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Research Article

Challenges of selecting rice mutants for salinity tolerance at early vegetative stage

Desafios da seleção de mutantes de arroz para tolerância à salinidade no início do estádio vegetativo

Aguiar Afonso Mariano[1,2](ORCID 0000-0001-7046-6816), **Luís Hermínio Chairez Tejeda**[1](ORCID 0000-0003-3503-4799), **Viviane Kopp da Luz**[1](ORCID 0000-0001-5003-3327), **Gabriel Brandão das Chagas**[1](ORCID0009-0007-4378-3243), **Ariano Martins Magalhães Júnior**[3] (ORCID 0000-0002-0756-4648), **Antonio Costa de Oliveira**[1](ORCID 0000-0001-8835-8071), **Luciano Carlos da Maia**[1](ORCID0000-0002-4371-261X), **Camila Pegoraro**[1*] (ORCID 0000-0003-1376-9751)

[1]Universidade Federal de Pelotas, Capão do Leão, RS, Brazil. *Author for correspondence: pegorarocamilanp@gmail.com
[2]Instituto de Investigação Agrária de Moçambique, Lichinga, Niassa, Moçambique.
[3]Embrapa Clima Temperado, Capão do Leão, RS, Brazil.

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population, but its production is threatened by salinity, which affects its development in both early and reproductive stages. Gamma radiation-induced mutation has been used to generate genetic variability and develop cultivars better adapted to saline conditions. However, selecting tolerant mutants is challenging due to the genetic complexity of salinity response and the need for large populations. In this study, 100 rice mutants (M_5 and M_6) and two control cultivars (sensitive and tolerant) were evaluated under salt stress (NaCl 120 mM) in a greenhouse, assessing shoot and root growth and dry weight. Despite the variability generated, no mutant outperformed the tolerant cultivar in all traits analyzed, highlighting the difficulty of selecting promising individuals from small populations. Furthermore, environmental factors may have contributed to inconsistencies between generations, reinforcing the need for large-scale screening. The most effective strategy involves initial field selection, validation under controlled conditions, and further agronomic reassessment. Technologies such as remote sensing-based phenotyping could improve efficiency, but they remain costly. Future studies should integrate new methodologies and keep the selection of salt-tolerant mutants in early generations (M_2 and M_3) from large populations, alongside yield evaluation to confirm their agronomic applicability under salinity conditions.

KEYWORDS: Plant breeding. Abiotic stress. Gamma radiation. Genetic variability. *Oryza sativa* L.

RESUMO

O arroz (*Oryza sativa* L.) é um alimento essencial para mais da metade da população mundial, mas sua produção é ameaçada pela salinidade, que afeta seu desenvolvimento nos estádios iniciais e reprodutivos. A indução de mutações por radiação gama tem sido utilizada para gerar variabilidade genética e desenvolver cultivares mais adaptadas a condições salinas, porém a seleção de mutantes tolerantes é um desafio devido à complexidade genética da resposta à salinidade e à necessidade de grandes populações. Neste estudo, 100 mutantes de arroz (M_5 e M_6) e duas cultivares testemunhas (sensível e tolerante) foram avaliados sob estresse salino (NaCl 120 mM) em casa de vegetação, considerando o crescimento e o peso seco da parte aérea e raiz. Apesar da variabilidade gerada, nenhum mutante apresentou desempenho superior a cultivar tolerante em todas as características analisadas, evidenciando a dificuldade de selecionar indivíduos promissores em pequenas populações. Além disso, o efeito ambiental pode ter contribuído para a inconsistência entre gerações, reforçando a necessidade de triagem em grandes escalas. A estratégia mais eficiente envolve seleção inicial em campo, validação em condições controladas e posterior reavaliação agronômica. Tecnologias como fenotipagem remota podem otimizar o processo, mas são de alto custo. Estudos futuros devem integrar novas metodologias e manter a seleção de mutantes tolerantes

à salinidade em gerações iniciais (M_2 e M_3) a partir de grandes populações, juntamente com a avaliação de rendimento, para confirmar sua aplicabilidade agronômica em condições de salinidade.
PALAVRAS-CHAVE: Melhoramento genético. Estresse abiótico. Radiação gama. Variabilidade genética. *Oryza sativa* L.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely cultivated crops and serves as a staple food for more than half of the world's population (QIN et al. 2020, SINGH et al. 2024). The growing global population demands a significant increase in rice production to ensure food security (QIN et al. 2020, HAQUE et al. 2021). However, climate change exacerbates abiotic stresses such as extreme drought, prolonged submergence, extreme temperatures, and high salinity, all of which threaten rice yields (HAQUE et al. 2021, HASSAN et al. 2023). Among these stresses, salinity is a major constraint, reducing rice productivity and affecting crop quality, particularly in salt-affected regions where food security is already at risk (CHAPAGAIN et al. 2021).

Salinity, which affects 20% of irrigated agricultural land, is a significant challenge (AFZAL et al. 2023). Rice is particularly sensitive to this stress, with notable impacts from an electrical conductivity of around 3 dS m^{-1} (YEO et al. 1990, QIN et al. 2020, RODRÍGUEZ COCA et al. 2023, MHENI et al. 2024). This sensitivity varies among genotypes and throughout the rice growth cycle (ZENG et al. 2001, RODRÍGUEZ COCA et al. 2023). This species is highly vulnerable at the early vegetative stage, reducing growth rate and photosynthetic efficiency, while high salinity at the reproductive stage leads to panicle sterility, lower seed production, and inhibited starch synthesis (SINGH et al. 2021, YAO et al. 2022). However, salt tolerance in the early vegetative stage does not correlate with tolerance at the reproductive stage (TIWARI et al. 2022).

Developing new salinity-tolerant rice cultivars is a key strategy to mitigate yield losses, but breeding efforts have also narrowed the genetic base, making elite cultivars more susceptible to biotic and abiotic stresses (MHENI et al. 2024, TEMESGEN 2021). Genetic variability can be increased through conventional breeding or induced mutations, with gamma radiation being one of the most widely used physical mutagens to generate genetic variations (SAEED AWAN et al. 2021, BAGHERI et al. 2022). As a type of ionizing radiation, gamma rays can induce beneficial mutations for traits such as salt tolerance, but selecting desirable mutants remains challenging due to the need for large populations and extensive screening under controlled conditions and in the field (MBA et al. 2007, CHOI et al. 2021, SARSU et al. 2023, HAQUE et al. 2021, AFZAL et al. 2023). Despite these difficulties, mutation breeding remains a valuable tool for enhancing genetic diversity and improving stress tolerance in rice.

Improving the efficiency of selection strategies is essential to overcoming these challenges and facilitating the identification of superior mutant individuals for abiotic stress tolerance. In this context, this study aimed to explore the challenges and discuss specific strategies for selecting rice mutants with salt tolerance at the early vegetative stage applying to a small population. A collection of rice mutants obtained through gamma irradiation (250 and 300 Gy) in M_5 and M_6 generations was subjected to salt stress for this purpose.

METHODS

Plant material

The seeds of the BRS Pampeira cultivar were subjected to gamma radiation (^{60}Co) at doses of 250 and 300 Gy, and generations M_1 to M_4 were grown in the field as described by TEJEDA et al. (2024). In generations M_5 and M_6, 100 mutants, randomly chosen from a population of 4000 genotypes, to simulate selection in a small population, were characterized for salinity tolerance, with 50 genotypes from each radiation dose. The screening included these 100 mutants along with the control cultivars BRS Pampeira (salinity sensitive), from which the mutants originated, and BRS Bojuru (salinity tolerant). The experiment was conducted during the 2021/2022 and 2022/2023 harvest seasons in a greenhouse at the Universidade Federal de Pelotas, in Capão do Leão, RS, using an intercalary control experimental design with three replicates.

The plants were grown in trays containing irrigated rice field soil, with 10 lines of 10 seeds each, where each line corresponded to a different mutant or control cultivar. Salinity stress was applied when at least 50% of the plants reached stages V_2 and V_3 by replacing the water layer with a 120 mM NaCl solution for seven days, until more than 50% of the plants exhibited salt stress symptoms (LIU et al. 2017, followed by morphological characterization. For growth assessment, five plants of each mutant or control cultivar were randomly selected from each replicate (representing 50% of the available plants per line) to minimize variability while ensuring a representative evaluation of the population. Shoot length (SL) and root length (RL) were measured in these five plants per replicate, totaling 15 plants per mutant or control cultivar. The same plants were used to determine shoot dry weight (SDW) and root dry weight (RDW), with tissues dried at 65 ºC for 72 hours and weighed on a precision scale, ensuring standardized evaluation of growth and biomass accumulation.

Data analysis

The obtained data were checked for normality using the Shapiro-Wilk test and then subjected to analysis of variance to check for significant differences among genotypes. The means were then grouped using the Scott-Knott test ($p<0.05$). The Genes program (CRUZ 2013) was used to carry out the analyses. Box plots were then constructed in the R Studio V.2024.04.2+764 program (R CORE TEAM 2020) based on the groupings resulting from the Scott-Knott mean comparison test.

RESULTS

The analysis of variance revealed a genotype effect for all variables analyzed, indicating an effect of the gamma-induced mutation (Figure 1). When shoot length (SL) is considered, in the M_5 generation, mutants were classified into four groups (A–D), where Group A contained those with SL superior to BRS Bojuru (tolerant), Group B included BRS Bojuru and twenty similar mutants, and Group D clustered BRS Pampeira (sensitive) with twenty-four mutants showing the shortest SL. In the M_6 generation, ten groups (A–J) were formed, with Groups A, B, C, and D including mutants superior to BRS Bojuru, which was classified in Group E, while BRS Pampeira and other mutants with the shortest SL were placed in Group J. These results confirm the variability induced by gamma radiation and highlight the presence of mutants with improved SL compared to the original cultivar.

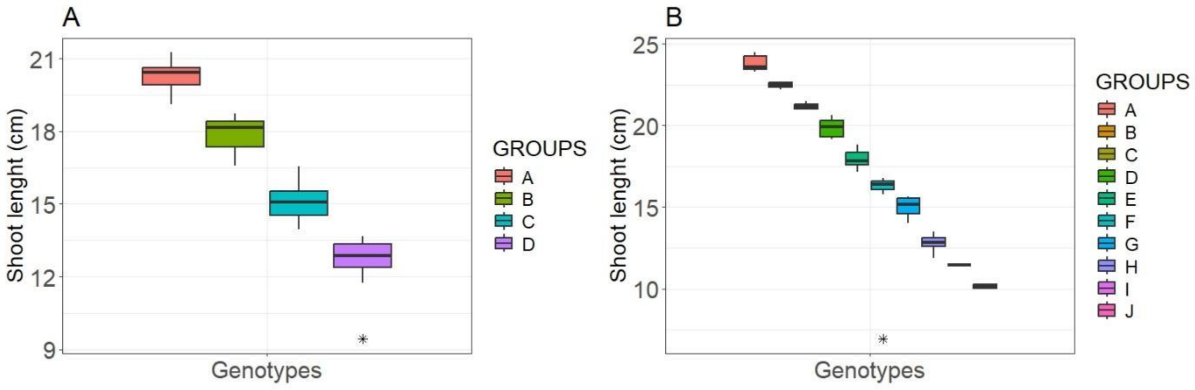

Figura 1. Shoot length (SL) of 100 mutant rice in M_5 (A) and M_6 (B) generations and BRS Pampeira and BRS Bojuru control cultivars, under salinity stress (NaCl 120mM) at early vegetative stage. FAEM/UFPel, 2023.

For root length (RL) (Figure 2), the M_5 generation mutants were divided into four groups (A-D), with BRS Bojuru and nineteen mutants in group A, showing superior performance. BRS Pampeira, along with eleven mutants, was placed in group D, exhibiting the lowest RL. In the M_6 generation, mutants were classified into eight groups (A-H), with BRS Bojuru positioned in group D, having lower RL than the top groups but higher than the remaining ones. BRS Pampeira and fourteen mutants, with the shortest RL, were grouped in category H.

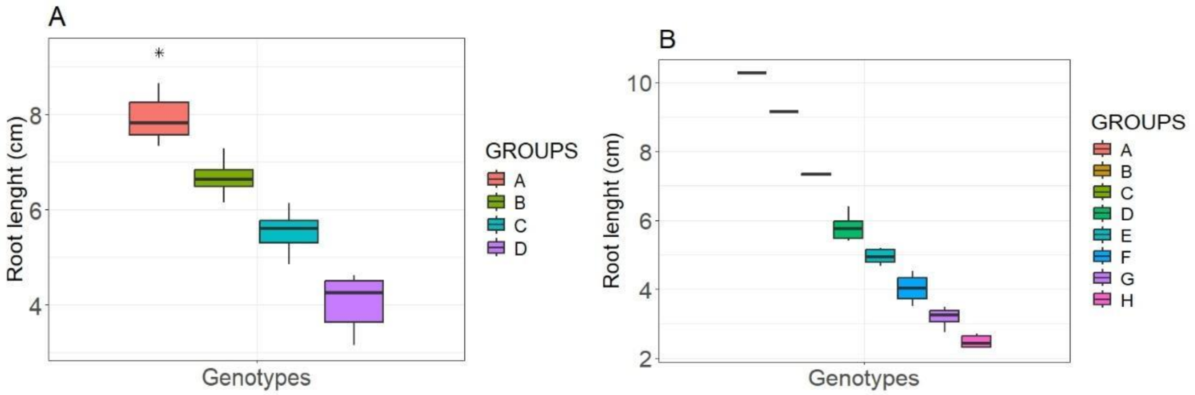

Figure 2. Root length (RL) of 100 mutant rice in M_5 (A) and M_6 (B) generations and BRS Pampeira and BRS Bojuru control cultivars under salinity stress (NaCl 120mM) at early vegetative stage. FAEM/UFPel, 2023.

The M_5 generation mutants were divided into five groups (A-E) based on shoot dry weight (SDW), with BRS Bojuru and forty-two mutants placed in group D, having lower SDW than those in groups A, B, and C (Figure 3). BRS Pampeira, along with twenty-two mutants, had the lowest SDW and was assigned to the last group. In the M_6 generation, mutants were classified into ten groups (A-J), with BRS Bojuru and two mutants positioned in group B, showing lower SDW than only those in group A. BRS

Pampeira and twenty-eight mutants were grouped in category G, exhibiting lower SDW than the previous groups but higher than those in groups H, I, and J.

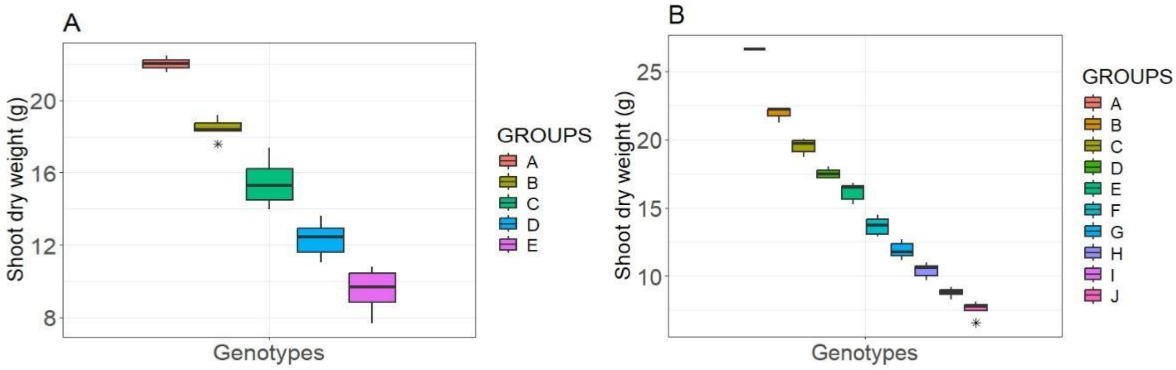

Figure 3. Shoot dry weight (SDW) of 100 mutant rice in the M_5 (A) and M_6 (B) generations and BRS Pampeira and BRS Bojuru control cultivars under salinity stress (NaCl 120mM) at early vegetative stage. FAEM/UFPel, 2023.

Genotypes in the M_5 generation were classified into four groups (A-D) based on root dry weight (RDW), with some mutants in group A exhibiting higher RDW than BRS Bojuru (Figure 4). This cultivar was placed in group B along with thirty-eight mutants, while BRS Pampeira and eighteen genotypes were positioned in group D, having the lowest RDW. In the M_6 generation, nine groups (A-I) were identified, with BRS Bojuru and two mutants in group A, showing the highest RDW among all groups. BRS Pampeira was placed in the last group, displaying the lowest RDW compared to the other genotypes.

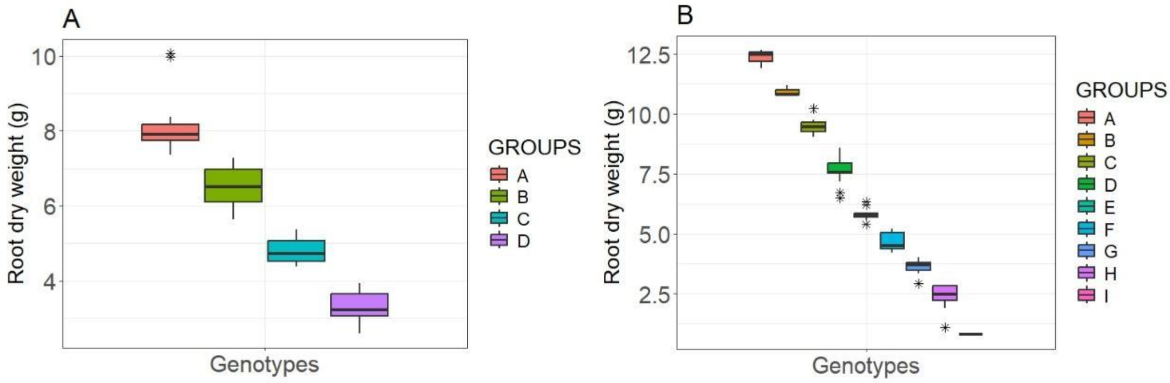

Figure 4. Root dry weight (RDW) of 100 mutant rice in generations M_5 (A) and M_6 (B) and BRS Pampeira and BRS Bojuru control cultivars under salinity stress (NaCl 120mM) at early vegetative stage. FAEM/UFPel, 2023.

DISCUSSION

Mutation induction has been widely used in rice breeding, with nearly 823 mutant rice events released globally, particularly in Japan and China (KHANH et al. 2021). While breeding and domestication have historically been based on phenotypic selection, modern mutation breeding requires a structured and efficient selection process, combining field expertise with controlled screening to identify promising mutants (FAO/IAEA 1968, KUMAR et al. 2021). In the case of salinity tolerance, selecting mutants at the early vegetative stage presents significant challenges due to the polygenic nature of the trait and its dependence on multiple physiological and genetic factors (WALIA et al. 2005, HOANG et al. 2016, LIU et al. 2022). Additionally, the success of mutation breeding depends not only on having enough plants to increase the chance of finding a desirable mutation but also on using well-adapted genetic backgrounds to ensure that salinity tolerance does not come at the expense of other agronomic traits (SINGH et al. 2021).

In this study, some mutants performed as well as or better than the tolerant control, but no genotype demonstrated consistent tolerance across both generations. This variability is expected, as salinity tolerance is controlled by multiple genes, and single mutations are unlikely to confer full tolerance without additional genetic and physiological interactions (GALHARDO et al. 2007). Furthermore, environmental effects, gene expression instability, and possible residual heterozygosity from the mutagenic process may have contributed to differences between generations, making careful selection even more critical (SINGH et al. 2021). Given these challenges, a large population, a well-structured selection approach, including experienced scientists assessing plants in the field, remains essential to ensure that potential mutants are identified efficiently.

Continued research combining induced mutagenesis and advanced screening techniques is essential to overcoming the challenges of salinity tolerance in rice, as the selection process requires evaluating large populations to increase the probability of obtaining desirable traits. However, field evaluation of salinity tolerance remains complex due to confounding abiotic stress factors, reinforcing the need for controlled environment screening, which is often limited by scale, time, and cost (GREGORIO et al. 1997, KIM et al. 2020). Therefore, an effective approach combines large-scale field screening with controlled validation, balancing genetic gains, agronomic stability, and the practical constraints of rice breeding programs.

CONCLUSION

This study demonstrated the potential of gamma radiation-induced mutation as a tool for generating genetic variability for salinity tolerance in rice. However, despite the observed variability, no genotype exhibited consistent tolerance across two generations, reinforcing the complexity of this trait and the limitations of small sample populations. Since salinity tolerance is a polygenic trait with strong environmental interactions, identifying truly superior genotypes requires not only larger populations and more refined screening methodologies but also yield assessments to confirm agronomic viability. Given that tolerance can only be effectively validated when yield performance is evaluated under stress conditions, future studies should integrate comprehensive phenotyping approaches that assess both vegetative and reproductive traits to enhance selection accuracy. Ultimately, a multi-tiered strategy combining large-scale mutation induction, field and controlled environment screenings, and yield trials will be essential to effectively develop resilient rice cultivars adapted to saline conditions.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, C.P., A.C.O., L.C.M., A.M.M.J. and A.A.M.**; investigation**, A.A.M., L.H.C.T., G.B.C. and V.K.L**; resources and data curation**, A.C.O. and C.P.**; writing-original draft preparation**, A.A.M.**; writing-review and editing**, A.A.M., L.H.C.T., G.B.C., V.K.L., A.M.M.J., A.C.O., L.C.M. and C.P.**; visualization**, A.A.M., L.H.C.T., G.B.C., V.K.L., A.M.M.J., A.C.O., L.C.M. and C.P.**; supervision, **C.P.**; project administration, **A.C.O. and C.P.**; funding acquisition, **A.C.O. and C.P**. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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