

# IDENTIFICATION OF RICE MUTANTS (*Oryza sativa* L.) FOR AGRONOMICAL AND ROOT SYSTEM TRAITS

## IDENTIFICAÇÃO DE MUTANTES DE ARROZ (*Oryza sativa* L.) PARA CARACTERÍSTICAS AGRONÔMICAS E DO SISTEMA DE RAÍZES

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### ABSTRACT

*Mutation techniques are very important tools to study genetic variability, function, action and regulation of genes. Moreover, in plant breeding the root system is traditionally selected in an indirect way. In this paper we propose the use of mutation techniques to directly select mutants affecting root morphophysiological traits for future mapping and cloning of the genes involved. After preliminary tests to identify the best radiation dose, 2,500 and 3,500 seeds from cultivar Taim, indica sub species, were irradiated with doses 200 Gy and 250 Gy, respectively, and grown in a protected area. After harvesting, M<sub>2</sub> seeds were dried, stored and plantlets were evaluated in hydroponic culture, aiming to identify potential root mutants. The dose of 200 Gy seems to be associated with increases in number of seminal roots. From 9,737 seeds evaluated we selected 623 putatively root mutants. Moreover, we selected plants with high and low number of seminal roots. Which can be important to evaluate the root effect on plant establishment. The root mutants described will be available to the scientific community through the rice mutation club / FAO / IAEA after their proper confirmation and characterization.*

*Key words: seminal roots, irrigated rice, irradiation, <sup>60</sup>Co, genetic variability.*

### INTRODUCTION

Many studies discuss the needs and difficulties to increase world grain production (BORLAUG, 1983; ZAPATA & IZQUIERDO, 1994; BORLAUG, 1997; MANN, 1997 and 1999). Many recent tools have been described as very important not only for the goal of increasing production, but also for improving quality traits, i.e., the application of molecular markers looking for genetic potential from the wild (TANKSLEY & MCCOUCH, 1997), implementation of QTL – Quantitative Trait Loci – transfer methods by MAS – Marker Assisted selection – (FRARY et al., 2000) and the possibility to increase provitamin A in rice endosperm by GMO – Genetically Modified Organism - production (YE et al., 2000).

To increase yield potential, it is necessary the assembly of many techniques that form modern genetic breeding programs such as empiric selection, MAS and genomic methods (STUBER et al., 1999). Also changes in the paradigm of phenotypic selection to gene selection and disregarding differences between classical and modern breeding, as well as

between transgenic and non-transgenic plants have been suggested (KOORNNEEF & STAM, 2001).

Induced mutation is an important tool in rice breeding worldwide (MALUSZYNSKI, 1998; MALUSZYNSKI et al., 1998). In Brazil, breeding programs have used this technology in wheat as an efficient method to correct a deficiency in a cultivar (TULMANN NETO et al., 1995 a) and in soybean to increase the earliness (TULMANN NETO et al., 1995 b). PANDINI et al. (1997) obtained very close results when comparing two strategies, induced mutation and reciprocal crosses aiming to obtain genetic variability in triticale for plant height. Worldwide, many studies have contributed to increase the world grain production and to produce mutants for many traits (MALUSZYNSKI et al., 1998). Although, except to maize, barley and tomato, mutants generated and not included into breeding programs were lost. Nowadays, mutants for all traits are very important for variability studies, function, regulation and action of genes, mainly because of the new technologies available. To facilitate genetic variability studies, it is necessary to decrease the phenotypic gap; therefore, genomes might be studied in greater depth – by knowing new mutants in the same loci – and greater breath – by knowing new mutated loci (MALUSZYNSKI, 1998).

Genomics has contributed to comparative studies especially after the recent development of molecular marker technology and bioinformatics (LIU, 1998). Moreover, the utilization of small genomes as models, such rice, contributes to genomic studies, mainly because of the syteny among cereals (BENNETZEN & FREELING, 1993; GALE & DEVOS, 1998; MALUSZYNSKI, 1998; WILSON et al., 1999; MCCOUCH, 2001).

In this context, our goal was to obtain mutants for the rice root system and other agronomical traits by irradiation seeds with <sup>60</sup>Co.

### MATERIAL AND METHODS

Rice seeds from cultivar Taim, indica sub specie, were irradiated with <sup>60</sup>Co in an Eldorado 78 apparatus (*Centro regional de oncologia/UFPel*). Seeds were placed 10 centimeters distant from the radiation source, at this distance radiation is 24.25 Gy per minute. Therefore, for the dose of 200 Gy, seeds were exposed for 8 minutes and 15 seconds, and for 250 Gy, seeds were exposed for 10 minutes and 19

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seconds. For one hour before and after irradiation, seeds were subjected to distilled water.

To establish the radiation doses used in this work we carried out a preliminary experiment with different doses: 0, 25, 50, 100, 200 and 400 Gy. Each dose was composed of 200 seeds, the evaluation of the best dose was performed comparing the number of plants 20 days after germination.

Two doses were used to obtain M<sub>1</sub> plants: 2,500 seeds were irradiated with 200 Gy (first area) and 3,500 seeds were irradiated with 250 Gy (second area). After radiation, M<sub>1</sub> seeds were sowed and grown in greenhouse for one month and then planted in the field. M<sub>1</sub> plants were grown in field conditions until seed maturity.

After harvesting of M<sub>2</sub> seeds screening of the mutants started. A total of 9,737 M<sub>2</sub> seeds were evaluated. Initially, seeds were treated per 10 minutes with 0.2% sodium hypochlorite, avoiding fungi contamination. After this treatment seeds were washed immediately with sterile distilled water and put directly over pots having nylon net in contact with the hydroponic solution, to germinate. Hydroponic solution was performed as described by CAMARGO & OLIVEIRA (1981). Fifteen days later, the root system was evaluated. Traits evaluated were: number of seminal roots, earlier main root degeneration, main root absent or degenerated, albinism, disoriented growth, total collapse, dwarfing and others. All putative mutants and wild types were transferred to field to score for other traits. On field, plants were also evaluated for: cycle, plant height, dwarfness, plant and leaf architecture, absence of tiller and others with abnormal behavior. Putative mutants were identified and separately harvested.

Because of the great segregation of seminal root number trait, a comparative study was performed among control, 200 Gy M<sub>2</sub> seeds and 250 Gy M<sub>2</sub> seeds. One thousand seeds for each treatment were evaluated. Seeds were germinated in orientated growing as described in RAS (1992). Root system was evaluated according to seminal root number in eight classes. Starting from the frequencies of each class a polynomial regression was built for each treatment with the Microsoft Excel software.

## RESULTS AND DISCUSSION

Preliminary results did not show germination differences between zero to 200 Gy (approximately 80% - data not shown). However, 400 Gy affected drastically the germination. Besides these results, MALUSZYNSKI<sup>\*</sup> suggested the use of 200 and 250 Gy to irradiation seeds looking for root mutants. It pointed out to the range of radiation doses to choose for obtaining M<sub>1</sub> plant populations, 200 Gy (first area) and 250 Gy (second area). Furthermore, Shwewartun, a rice variety released in Myanmar in 1975 by U Tim Myint and U Khin Win, was obtained by irradiation of the variety IRS with 250 Gy (MALUSZYNSKI et al., 1998), which is consistent with the doses used in the present study. In the first area (200 Gy) 1,221 M<sub>1</sub> plants were harvested from 2,500 irradiated seeds, representing about 49% of productive or fertile plants. In the second area (250 Gy) 1,410 M<sub>1</sub> plants were harvested from 3,500 irradiated seeds, representing almost 40% of productive or fertile plants. These results characterize the negative effects of the higher radiation doses in plant survival, although plants irradiated with 200 Gy and 250 Gy produced similar number of seeds (data not shown).

Root system analyses were performed in hydroponic culture. From 9,737 seeds evaluated, 8,751 plantlets were obtained (~90%) and were planted in the field. A total of 623 (7.11%) possible mutants were identified (Table 1). From this total, 120 (1.37%) had no seminal roots, 192 (2.19%) were either rootless or earlier root degeneration, 5 (0.06%) plantlets showed a collapsed root system and 14 (0.16%) had disoriented growing (Table 1). These results are in agreement with SZAREJKO & MALUSZYNSKI (1980), who obtained 3.3% of root mutants by seed treatment with a combination of NaN<sub>3</sub> and Nmit (N-methyl-N-nitrosourea). Some putative mutants may be observed in Figure 1.

Table 1 – Putatively mutants identified from 8,751 M<sub>2</sub> rice plantlets evaluated in a hydroponic system.

Description	Number	% evaluated
Whole plantlets	8751	100
Whole putatively mutants	623	7.11
Absence or degenerated of main root	192	2.19
Absence of seminal root	120	1.37
Cycle	97	1.11
Plant height	29	0.33
Plants tillerless	13	0.15
Albinism	30	0.34
Dwarfness	62	0.71
Root system collapsed	5	0.06
Shoot collapsed	2	0.02
Plant collapsed	16	0.18
Disoriented growing	14	0.16
Other traits	43	0.49

Many root system mutants were identified and characterized in *Arabidopsis*: *alf1*, *alf2*, *alf4* (CELENZA et al., 1995), *rml1* and *rml2* (CHENG et al., 1995), *rt1* (KING et al., 1995) *sur1* (BOERJAN et al., 1995); and maize: *rt1* (JENKINS, 1930; OLIVEIRA & BENNETZEN, 1998) *des21* (GAVAZZI et al., 1993), *rth1-3* (WEN & SCHNABLE, 1994), *irt1* (HOCHHOLDINGER & FEIX, 1998 a; HOCHHOLDINGER et al., 2001) *rtcs* (HERTZ et al., 1996; HOCHHOLDINGER & FEIX, 1998 b). But in rice, projects looking for identifying and organizing mutant germplasm banks are just beginning. It is very important to search new mutated loci for contributions to narrowing the phenotypic gap in rice and other crop species. Essentially, rice is a genetic model for all cereals and the main feature that made it as model specie is the synteny among grass species (GALE & DEVOS, 1998; BENNETZEN, 2002), the relatively small genome (ARUMUGANATHAN & EARLE, 1991) and the economic and social importance. Identification and characterization of new rice mutants aiming at constructing a germplasm bank are necessary efforts to improve breeding programs and genetic basic studies.

Besides plantlets with anomalies in the root system, many other traits were identified, such as albinism, dwarfness and collapse of the shoot (Table 1). Furthermore, field observations also allow selecting plants with other altered traits such as: 97 plants for cycle, 29 plants for plant height and 13 plants tillerless. To select soybean plants for cycle, TULMANN NETO et al. (1995 b) also applied induced mutation in their studies.

A study carried out to verify the radiation effects on the number of seminal roots, presented attractive results. The 200 Gy dose increased the number of seminal roots in comparison to the control and 250 Gy. (Figure 2), indicating a potential increase in the frequency of alleles favor a high number of seminal roots. This tendency was also observed in mutated oat

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(400 Gy), although for this species the standard number of seminal roots was three (FONSECA et al., 2002). The great variation in number of seminal roots in rice, including all doses and the control, suggests that this trait still segregating, according to MALUSZYNSKI (1998), who described that the

root system has not been directly selected. We suggest that the selection of plants with high and low number of seminal roots could be important to evaluate the effect of this kind of root on plant establishment in future studies.



Figure 1 – Some putative mutants selected in hydroponic culture. 1 – Absence of seminal roots, 2, 4 and 6 – control, 3 – root system collapsed, 5 – albinism, 7 and 8 – two seminal roots, 9 – early main root degenerated, 10 – plantlet with two apical meristems.

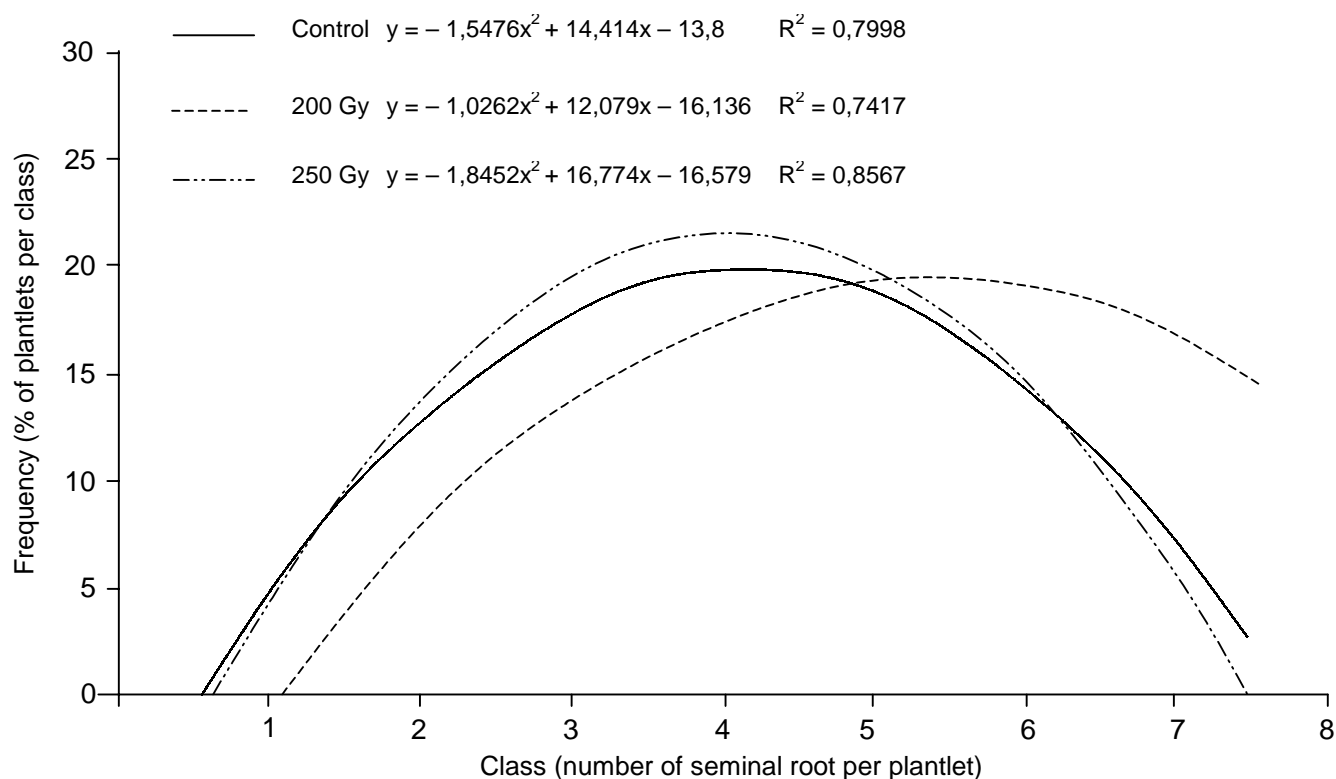


Figure 2 – Seminal root system affected by different radiation doses – data from 1,000 plantlets evaluated in each dose.

## CONCLUSIONS

Induced mutation with  $^{60}\text{Co}$  is efficient to promote different root system anomalies and to alter other plant agronomic traits.

## RESUMO

As técnicas de mutações são ferramentas fundamentais para estudos de variabilidade genética, função, ação e regulação de genes. Além disso, no melhoramento genético de plantas, o sistema de raízes é tradicionalmente selecionado de forma indireta. Neste trabalho, propõe-se a utilização de técnicas de indução de mutações para selecionar diretamente mutantes que afetam características morfológicas do sistema de raízes do arroz, para futuramente mapear e clonar os genes envolvidos. Após estudos preliminares para identificar a melhor dose de irradiação, foram irradiadas 2.500 sementes com 200 Gy e 3.500 sementes com 250 Gy. Após a irradiação as sementes foram semeadas em área protegida para obtenção da geração  $M_2$ . Após a colheita, as sementes  $M_2$  foram secas e estocadas. Os mutantes para o sistema de raízes foram identificados em cultivo hidropônico e as características ciclo e estatura foram avaliadas no campo. A dose de 200 Gy parece estar associada com o incremento do número de raízes seminais, indicando um aumento na frequência de alelos favoráveis a esta característica. De um total de 9.737 sementes  $M_2$  avaliadas, selecionou-se 623 possíveis mutantes. Além disso, foram selecionadas plantas com alto e baixo número de raízes seminais, sendo que estes mutantes poderão ser importantes para estudos relacionados ao efeito das raízes no estabelecimento das plantas no campo. Os mutantes identificados serão disponibilizados para a comunidade científica mundial através do Clube de Mutantes de Arroz / FAO / IAEA, após as devidas confirmações e caracterizações.

Palavras - chave: raiz seminal, arroz irrigado,  $^{60}\text{Co}$ , variabilidade genética.

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