 40-50% from the durum wheat cultivated area in the country. It was recognized as an original cultivar in 1990. The stem height is 85-98 cm with good lodging resistance. Progress is distinguished by very large grains with pleasant amber color – the 1000 grain weight is 58-61 g, test weight – 77 to 79.6 kg/ha (Figure 1). The vitreousness of the grains is 90-95%. Progress possesses the same biochemical and technological traits as Zagorka (Figure 2). It is suitable for pasta making. The results of the ten year testing period in Cotton and Durum Wheat Research Institute indicate that this cultivar has 10% higher productivity than the old standard Zagorka (Figure 3). Progress, with its high ecological plasticity and

adaptability, is suitable for different soil types and climatic regions. Maximum reached productivity of variety progress was 8340 kg/ha.
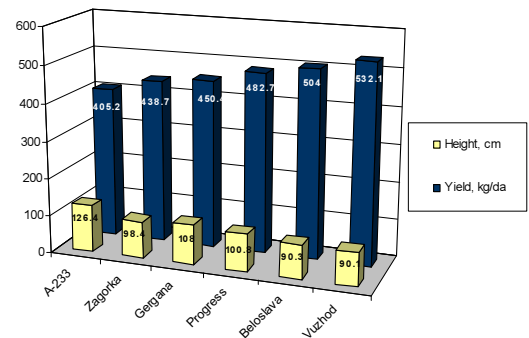

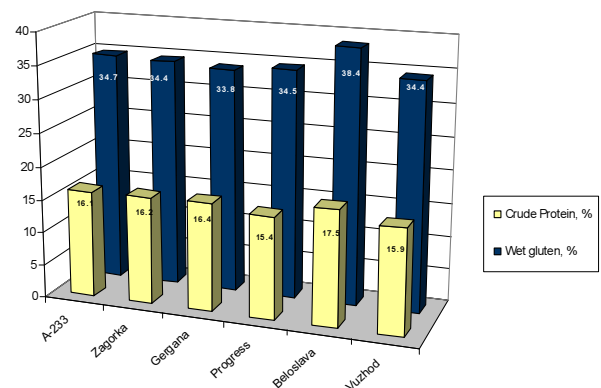*Figure 1.* The plant height and yield of mutant durum varieties

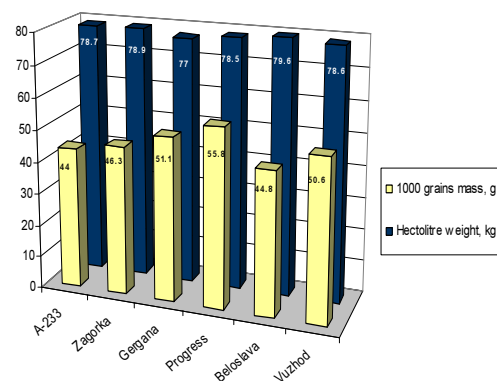*Figure 2.* The grain quality characters of mutant durum wheat varieties

Figure 3. The grain weight of mutant durum wheat varieties

Variety "Beloslava"

Variety Beloslava was recognized as an original cultivar, appropriate to the whole country in 1997. Beloslava originates from the crossing between two mutant lines: M-224 and M-155. The stem height is 80-88 cm with very good lodging resistance. The ear is large and produces 14.3% more grains and has 25% more grain weight per spike than the standard variety Progress. The vitreousness of the grains is 95-98%. The results regarding productivity, obtained in the course of the ten year long period in the Cotton and Durum Wheat Research Institute show that the new variety overweighs with 14.9%, the old standard cultivar Zagorka and with 4.4% - the new standard Progress.

The most essential trait of the new durum wheat variety is its grain quality. The amount of the crude protein varies between 17-19% as compared to 14-16.5% in the standard. The wet gluten is between 40-42% as compared to 32-36% in the standard, which signifies 1.17 to 4.43% more protein as a whole. Maximum productivity of the variety Beloslava is 8000 kg/ha.

Variety "Vuzhod"

This is one of the newest durum wheat cultivars, originated from the crossing between Gergana and Zagorka. The F_2 hybrid seeds from the above cross were irradiated with gamma rays – 5 krad again. It was recognized as an original cultivar in 1999. The spike is erect with very good productivity. The grains are middle to large with pleasant amber color; the 1000 grain weight is 50-53 g.

The stem height is 85-90 cm with good lodging resistance. The variety Vuzhod has very good winter hardiness and good disease resistance (resistant to *Puccinia graminis* and *Puccinie recondite tritice)*. The results of the ten year testing period in Cotton and Durum Wheat Research Institute indicate that this cultivar has 21.3% higher productivity than the old standard Zagorka, 10.2% higher than Progress and 5.6% higher than Beloslava. Vuzhod is the same as Progress regarding the biochemical and technological traits.

Variety "Yavor"

Yavor was recognized as an original cultivar in 2000, originated from crossing mutant line M-192 and Zagorka. The F_2 hybrid seeds from the above cross were irradiated with gamma rays – 10 krad. The stem height is 90-95 cm with good lodging resistance. The grain is middle in size with pleasant amber color and with a 1000 grain weight of 44-46 g. Vitreousness is very good - 95-98%. The new variety Yavor is resistant to *Puccinia graminis* and *Puccinie recondite tritice.*

Regarding the biochemical and technological traits, Yavor is the same as the standard variety.

Variety "Impuls"

Variety Impuls originates from the crossing between mutant line M-695 and variety Zagorka. The F_2 hybrid seeds from the above cross were irradiated with gamma rays – 10 krad. It was recognized as an original cultivar in the year 2000. The stem height is 90-100 cm with good lodging resistance. The spike is long and productive; the grain is large; its 1000 grain weight – 49-51 g.

Mutant Variety

Rice Mutant Cultivar SCS114 Andosan

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Abstract

The development process and its yield, quality performance of the mutant rice variety SCS Andosan 114 was described. SCS Andosan 114 was selected from the mutant progeny of IR 841 after treatment of 150 Gy gamma rays; It had a 7.4 ~ 9.6% yield increase over IR 841 and a higher amylase content (28%) than IR 841 (19%). The mutant variety also showed high tolerance to iron toxicity and resistance to blast disease.

Key words: Rice, gamma rays, mutant variety, SCS Andosan 114

The new rice cultivar SCS Andosan 114 was developed through gamma irradiation of the original cultivar IR 841 in Epagri Experiment Station of Itajai, Santa Catarina State, Brazil.

In 1993, 300 grams of breeder seeds of the rice cultivar IR 841 were irradiated with 150 Gy of gamma rays at CENA - Centro de Energia Nuclear na Agricultura, Piracicaba, SP- Brasil (Nuclear Energy Center for Agriculture in Brazil).

The M_1 population was formed by approximately 8000 plants. Three panicles of each M_1 plant were harvested, from each of these panicles five grains were collected and mixed to form the M_2 family. Mutants were selected from M_2 population composed of about 10,000 plants and verifies in progeny in M_3 to M_5 generations. Blast resistance and iron toxicity were evaluated under adequate field conditions to favor the occurrence of *Pyricularia* sp. and iron toxicity symptoms. From M_6 generation, selected lines were evaluated for grain yield potential, resistance to lodging and grain quality in preliminary evaluation and advanced evaluation experiment. Regional trials were conducted using the selected mutant lines at M_8 and above generations. They were compared with early, medium and late maturing check cultivars for four years under different areas of soil and climate of the state of Santa Catarina. At the same time, a lot of seeds of the candidate new cultivar were sowed to check for industrial behavior and consumer's approval.

After being tested and approved for the agronomic characteristics, milling and market, the mutant line was released for the rice growers as the cultivar SCS114 Andosan.

The results obtained in the preliminary evaluation, advanced evaluation and regional trials and some observed features of SCS114 Andosan, the check cultivar Epagri 108 and the original cultivar IR 841 are presented in Table 1.

SCS114 Andosan has similar maturity and plant height to Epagri 108, but has higher milling yield than the checks. SCS114 Andosan is shorter in grain length than Epagri 108; it has similar amylose content to Epagri 108 but higher than IR841. SCS114 Andosan presented average grain yield superior to the check and to other lines in regional trials (for 6 times in 4 years). Industrial tests performed with SCS114 Andosan showed that this cultivar is adequate for parboiling and it can substitute other varieties.

Table 1. Agronomic characteristics and grain quality of the cultivars SCS114 Andosan, the check cultivar Epagri 108, and IR 841. Epagri/EEI, Itajaí, 2000

Germplasm	Experiments[1]	Maturity (days)	Plant Height (cm)	Milling yield (%)[2]		Grain size (mm)[3]				Chalkiness[4]	Amylose %	Gel temp[5]	Yield (t/ha)
				W	B	L	W	T	L/W				
SCS114 Andosan	Preliminary	135	97	63,4	6,7	-	-	-	-	1	-	-	7.4
	Advanced	137	104	63,8	6,5	7,59	2,07	1,70	3,67	1	-	-	9.0
	Regional[1]	135-150	95-100	63,2	6,7	-	-	-	-	1	28	I	8.9
Epagri 108	Preliminary	138	94	60,3	8,0	-	-	-	-	1	-	-	7.4
	Advanced	136	101	63,7	6,9	7,66	2,04	1,69	3,75	1	-	-	9.6
	Regional[1]	135-150	97-109	60,7	8,1	-	-	-	-	1	28	I	8.5
IR 841[5]	Foundation seed	150	95-100	61,0	10,6	7,2	2,0	1,7	3,60	3	19	L	-

[1] Average data of four years in six rice areas;
[2] W - Whole, B - Broken;
[3] L - Length, W – Width, T – Thickness, L/W – Length-Width ratio;
[4] 0 – 5; 0 = No chalk;
[5] I – Intermediate; L - Low

Short Communication

In Vitro Mutagenesis of Chrysanthemum for Breeding

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Abstract

A protocol of *in vitro* mutagenesis for chrysanthemum was established. The 50% lethal dose (LD_{50}) is about 5.0 kR for calli irradiation. Various growth, developmental, morphological, colour and abnormal shape mutations were identified in M_1V_4 generation.

Key words: Chrysanthemum, *in vitro* mutagenesis, flower mutation

Introduction

Diversity of flower color is one of the most important breeding objectives as well as market preferences. Sexual cross is a basic breeding technique, which is carried out via combination between different traits of varieties to produce expected combinations in the off-spring. The history of mutation breeding shows that mutation technique is also effective for plant improvement, especially, when the genetic diversity is limited. Therefore, induced mutagenesis through irradiation or chemical treatment has become a very important method for plant breeding, including flower breeding. By 2005, 2335 varieties were released through mutagenesis in the world, in which ornamental crops and decorative crops are 552 varieties (IAEA, 2005). In 1976, Broetjies assumed that *in vitro* culture could remove chimerism after several cycles of regeneration. Therefore, mutation technique combined with *in vitro* culture could be successfully applied for the breeding of vegetative crops. Chrysanthemum *in vitro* culture is extremely useful for producing a huge number of explants in a short time. The purpose of this research was to combine *in vitro* culture with callus irradiation of gamma rays of Co^{60} source to enhance the diversity of flower color in chrysanthemum.

Materials and methods

CN43, a white chrysanthemum variety introduced from Holland, was used for *in vitro* mutagenesis. Flower buds were washed with soap at first, and then rewashed with sterilized distilled water and rinsed in alcohol for 30 seconds. The explants were further treated in 1% $HgCl_2$ for 6min. After sterilization, explants were washed again carefully (3-4 times) by using distilled water. The flower buds were used as explants for callus induction on Murashige & Skoog (1962) medium supplemented with growth hormones and 10% coconut water, pH = medium 5.7. The calli was transferred in petri dishes for irradiation at the various dosages of 1.0; 3.0; 5.0; 7.0; 15.0 kR; 20 Petri dishes for each dose.

After irradiation, the calli was transferred to fresh medium for calli multiplication for 30 days. The calli were sub-cultured for shoot induction on Murashige & Skoog (1962), medium supplemented with several growth hormones and 10% coconut water, pH = 5.7. At a temperature of $25 \pm 2^{o}C$, under light intensity of 3000 lux for 16h/day. After subculturing for three times, the explants were transferred to root induction medium (Murashige & Skoog 1962) with the supplements of mineral components, NAA, and 10% coconut water, pH = 5,7. After three weeks, all the plantlets were planted in the nursery for hardening and attentive care was made before transplanting in the open field. Screening of variations was done in the field at the M_1V_1 generation.

Results and discussions

After 10 days of irradiation, the calli of the control, and those treated with 1.0 and 3.0 kR gamma rays were green and compact, while those treated with higher dosages turned brown and friable. After 30 days, more calli turned into light and dark yellow treated with gamma rays at 5.0 kR and higher dosage and could not survive.

According to previous research, green, compact calli were able to regenerate to entire plant, but white yellow, friable type was not.

In the control and 1.0 kR dose class, regeneration rate reached 97.5% and 90% respectively. In the 3.0 kR group, it was 75%, while in 5.0 kR dose class group it was 20%, and explants grow slowly and weakly. Therefore, the lethal dose (LD_{50}) of this variety was determined as less than 5.0 kR.

Various growth, development, morphological, color and abnormal variation characteristics were observed in M_1V_4 plants (Figure 1).

In the control, population, all flowers were white, no variation was observed in 200 flowers. In the mutant populations treated with 1.0 and 3.0 kR gamma rays, several different variations including a range of abundant color variations and in number of petals were observed (Table 1).

Figure 1. Some typical variation forms
1. Left top: white (control), right top: pink mutant; 2. Yellow mutant; 3. Green on the top of petal; 4. Many petal; 5. A few petal; 6. A few petal; 7. Half of petal; 8. Few petals in flower; 9. Abnormal bud.

In many cases, a mutant has more than one mutated characteristic, i.e. a flower, has both light yellow and dark yellow petals in one flower. Some flowers lose half of outside petals, have half petal in pink, other petal color was white inside and light green on the top.

Table 1. Effect of irradiation on the frequency of various variations in chrysanthemum

Variation types	Irradiation dosage		
	Control	1.0 kR	3.0 kR
Flower color (yellow, pink, green)	0	120	98
Number of petal (many petal, a few petal, lose a half of petal)	0	89	64
Bud shape and not blossom	0	5	0
Leaf shape (without lobe, splitting many lobe)	0	13	17
Plant shape (stunted, growth slowly, not blossom)	0	50	36
Chlorophyll variation	0	4	11

Total plants: 200

Short Communication

High Yielding Opium Poppy (*Papaver somniferum* L.) Mutant Lines

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Abstract

Five opium poppy mutant lines were selected from the progeny of the poppy variety, De Botosani, after treatment with either gamma rays of additionally with EMS. Most of the lines showed significant increase of both seed yield and morphine content over commercial varieties in two locations.

Key words: *Papaver somniferum*, experimental mutagenesis.

Opium poppy (*Papaver somniferum* L., *Papaveraceae-* Fam.) is a plant of pharmaceutical importance for its alkaloids, among which morphine, codeine and thebaine [1, 2]. A Romanian popular local poppy variety, De Botosani, was treated by gamma rays, or additionally with EMS. Five mutant lines were selected with bigger capsules, shells and seed masses, as well as with higher morphine content in capsules in comparison with the initial *De Botosani* variety and the *Extaz* Romanian poppy cultivar, created by S.C.P.M.A. Fundulea (Romania) [3]. *Extaz* is the best performing cultivar present on the Romanian market.

In the two Romanian official centres for poppy cultivar's experimentation – Bacau and Harman - the selected plants showed, compared to the *Extaz* cultivar, bigger capsules (3 – 43% increase in length and 13 – 31% in width) (Table 1). Most of the mutant lines had also higher shell, seed mass and morphine content than *Extaz* cultivar (Table 1).

Table 1. Yielding and morphine content mutant lines

Variety	Treatment	Capsule dimensions (mm)				Shell mass (kg/ha)		Seed mass (kg/ha)		Morphine (% d.w.)	
		Length		Width							
		Mean	± %	Mean	± %	Mean	± %	Mean	± %	Mean	± %
A. Bacau location											
Control – *Extaz* cultivar		28.4		30.1		743		1338		4.235	
PN-10	2.5 kR	38.9	+36.9	45.2	+50.2	660	-11.2	878	-34.4	4.158	-1.8
PN-13	10 kR	37.2	+30.9	40.1	+33.2	1100	+48.0	1430	+6.9	5.610	+32.5
PN-15	10kR	43.2	+52.1	38.0	+26.6	1150	+54.8	1550	+15.8	5.980	+41.2
PN-18	15 kR	43.4	+52.8	36.3	+20.6	760	+2.3	1113	-16.8	3.952	-6.7
PN-19	10 kR + 0.05% EMS	40.6	+42.9	37.2	+23.6	828	+11.4	1140	-14.8	4.885	+15.3
B. Harman location											
Control – *Extaz* cultivar		45.4		32.6		562		604		2.642	
PN-10	2.5 kR	49.1	+8.0	37.9	+16.5	854	+51.9	701	+16.0	3.672	+39.0
PN-13	10 kR	46.8	+3.1	37.9	+16.5	660	+17.4	646	+6.9	2.970	+12.0
PN-15	10kR	47.9	+5.6	34.7	+6.4	868	+54.4	875	+39.9	3.646	+38.0
PN-18	15 kR	43.9	-3.3	36.7	+12.6	854	+51.9	812	+34.4	3.587	+35.8
PN-19	10 kR + 0.05% EMS	46.0	+1.3	36.9	+13.2	660	+17.4	736	+21.8	2.838	+7.4

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Short Communication

Induced Red Purple Mutants (*RP-R*) in *Datura innoxia* Mill. by Ethyl Methanesulphonate

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Abstract

Torn apple (*Datura innoxia* Mill.) was treated with methanesulphonate (EMS), diethylsulfate (DES), gamma rays, singularly or in combination. From a mutant with purple-red stem colour, petiole and foliar nervures identified in M_2, several homozygous lines were developed in M_5, which showed altered agronomic performance and increased alkaloid yield (scopdamine). The results suggested induced mutations could be used for genetic improvement of torn apple.

Key words: *Datura innoxia* Mill., ethyl methanesulphonate (EMS), pigment mutation, alkaloid content

Torn apple, *Datura innoxia* Mill., (*Solanaceae* Fam.) originating from Mexican Tableland, is one of the medicinal plant species cultivated in Romania. Tropanic alkaloids including scopolamine are extracted from herb and could be used to prepare spasmolytic drugs [1, 2].

Alkylating agents, i.e. EMS and DES and ^{60}Co gamma rays were used, singularly or combined, for treatment of cultivar Laura from the M_2 population treated with 0.3% EMS (24 hours), a mutant with purple – red colour of the stems, petiole and foliar nervures was isolated. However, the progeny of this plant segregated in M_3-M_4 generations, therefore, various homozygous lines were obtained in M_5 generation.

The total alkaloid content (expressed in % scopolamine) was quantified according to the method described in the Romanian Pharmacopoea [3]. The agronomic traits were also evaluated in various locations. In general, the mutant lines had similar plant height and degree of branching to Laura, but the alkaloid content of the *RP-R* mutant lines was 10-24% higher than the control (Table 1), therefore, the mutant *RV-R* lines can be used for the increasing alkaloid yield of scopolamine and genetic improvement of other agronomic traits.

Table 1. Mean performance of the RP-R mutant lines of *Datura innoxia* Mill. at Piatra Neamt location[1]

Mutant lines	No of capsules/plant		Alkaloid content (% scopolamine)	
	Mean	± %	Mean	± %
Control – *Laura cultivar*	11.9±0.6		0.50	
13-3-PN-85	11.4±0.6	-4.4	0.57	+14.0
14-14-PN-85	11.9±0.7	-7.4	0.59	+17.0
16-5-PN-85	13.8±0.7	+16.1	0.55	+10.0
19-2-PN-85	11.6±0.5	-2.6	0.62	+24.0
20-1-PN-85	10.6±0.5	-10.4	0.60	+20.0
21-N-85	10.8±0.7	-8.8	0.58	+16.0
3-3-SC-85	11.8±0.5	-0.3	0.59	+18.0
10-SC-85	13.5±0.7	-13.5	0.58	+16.0
16-SC-85	11.6±0.6	-2.5	0.56	+12.0

[1]Data is the average of three generations M_{6-9}

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Short Communication

Valuable Fenugreek (*Trigonella foenum-graecum* L.) Mutants Induced by Gamma Rays and Alkylating Agents

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Abstract

Seeds of fenugreek (*Trigonella foenum-graecum* L.) were treated with either gamma rays (25-15 kR) or ethyl methanesulphonate (EMS 0.1-0.5%, 2h) or ethylene imine (EI, 0.01-0.1%, 2h). After repeated selection for better morphological performance from M_2 to M_4 generation, 11 mutant lines were identified with better yield potential and higher diosgenin content than their parent.

Key words: *Trigonella foenum-graecum*, gamma rays, alkylating agents, induced mutation, genetic variability

Introduction

The seeds of fenugreek, *Trigonella foenum-graecum* L. (*Fabaceae*, *Papilionaceae* Family), have pharmaceutical interest thanks to their content in monohydroxy-sapogenines (i.e. diosgenin, yamogenin), which are precursors used in steroid hormones semisynthesis [1, 2, 3]. As a result of its complex chemical composition, fenugreek seeds have numerous pharmacological properties: emollient, anti-inflammatory, internal healer, antiulcer, CNS and sexual stimulant [4], hypocholesterol [5], glycemia lowering [6], antitumor [7], lowers hepatic toxicity [8], as well as diuretic [9], antioxidant [10], etc.

The purpose of this study was to increase genetic variability and to develop fenugreek lines high in steroid sapogenins content and perfectly adapted to the Romanian pedo-climatic conditions, using experimental mutagenesis, as it was done for agronomic traits.

Materials and methods

Seeds of a Romanian local population were treated singularly with gamma rays (2.5 – 15 kR) and alkylating agents – Ethyl methanesulphonate (EMS) (0.1 – 0.5%, 2h) and Ethylene imine (EI) (0.01 – 0.1%, 2h). The experiment was conducted in two experimental fields with different environmental conditions in Piatra Neamt and Secuieni of Neamt County.

Repeated selection of the mutagenized populations at M_2 to M_4 generations was applied morphological criteria. Consequently, 11 valuable vines with high diosgenin content over the local population were selected. The morphological characteristics were analyzed using 25 most vigorous individuals of each vine from the investigated local population. The diosgenin was qualitatively analyzed in CH_2Cl_2 extracts, by TLC, using cholesterol and diosgenin as standards and quantitatively measured.

Results and discussion

The mutagenic effects on M_1 fenugreek plants were reported earlier [13, 14]. Mutant lines were selected from populations derived from mutagenic treatment of either gamma rays or EMS. The number of pods per plant and the diosgenin content of 11 selected mutant lines were shown in Table 1.

The highest diosgenin level was recorded in the *Tfg-6-Sc* line with 0.38 mgg^{-1} d.w. versus 0.26 mg g^{-1}dw in control. In general, the diosgenin content of the selected fenugreek lines increased by 30%, with a maximum by 173% in the *Tfg-15-PN* variety (0.36 mg g^{-1}d.w. versus 0.13 mg g^{-1}d.w. in the control). The lowest level of diosgenin was in the *Tfg-13-PN* variety, with 77% increase over the *De Muntenia* population.

Table 1. Valuable varieties of *Trigonella foenum-graecum* selected from mutagenized populations ($M_2 – M_4$)

Mutant lines	Treatment	No. of pods / plant		Diosgenin content (mgg^{-1} d.w.)	
		Actual	Relative (*)C=100	Actual	Relative (*)C=100
Tfg-6-SC	0.05% EI, 2h	45	162	0.38	144
Tfg-5-PN	15 kR	37	132	0.36	185
Tfg-15-PN	0.5% EMS, 2h	58	154	0.36	273
Tfg-18-PN	0.5% EMS, 2h	59	158	0.34	256
Tfg-4-PN	10 kR	36	128	0.31	160
Tfg-17-PN	10 kR	55	148	0.30	227
Tfg-14-PN	15 kR	48	127	0.28	214
Tfg-6-PN	20 kR	42	146	0.28	142
Tfg-3-PN	5 kR	46	160	0.28	137
Tfg-16-PN	0.5% EMS, 2h	55	146	0.27	202
Tfg-13-PN	0.5% EMS, 2h	57	151	0.23	177

(*) Control (*De Muntenia* local population)

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Research Article

Radiosensitivity and *in vitro* mutagenesis in African accessions of cassava, *Manihot esculenta* Crantz

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Abstract

Induced mutagenesis holds promise for the subtle manipulation of traits of interest in crop plants. For a vegetatively propagated crop like cassava with severe constraints posed on its genetic improvement by inherent biological systems, the adoption of this methodology seems even the more appealing. However, there is scant information on protocols for inducing mutations in this crop. We present in this report the preliminary data on the determination of radiosensitivities for some African cassava accessions. The optimal doses of gamma ray irradiation varied from as low 12 Gy to 25 Gy. The probable implication of genotypic variation in response to gamma irradiation as was found in this study buttresses the need to carry out this larger scale study in order to avail cassava scientists intending to adopt induced mutagenesis of requisite information in this regard. A modified *in vitro* culture medium, half strength MS without growth hormones, was also shown to greatly enhance the growth of the plantlets without producing callus.

Key words: Cassava, gamma irradiation, *in vitro* mutagenesis

Introduction

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae) is a dicotyledonous tropical crop cultivated primarily for its edible storage roots on about 16million hectares with a total annual root production of 184 millions tons globally. As a staple crop, it accounts for the daily calorie intake of over 500 million people in the world (Cock, 1985.) Cassava is an ideal subsistence crop for the humid and sub humid tropics because it is well adapted to marginal soils (low fertility, high acidity), has the ability to tolerate environmental stress, has an unrivalled ability to recover from damage by pests and diseases, gives relatively high yields compared to other staple crops, and can be kept underground from 6 - 36 months after planting and is thus always available to the farmer (Uriyo, 1982). Since most processing of cassava into food is done on a small scale in rural areas, it is an important source of employment and income, especially for women. In some parts of the world the leaves are consumed as a vegetable. The starch from the roots is used in a wide variety of products, including paper, textiles, pharmaceuticals, and various foods, such as crackers, flavoring agents, noodles, and cheese breads.

The current and projected future trends in end-user preferred varieties in food, feed and industrial applications indicate a progressively increasing emphasis on the cultivation of cassava varieties whose end-products meet strict industrial and nutritional requirements. The genetic improvement of cassava through conventional means is however severely constrained by the asynchronous and shy flowering nature of the crop (Jennings and Hershey, 1984). Also, being an outcrossing species that is not amenable to inbreeding, the high levels of heterozygousity impact on the ability of breeders to develop lines for use in hybridization schemes. These scenarios therefore make the adoption of alternative means for improving the crop imperative. One such option is induced mutagenesis which accords with the current trends for the crop: maintaining the favorable traits (such as high yield) in elite clones while simultaneously effecting subtle changes to the quality traits in order to develop variants that would be suitable for specific industries (Broertjes and Van Huan, 1978; Broertjes and Van Huan, 1988; Donini and Micke, 1984; Konzac, 1984; Micke et al., 1987). The aim therefore is to produce induced cassava mutants whose end products will be suitable for bioethanol production; have contents of waxy starch; root and foliage protein content and profile; high amylose contents; and reduced anti-nutritional factors. One such strategy involves inducing elite well-adapted African cassava varieties to mutate by exposure to gamma rays.

The choice of irradiation doses is critical for the success of mutation induction. For instance, while a high dose may have high mutation frequencies; this is usually accompanied by lots of undesirable mutations in several segments of the genome implying a necessity for elaborate strategies to break the linkage drags. As there is a paucity of data for induced mutations in cassava, this was the first step in establishiung an induced mutagenesis platform for the genetic improvement of cassava.through the irradiation of cassava *in vitro* propagules. The decision to use *in vitro* propagules derives from the aforementioned difficulty to generate sexual botanical seeds from most of these varieties. *In vitro* propagation from axillary buds and meristems tips is one such method applicable to cassava improvement and also offers possibilities for further manipulation such as for the dissociation of chimeras. The method rely basically on the premise that vegetative explants are treated with mutagens; resulting population of putative mutants are multiplied to dissolve chimerism; and homohistont plants are established and finally screened for the useful traits.

Additionally, mutation induction aims to optimize genetic variation with minimal plant injuries meaning that a balance has to be found between achieving mutagenesis and maintaining the integrity of the majority of the genome constitution of the mutated material. The present study was aimed at determining

the optimal doses for irradiation using assessable parameters of primary injury in tissue cultures of different African cassava accessions. These parameters would then be relied upon as being indicative of mutagenic responses and thereby permits the estimation of optimal doses for mutation induction in this crop.

Materials and methods

Plant materials and source of explants

Three virus-tested and *in vitro* plantlets of 17 elite IITA-derived cassava varieties and two popular grown landraces in Nigeria were used for the study (Table 1). They were selected on the bases of validated agronomic traits such as high yield (dry matter and starch contents); disease and pest resistance; plant architecture; etc.

Rapid in vitro propagation of the cassava accessions

The ex-plants for initiating the cultures were two-node segments obtained by cutting the stems of the *in vitro* plantlets. These segments were transferred to conical flasks containing liquid culture medium. To get the best media composition, the Murashige and Skoog (1962) medium containing basal salts and vitamins and 20g sucrose at pH 5.8 along with media compositions of Roca et. al.(1984); Konan et al.(1997); and Danso and Ford-Lloyd (2002) that had been reported for meristem culture of cassava were evaluated for efficiency in the multiplication of different *in vitro* accessions of cassava.

Table 1. Characteristics of elite African cassava accessions for which optimal gamma irradiation doses were determined

Genotype	Superior characteristics
I30572	High yield and resistance to biotic stresses
I4(2)1425	Good nutritional quality and plant architecture
I82/00058	High yield , resistance to biotic stresses, and good plant architecture
I92/0057	High yield , resistance to biotic stresses, and good plant architecture
98/0505	High yield , resistance to biotic stresses, and good plant architecture
98/0581	High yield , resistance to biotic stresses, and good plant architecture
98/2101	High yield and resistance to biotic stresses
I95/0289	High yield , resistance to biotic stresses, and good plant architecture
I96/1632	High yield , resistance to biotic stresses, and good plant architecture
01/1277	High yield , resistance to biotic stresses, and good plant architecture
94/0330	High yield , resistance to biotic stresses, and good plant architecture
01/1371	High yield , resistance to biotic stresses, and good plant architecture
98/0002	High yield , resistance to biotic stresses, and good plant architecture
TME 1	Popularly grown landrace good nutritional and culinary qualities and resistance to biotic stresses
TME 2	Popularly grown landrace good nutritional and culinary qualities and resistance to biotic stresses,
TME 203	Popularly grown landrace good nutritional and culinary qualities and resistance to biotic stresses
TME 419	Popularly grown landrace good nutritional and culinary qualities and resistance to biotic stresses

Determination of optimal doses for irradiation

Radiation sensitivity tests were carried out to determine the optimal doses of irradiation for the exposure of the explants of the 17 cassava accessions. The grown explants were de-leafed and cut into explants containing 2 nodes each. These were placed in Petri dishes containing sterile distilled water, with each Petri dish containing 10 explants. These Petri dishes were sealed with laboratory parafilm. Each Petri dish was irradiated with different doses (5, 10, 15, 20, 25 and 30 Gy) while a control batch was not irradiated. Under aseptic conditions (in an airflow cabinet), these irradiated samples were then introduced into conical flasks containing liquid growth medium described above. These were allowed to grow on a horizontal gyratory shaker at about 30 rotations per minute. The culture was maintained at 26 C under continuous light.

After 4 to 5 weeks of growth, 3 parameters, weight of the explants, the average number of nodes, and plant height were measured and scored as a percentage of the control, i.e. untreated material of the same genotype. Data from these parameters were plotted against the corresponding doses of Gamma ray exposure. Based on the differences in height and weight between the irradiated and non-irradiated control, the dose leading to an average of 30 percentage damage was determined as the optimal dose. This is usually designated as LD_{30} with LD being the abbreviation for lethal dose.

Results and discussion

Culture medium

Unintended formation of callus is one of the handicaps that are usually encountered in *in vitro* multiplication of some cassava varieties. In the present study, several previously reported media compositions were evaluated for their efficiency to achieve good multiplication rates without forming callus for the 17 different accessions of cassava from IITA. The best results were achieved through the modification of the Murashige and Skoog (1962) basal medium involving alterations in strength, and contents of hormonal and other supplements. The best culture medium was made with half strength of Murashige

and Skoog basal medium supplemented only with 20gm sugar. The growth hormones were eliminated completely. The medium was constituted as follows:

For one litre of liquid medium, the following was used:

- *MS basal medium(Sigma M5519) = 4.4g*
- *Sucrose (Grade1, Sigma) = 20g*
- *Sterile double distilled water used to make up the volume to 1000ml*
- *pH adjusted to 5.8*

For one litre of solid medium, the following was used:

- *MS basal medium (Sigma M5519) = 4.4g*
- *Sugar (Grade1, Sigma) = 20g*
- *Gelrite = 1.8g*
- *Sterile double distilled water used to make up the volume to 1000ml*
- *pH adjusted to 5.8*

Optimal doses for gamma irradiation

Figure 1 shows the reaction to variations in irradiation dosage by cassava genotype TME 203. This was estimated as percentage departure of the height and weight of plantlets from irradiated *in vitro* nodal segments from the values of the non-irradiated control. The point corresponding to 30% damage, i.e. 70% mark on the Y-axis (read off from the line of best fit) is considered as the LD_{30}. For this genotype and using these 2 parameters, these points fall around 15 Gy and 18 Gy. This could also be calculated more precisely using the linear regression equation. In practice however, irradiation for generating mutants in crop improvement programmes is carried out over a range of plus/minus 5 of this determined optimal dose. The values for LD_{30} have also been determined for the other cassava accessions (data not shown) and based on these, bulk irradiation of these cassava genotypes have been carried out at 3 different gamma irradiation doses of 12 Gy, 25 Gy and 20 Gy, respectively. This variation would indicate a genotypic effect in the reaction of cassava genotypes to irradiation. In order to substantiate this, a more detailed study is ongoing and involves the evaluation of the reaction of both African and South American cassava accession to gamma irradiation based on these 3 parameters.
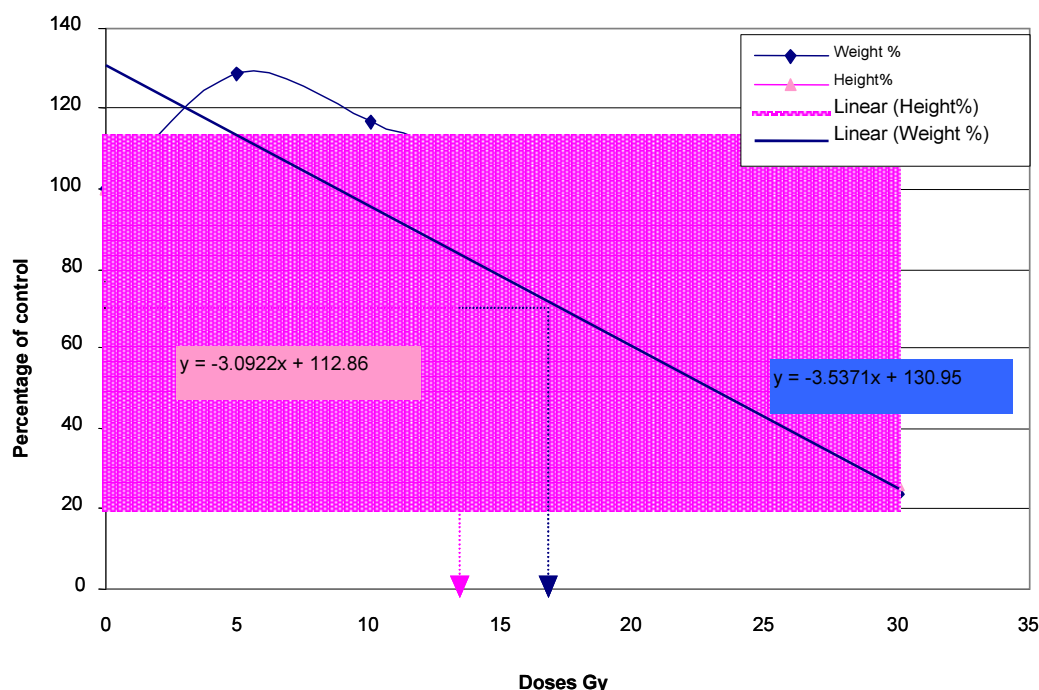

Figure 1. Differences (%) in growth parameters between plantlets arising from non-irradiated and irradiated in vitro nodal segments of cassava genotype TME 203 plotted against irradiation dosage

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[*] Control (*De Muntenia* local population)

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