

Assessment of mutant rice genotypes on growth cycle length and response to reduced water availability

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Edited by: Leonardo Oliveira Medici

Received December 19, 2022

Accepted May 02, 2023

ABSTRACT: Rice (*Oryza sativa*) is among the most important crops worldwide; however, rice crops demand high water consumption. Future projections indicate reduced water availability and severe drought events, which may affect rice crops as the cereal is highly sensitive to drought stress. Thus, cultivars with lower water demand for irrigation or drought escape capacities are among the strategies to address this issue. This study aimed to assess five mutant rice genotypes in terms of growth cycle length, other agronomic interest traits, and drought response in the reproductive stage. The mutant genotypes evaluated showed a shortening of the growth cycle compared to the original cultivar, BRS Pampeira, representing a drought escape strategy. In addition, mutations did not negatively affect plant height and crop yield. However, similar to the original cultivar, mutants are not tolerant to water deficit in the reproductive stage. The genotypes evaluated have potential to be released as early cycle cultivars, which can reduce water demand during the harvest season while presenting a drought escape strategy.

Keywords: *Oryza sativa* L, drought stress, early cycle, mutation breeding

Introduction

Rice (*Oryza sativa* L.) is highly sensitive to drought stress in the germination, seedling (Panda et al., 2021), vegetative, and reproductive stages (Yang et al., 2019), causing yield losses. Drought duration and severity and the plant growth stage during stress impact the yield of rice crops. Stress during the reproductive stage can affect spikelet fertility and grain number, reducing yield. Likewise, stress at terminal or lateral crop stages can cause severe yield losses, as drought affects the source-sink distribution (Hussain et al., 2022).

The state of Rio Grande do Sul is Brazil's leading rice producer and commonly faces drought periods in the summer, which coincides with crop flowering. Recently (2022-2023 harvest season), drought reduced water availability for irrigation, severely affecting the rice crop yield (CONAB, 2023).

In cultivated plants, the three main mechanisms to deal with drought are escape, avoidance, and tolerance (Turner, 2003). Drought tolerance is a complex trait controlled by many genes, which hinders the work of breeders (Ghazy et al., 2021). Another alternative is the development of early cultivars, as in the escape strategy, plants complete their cycle before the onset of the drought (Singh et al., 2021). In addition, there is evidence that shorter cycle cultivars favor water saving (Vergara et al., 1966; Magalhães Júnior et al., 2021).

Plant breeding depends on genetic variability and new variations are crucial to introduce new traits during the development of cultivars. Other options can be used to obtain genetic variability when a trait is not readily available in crosses, such as the induction

of mutations using chemical and physical mutagens (Holme et al., 2019).

Gamma radiation is a physical mutagenic used to induce random mutations because this ionizing radiation can cause DNA changes. Gamma rays can induce base modifications and cause the break of one or both DNA strands. Mutations may occur during repair, leading to phenotype changes (Du et al., 2022; Riviello-Flores et al., 2022).

Therefore, this study aimed to assess five mutant genotypes of rice obtained by gamma radiation regarding growth cycle length, other agronomic traits, and drought response in the reproductive stage.

Materials and Methods

Mutation induction

Seeds of BRS Pampeira cultivar (Magalhães Júnior et al., 2017) were subjected to gamma radiation (⁶⁰Co) at doses of 250 and 300Gy to obtain mutant genotypes. The irradiation was conducted at Centro de Energia Nuclear na Agricultura (CENA) at the Universidade de São Paulo (USP).

Plant material

Seeds that underwent mutation induction were sown in the field and approximately 2000 M₁ plants were selected in the 2017/2018 harvest season. The seeds of each plant selected in M₁ arose a line in M₂ generation (2018/2019 harvest season), which underwent a selection of individual plants within the line, totaling approximately 5000 plants, which in turn originated

M_3 lines (2019/2020 harvest season). In M_1 , M_2 , and M_3 generations, the selection was based on the superiority of plants grown under water deficit in the reproductive stage. Furthermore, in M_3 generation, five mutant lines (M267, M425, M498, M519, and M636) were selected due to their apparent early cycle. These selected mutant genotypes, along with BRS Pampeira, were cultivated in the 2020/2021 and 2021/2022 harvest seasons (M_4 and M_5 generations) and were used to confirm cycle length reduction, response water deficit to in the reproductive stage, and other agronomic importance traits.

All experiments (M_1 , M_2 , M_3 , M_4 , and M_5 generations) were conducted in the field of Embrapa Terras Baixas experimental station, at Embrapa Clima Temperado, located in the municipality of Capão do Leão, Rio Grande do Sul State, Brazil (31°48'16" S, 52°24'46" W, altitude of 15 m, Köppen climate classified as predominantly temperate (with modifications by Maluf) (Wrege et al., 2012). A flood irrigation system was used, with water interruption from R_2 to R_4 (around 13 days), except for the control treatment (M_4 and M_5 generations), which had irrigation throughout the cycle. Management followed technical recommendations for rice cultivation (SOSBAI, 2018).

Assessment of mutant rice genotypes under control and water deficit

Five mutants and the original cultivar were subjected to two conditions in M_4 (2020/2021) and M_5 (2021/2022) generations. For the control, plants remained at a water depth of V_4 (collar formation on leaf four on the main stem) up to 7-10 days before harvest. In water deficit treatment, irrigation was suspended for around 13 days, from R_2 (flag leaf collar formation/booting) to R_4 stage (one or more florets on the main stem panicle have reached anthesis) (Counce et al., 2000). No rainfall was recorded in the period. Analog tensiometers (Hidrosense, model HID38) installed at a depth of 0.10 m and 0.15 m were used to monitor soil water. Soil tension at -100 KPa was obtained during the water deficit stress. After R_4 stage, irrigation was re-established. The experimental design was randomized blocks with three replications. Each repetition consisted of one 0.50 m row with a spacing of 0.20 m. The sowing density used was 60 viable seeds per linear meter.

In M_4 , days to flowering of each mutant genotype were analyzed during the period between S_3 (emergence of prophyll from coleoptile) and R_4 stage. Plant height (cm) was also evaluated when genotypes were in R_6 ; at last, one caryopsis on the main stain panicle elongated to the hull end. After harvesting, grain characterization was performed as thousand grain weight (TGW) (measured using an analytical scale (Mars) with a precision of 0.001 g in samples of 100 grains from each row, followed by extrapolation to 1000 grains), length, width and thickness grain (measured in 100 grains from

each row using a pachymeter (Starrett), with accuracy of ± 0.05 mm). Evaluations were made considering the entire row.

In M_5 , days to flowering, days to harvest, plant height, panicles number per plant, tillers number per plant, main panicle length, total grains number, sterile spikelet number, full grains weight, main panicle weight, plant yield, thousand grains weight, grain length, width and thickness were analyzed. Evaluations were made in individual plants, analyzing five plants per row.

Statistical analyses

The data on phenotyping were submitted to the analysis of variance (ANOVA) using `lm()` function from the stats library. Subsequently, the means were compared by the Tukey test ($p \leq 0.05$) using `glht` function from the multcomp library. The analyses were conducted in the "R" software.

Genotyping

A genotyping study was conducted to confirm that the mutants originated from BRS Pampeira. DNA was extracted from young leaves in a pool of ten seedlings from each M_5 mutant line (M267, M425, M498, M519 and M636), BRS Pampeira and other outgroup cultivars (BRS Pampa, BRS Bojuru, BRS Chui BRS Taim and BRS Querência) using CTAB protocol (hexadecyltrimethylammonium bromide). After verifying DNA quality and quantity in agarose gel electrophoresis and NanoVue spectrophotometer, the samples were sent to Genotyping Services Laboratory at International Rice Research Institute (IRRI), in the Philippines. Samples were genotyped with 7098 SNP markers (single nucleotide polymorphism) using 7K Infinium SNP platform (Illumina®) (Thomson et al., 2017).

Only SNPs/loci containing at least one allelic difference between the evaluated genotypes were kept.

Relatedness estimation among M_5 mutants and BRS Pampeira

After filtering the genotyping data (6656 SNP markers), relatedness was calculated based on Queller-Goodnight estimator (Queller and Goodnight, 1989). The analysis used Coancestry software, version 1.0.1.10 (Wang et al., 2011).

The remaining 6656 SNPs/loci were exported to a .vcf (Variant Call Format) file. The 6656 SNPs/loci were transformed into a matrix containing two columns using the Perl language scripts developed in-house, for each SNP, recoding the alleles to co-dominant numerical values (A = 100, C = 200, T = 300, G = 400"- "-" = 0) with the first column identifying the genotypes.

With the new matrix with co-dominant data, the analysis in the Coancestry software was performed using the parameters: 1) Unknown Frequency, 2) Calculate

Allele Frequency, and 3) Queller-Goodnight method. In this method, as described by Wang (2017), the relatedness estimator is given by:

$$\hat{r}_{QC}[a,b,c,d] = \frac{1}{2} \left[\left(\frac{\delta_{ac} + \delta_{ad} + \delta_{bc} + \delta_{bd} - 2(p_a + p_b)}{2(1 + \delta_{ab} - p_a - p_b)} \right) + \left(\frac{\delta_{ac} + \delta_{ad} + \delta_{bc} + \delta_{bd} - 2(p_c + p_d)}{2(1 + \delta_{cd} - p_c - p_d)} \right) \right]$$

where: for two individuals called X and Y, containing n loci with alleles a/b for X and c/d for Y, comparison values between the alleles of the two individuals is given by:

$\delta_{ab} = 0$ when X is heterozygous or 1 when X is homozygous; $\delta_{ac} = 0$ when alleles a and c are different and 1 when a and c are identical; δ_{ad} , δ_{bc} , $\delta_{bd} =$ same rule applied to δ_{ac} , p_a , p_b , p_c , $p_d =$ frequencies of alleles a , b , c and d in the analyzed population/sample.

Results

Assessment of mutant rice genotypes under control and water deficit in M_4 generation

In 2020-2021 harvest season, M_4 generation, days to flowering, and TGW were influenced by the genotypes. The genotype and the water deficit treatment affected grain width, length, and thickness. Plant height was changed by the interaction between the genotype and the treatment (ANOVA data not shown). In this harvest season, the main objective was to evaluate M_4 mutants regarding cycle length and drought response; therefore, yield plant was not considered.

All mutant lines showed a shortening of days to flowering. Even M425, which required a longer time to arrive at anthesis, decreased by 14 days compared to BRS Pampeira. In M267, the mutant with the shortest time to flowering, the reduction reached 26 days (Table 1). The TGW was higher in mutant lines, except for M519, which did not differ from the original cultivar (Table 1).

Table 1 – Genotype effect on days to flowering (DF) and thousand grain weight (TGW) in five rice M_4 mutants and the original cultivar, BRS Pampeira, in 2020-2021 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Genotype	DF	TGW
	days	g
BRS Pampeira	108.66 a ¹	24.66 d
M636	87.66 c	25.70 c
M519	88.66 c	25.10 cd
M498	84.66 cd	29.13 a
M425	94.33 b	27.96 b
M267	82.00 d	25.80 c

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

Mutant lines showed plant height equal to or below BRS Pampeira's (Table 2). The water deficit treatment caused a reduction in plant height in most genotypes (Table 2). The grain width of mutant lines was equal to or slightly lower than that of BRS Pampeira, while for grain length, mutant lines were longer, shorter and equal to the original cultivar. Mutant lines showed grain thickness equal to or moderately greater than BRS Pampeira's (Table 3). The water deficit treatment in the reproductive stage caused a reduction in grain width, length, and thickness (Table 4).

Table 2 – Interaction effect between genotype and treatment on plant height (PH) in five rice M_4 mutant genotypes and original cultivar, BRS Pampeira, grown with and without water deficit during the reproductive stage in 2020-2021 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Genotype	PH (cm)	
	Control	Water deficit
BRS Pampeira	83.18 aA ¹	74.83 abB
M636	77.11 bcA	67.30 cdB
M519	75.32 bcA	64.00 dB
M498	79.76 abA	76.13 abB
M425	82.8 aA	76.50 aB
M267	73.36 cA	69.96 bcA

¹Values followed by the same lowercase letter in the column and by the same capital letter in the row do not differ from each other by the Tukey's test ($p \leq 0.05$).

Table 3 – Genotype effect on grain length (GL), grain width (GW) and grain thickness (GT) in five rice M_4 mutant genotypes and original cultivar, BRS Pampeira, in 2020-2021 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Genotype	GL	GW	GT
	mm		
BRS Pampeira	10.13 b ¹	2.39 ab	1.97 b
M636	9.73 c	2.45 a	2.04 a
M519	9.60 c	2.40 ab	2.01 ab
M498	11.70 a	2.36 b	2.06 ab
M425	10.42 b	2.43 ab	2.06 ab
M267	10.44 b	2.37 b	2.01 a

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

Table 4 – Treatment effect on grain length (GL), grain width (GW) and grain thickness GT in five M_4 mutant rice genotypes and original cultivar, BRS Pampeira, grown with and without water deficit during a reproductive stage in 2020-2021 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Treatment	GL	GW	GT
	mm		
Control	10.48 a	2.42 a ¹	1.71 a
Water deficit	10.19 b	2.37 b	1.68 b

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

Assessment of mutant rice genotypes under control and water deficit in M₅ generation

In the 2021-2022 harvest season, M₅ generation, days to flowering and days to harvest as well as the main panicle length were influenced by the genotype. The genotype and the treatment affected panicle number per plant, tillers number per plant, sterile spikelets number, plant yield, grain length and grain thickness. The drought treatment changed the number of full grains, total grain weight, and grain width. The plant height and TGW were influenced by the interaction between the genotype and the treatment (ANOVA data not shown).

The growth cycle shortening of mutant genotypes was confirmed, reducing days to flowering and days to harvest (Table 5). The main panicle length varied among mutants but did not differ from that of BRS Pampeira (Table 5).

Mutants had plant height equal to or lower than BRS Pampeira, and water deficit further reduced plant height (Table 6). Three mutants had a TGW higher than the original cultivar; nevertheless, this trait was reduced in all genotypes under stress (Table 6).

Mutants showed variation in the number of panicles per plant and the number of tillers per plant, but they did not differ from those of BRS Pampeira. The number of sterile spikelets was equal to or lower than, while plant yield was equal to or higher than, in

Table 5 – Genotype effect on days to flowering (DF), days to harvest (DH), and main panicle length (MPL) in five M₅ mutant rice genotypes and original cultivar, BRS Pampeira, in 2021-2022 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Genotype	DF	DH	MPL
	days		cm
BRS Pampeira	102.66 a ¹	133.00 a	23.26 ab
M636	85.66 b	115.66 bc	21.91 b
M519	86.83 b	116.83 b	21.77 b
M498	84.83 b	114.83 bc	24.05 a
M425	87.00 b	117.00 b	22.72 ab
M267	82.66 b	112.00 c	23.22 ab

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

Table 7 – Genotype effect on panicles number per plant (PNP), tillers number per plant (TNP), sterile spikelets number (SSN), plant yield (PY), grain length (GL), and grain thickness (GT) in five M₅ mutant rice genotypes and original cultivar, BRS Pampeira, in 2021-2022 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Genotype	PNP	TNP	SSN	PY	GL	GT
	g			mm		
BRS Pampeira	23.03 ab ¹	28.80 ab	48.16 a	18.74 b	9.53 c	1.97 b
M636	28.96 a	33.66 a	31.73 b	24.50 ab	9.49 cd	1.99 ab
M519	23.83 ab	24.66 ab	39.90 ab	21.35 ab	9.27 d	1.98 b
M498	29.26 a	28.10 ab	44.00 a	25.67 a	11.41 a	2.02 a
M425	20.20 b	22.90 b	33.56 b	20.68 ab	10.05 b	2.02 a
M267	27.20 ab	29.63 ab	39.66 ab	22.35 ab	10.02 b	1.99 ab

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

the comparison of mutants with the original cultivar. Mutants showed larger and thicker grains than BRS Pampeira (Table 7). Water deficit caused a reduction in the number of panicles per plant, the number of tillers per plant, plant yield, as well as grain length and thickness. There was an increase in sterile spikelets under stress condition (Table 8). In water deficit treatment, the number of full grains, total grain weight, and grain width were reduced (Table 9).

Relatedness estimation among M₅ mutants and BRS Pampeira

As expected, mutant lines have relatedness close to that of BRS Pampeira cultivar than to most of the outgroup cultivars (Table 10). Interestingly, cultivar BRS Pampa (parents IRGA417/BRS Jaburu) demonstrated a relatedness with the BRS Pampeira (parents IR22/CNA8502) and of the mutant lines (Table 10).

Discussion

In general, rice cultivation is grouped into four ecosystems: irrigated, rainfed lowland, rainfed upland, and flood-prone (Singh et al., 2021). Irrigated rice has the highest water requirement of all cereals and when

Table 6 – Interaction effect between genotype and treatment on plant height (PH) and thousand grain weight (TGW) in five M₅ mutant rice genotypes and original cultivar, BRS Pampeira, grown with and without water deficit during a reproductive stage in 2021-2022 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Genotype	PH (cm)		TGW (g)	
	Control	Water deficit	Control	Water deficit
BRS Pampeira	91.80 aA1	73.73 bB	26.00 dA	24.00 cB
M636	84.80 bcA	70.53 bcB	26.00 dA	23.00 dB
M519	85.93 bcA	72.73 bcB	25.00 eA	23.00 dB
M498	86.20 bcA	74.13 bB	30.00 aA	28.00 aB
M425	89.20 aA	83.53 aB	27.00 cA	24.00 cB
M267	80.53 cA	68.13 cB	28.00 bA	26.00 bB

¹Values followed by the same lowercase letter in the column and by the same capital letter in the row do not differ from each other by the Tukey's test ($p \leq 0.05$).

rainfall is not sufficient to ensure water availability in flooding conditions, irrigated cultivation experiences drought effects. Rainfed cultivation is more subject to drought because it depends on irrigation facilities (Zu et al., 2017; Sandar et al., 2022).

Escape, avoidance, and tolerance are mechanisms used by plants to deal with drought stress. Escape is the capacity of the plant to complete the life cycle before the drought condition. This strategy requires cultivars with rapid germination, seedling establishment, early flowering and maturity. Avoidance refers to water maintenance during the stress period through changes, such as roll and shape modification of leaves, decreased number and size of leaves, besides increased waxiness

Table 8 – Treatment effect on panicles number per plant (PNP), tillers number per plant (TNP), sterile spikelets number (SSN), plant yield (PY), grain length (GL), and grain thickness (GT) in five M_5 mutant rice genotypes and original cultivar, BRS Pampeira, grown with and without water deficit during a reproductive stage in 2021-2022 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Treatment	PNP	TNP	SSN	PY	GL	GT
				g	mm	mm
Control	28.87 a ¹	30.80 a	33.96 b	25.97 a	10.14 a	2.06 a
Water deficit	21.97 b	25.12 b	45.06 a	18.46 b	9.78 b	1.97 b

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

Table 9 – Treatment effect on full grains number (FGN), full grains weight (FGW), and grain width (GW) in five M_5 mutant rice genotypes and original cultivar, BRS Pampeira, grown with and without water deficit during a reproductive stage in 2021-2022 harvest season, FAEM/UFPel – municipality of Capão do Leão, Rio Grande do Sul, Brazil.

Treatment	FGN	FGW	GW
		g	mm
Control	77.59 a ¹	1.96 a	2.22 a
Water deficit	68.09 b	1.63 b	2.14 b

¹Values followed by the same lowercase letter in the column do not differ from each other by the Tukey's test ($p \leq 0.05$).

Table 10 – Relatedness between M_5 mutants and the original cultivar, BRS Pampeira, as well as other cultivars used as an outgroup. IDB (identity-by-descent) coefficient range 0-1, where values close to 1 indicate greater relatedness and values close to 0 indicate less relatedness.

	BRS Pampeira	M519	M636	M498	M425	M267	BRS Pampa	BRS Bojuru	BRS Chui	BRS Taim	BRS Querência
BRS Pampeira	1.00	0.63	0.63	0.65	0.66	0.93	0.64	0.00	0.46	0.40	0.08
M519	0.63	1.00	1.00	0.51	0.49	0.71	0.52	0.00	0.00	0.02	0.00
M636	0.63	1.00	1.00	0.52	0.49	0.71	0.52	0.00	0.00	0.02	0.00
M498	0.65	0.51	0.52	1.00	0.70	0.62	0.41	0.00	0.21	0.20	0.00
M425	0.66	0.49	0.49	0.70	1.00	0.61	0.39	0.00	0.14	0.18	0.00
M267	0.93	0.71	0.71	0.62	0.61	1.00	0.60	0.00	0.02	0.01	0.00
BRS Pampa	0.64	0.52	0.52	0.41	0.39	0.60	1.00	0.00	0.07	0.04	0.00
BRS Bojuru	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
BRS Chui	0.46	0.00	0.00	0.21	0.14	0.02	0.07	0.00	1.00	0.55	0.55
BRS Taim	0.40	0.02	0.02	0.20	0.18	0.01	0.04	0.00	0.55	1.00	0.31
BRS Querência	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55	0.31	1.00

of leaves and root density and profound rooting. Finally, tolerance maintains cell turgor through osmoregulation, produces antioxidants, and accumulates compatible solutes, allowing plants to survive and grow under drought stress (Sing et al., 2021).

Developing rice cultivars to deal with drought is the way to reduce crop loss and mutation breeding is an approach to achieving this goal. Mutation breeding allows the creation of genetic variability followed by selection for one or a few traits without modifying the rest of the plant traits. The success of mutation breeding depends on the mutagen type, its dose, management and selection in the mutant population (Sao et al., 2022). Mutations can be induced by T-DNA or transposons and by chemical or physical mutagens (Morita et al., 2009).

According to FAO/IAEA Mutant Variety Database, 3402 mutant varieties have been developed worldwide through induced mutagenesis. For rice, 873 mutant varieties are registered, most developed using the physical mutagen gamma radiation. In Brazil, only four mutant rice varieties are registered, three developed by gamma radiation (FAO, 2023).

The gamma ray is an ionizing radiation extremely penetrating. Artificial gamma rays can be generated using ^{60}Co . Ionizing radiation can cause direct or indirect damage to DNA. Direct damage is associated radiation energy's effect on cells or DNA. On the other hand, indirect damage to DNA results from oxidative stress caused by reactive oxygen species generated by water radiolysis (Choi et al., 2021). Ionizing radiation cause base modifications in DNA, leading to base substitutions. In addition, this mutagen can produce DNA single or double strand breaks, which often result in mutations as deletions and insertions when repaired (Morita et al., 2009). Gamma rays are routinely used in plant breeding to improve different traits.

In this study, the assessment of M_4 and M_5 mutant rice lines obtained by gamma radiation was carried out in terms of growth cycle length, other agronomic traits, and drought response in the reproductive stage.

There was a shortening of days to flowering in all mutant lines in M_4 generation. The residual heterozygosity in the M_4 generation can explain the variation among mutant lines. In M_5 generation, a shortening of the vegetative growth phase of mutants was kept, evidencing its potential as new cultivars. A previous study also observed the gamma radiation effect on reducing days to flowering in rice (MT et al., 2021). Creating genotypes with a shorter growth cycle is advantageous for rice crops, as it results in less exposure to abiotic stresses, such as drought, as an escape strategy that can be used to deal with this adverse condition. In addition, early genotypes had less contact with biotic stresses, and the site is available to be used with other crops for a longer period. Besides, shorter cycle genotypes require less water per harvest season (Singh et al., 2021; Vergara et al., 1966) since the plants spend fewer days in the field, thus reducing the irrigation period. For example, BRS A705 cultivar developed by Embrapa showed less irrigation water use during harvest season and had a yield similar to late cultivars (Magalhães Júnior et al., 2021).

Plant height in mutant lines was equal to or less than that of the original cultivar in both generations. This behavior is desirable because reduced height is required to minimize the risks of lodging in rice, as yield and grain quality are limited by stem breakage or bending. Thus, there has been a search for low-sized (semi-dwarf) rice cultivars since the Green Revolution (Zhang et al., 2016). However, water deficit resulted in a more significant reduction of plant height in most mutant lines, which can affect crop yield. This performance indicates that these lines are not tolerant to water deficit. In rice, plant height is a major trait directly affecting yield potential. Short plants lead to insufficient growth and affect rice yield (Zhang et al., 2017). Drought stress reduces plant growth and crop yield in rice and its impacts vary according to the duration and stage at which water deficit occurs (Ahmadikhah and Marufinia, 2016; Panda et al., 2021). Water restriction affects biomass, plant height, leaf area, stomatal conductance, photosynthesis, starch metabolism, and the plant-water ratio (Ahmadikhah and Marufinia, 2016; Panda et al., 2021).

Mutant lines showed high TGW in M_4 and M_5 generations. Maintaining or increasing TGW is a requirement for new cultivars, as this trait directly influences plant yield (Li et al., 2019). The TGW is influenced by grain length, width, and thickness, controlled by many minor/major genes quantitatively inherited (Anilkumar et al., 2022), and can be influenced by environmental factors. Under water deficit stress, TGW was reduced in all lines, which was not expected since stress was kept until anthesis and irrigation was reestablished during grain filling. A previous study found that TGW was not affected by water deficit during the reproductive stage (Yang et al., 2019). However, not all plant panicles show the same developmental stage throughout the growth cycle and consequently during

the stress period, which could explain the negative effect of water deficit on TGW in this study.

In rice, yield is the most essential and complex trait and it is controlled by quantitative trait loci and strongly influenced by the environment. Yield is indirectly determined by plant height, growth cycle, tillering capacity, panicle length and seed length. Likewise, yield is directly affected by the number of panicles per plant, filled grains number per panicle, thousand grains weight, and grain-related attributes (Li et al., 2019). Mutants showed yield traits (number of panicles per plant, number of tillers per plant, and larger and thicker grains) and plant yield equal to or higher than the original cultivar. In contrast, the sterile spikelets number was equal or lower. The equal or higher yield and yield traits of mutants in relation to the original cultivar confirm the potential of these early genotypes as new rice cultivars to use in the drought escape strategy and reduce water requirements during the harvest season. Under water deficit, there was a reduction in plant yield and yield traits (number of panicles per plant, number of tillers per plant, and grain length and thickness) and an increase in the number of sterile spikelets. These results also show that mutant lines are sensitive to water deficit. Previous studies have shown that water deficit during the reproductive stage decreases crop yield (Yang et al., 2019). A decrease in the number of full grains and an increase in the number of sterile spikelets may be related to pollen fertility, which is drastically affected by water deficit due to aberrant anther development (Jin et al., 2013). The lower weight of full grains may be due to reduced starch accumulation in the grain caused by previous stress. Drought stress in the anthesis stage can interrupt flowering, floret initiation, pollen fertility, and grain filling. In this stage, drought stress restricts the availability of photosynthates and reduces grain yield (Ahmad et al., 2022). However, the reduction in the number of tillers was not expected since the end of tillering occurs in V_6 , before the reproductive stage. The hypothesis of non-synchronism in plant tillers may cause this behavior.

The main panicle length did not differ from that of BRS Pampeira. Apparently, mutations did not interfere with this yield trait. Interestingly, drought did not affect this trait, despite a reduction in the number of grains. Research shows that the panicle length affects several traits, such as the number of primary branches per panicle, the number of secondary branches per panicle, and the grain density per panicle (Luong et al., 2019).

Molecular marker data can be used to estimate relatedness in individuals where pedigree records are lacking or unreliable (Wang et al., 2011) or for confirmatory studies, as applied in this study. The Queller-Goodnight estimator (Queller and Goodnight, 1989) using identity by descent (IBD) was applied here to determine the relatedness between M_5 mutant lines (M267, M425, M498, M519, and M636) and BRS Pampeira (degree of relatedness for pairs of individuals).

As reviewed by Wang (2017), related individuals have more similar genotypes at each locus because their alleles have a higher probability of IBD than unrelated individuals. Identity by descent is the shared inheritance of an identical portion of the genome between two individuals (Sticca et al., 2021); in other words, IBD probability means that a pair of individuals inherited two (or more) copies of the same allele from a common ancestor (Conomos et al., 2016). Identity by descent is not the same as identity-by-state (IBS), in which a portion of two genomes of an individual may appear identical; however, it may not be due to recent shared co-inheritance (Sticca et al., 2021). Mutant genotypes are closely related to BRS Pampeira, confirming that the mutant genotypes originated from this cultivar, excluding the possibility of seeds contamination of other cultivars (soil seed bank) during the generation advancement. On the other hand, the relatedness between BRS Pampeira and BRS Pampa observed can be a consequence of shared genealogical history (Wang, 2017), but not the parents. However, this result may show a slight change in the next generations since the mutants still show segregation; nevertheless, it brings a preliminary view of the relatedness between M_5 mutants and BRS Pampeira.

The five mutant rice lines showed a growth cycle reduction of up to 20 days in relation to the original cultivar, without compromising plant height and grain yield. However, these mutant lines and BRS Pampeira are not tolerant to water deficit during the reproductive stage. Despite their sensitivity to drought stress, mutant genotypes are potential candidates for release as new cultivars, as the shortening of the growth cycle allows the use of the escape strategy to deal with drought and represent lower water demand during the harvest season.

Acknowledgments

To Centro de Energia Nuclear na Agricultura da Universidade de São Paulo for their help in mutation induction. This research and fellowships were supported by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), Consejo Nacional de Ciencia y Tecnología Mexico (CONACYT), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) grant n° 001 and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

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