Screening of eight mutants of Sinjai lokal red rice (*Oryza sativa*) to salinity stress

N Kasim, Y Musa, K Mustari, S A Syaiful, M Riadi, R Sjahri and N Ahyani

Departement of Agronomy, Faculty of Agriculture, Universitas Hasanuddin, Makassar, 90245, Indonesia.

E-mail: nina_nurlina@yahoo.com

**Abstract.** The research aimed to determine the growth and tolerance of M7 brown rice mutant lines against stress by application of several doses of NaCl. The research was conducted at the Bio-Science and Biotechnology Laboratory for Plant Production, Agricultural Cultivation, Faculty of Agriculture of Universitas Hasanuddin. It was conducted from October 2018 to February 2019, which was compiled based on 2 factorial design patterns and a randomized block design as its environmental design. The first factor consisted of 9 genotypes, i.e. 8 red rice mutant lines namely g1, g2, g3, g4, g5, g6, g7, g8 and one comparison line g9 as red rice parent which was not mutated. The second factor consisted of 4 NaCl dosage levels namely 0 dS/m, 4 dS/m, 8 dS/m, 12 dS/m. The results of the NaCl treatment on several Sinjai local red rice mutant lines show that the g2 genotype had the highest average value on the parameters of the weight of the plants on a fresh and dry condition. It indicated the g2 genotype as a mutant genotype that has tolerance to salinity stress at the vegetative growth phase.

1. **Introduction**

Red rice is a local rice variety that has a much higher nutritional content compared to white rice varieties. The rice type has a high fiber content so it can nourish the body and digestive system, as well as a source of protein and minerals such as selenium which can increase endurance and a source of vitamin B. However, like local rice in general, red rice has several disadvantages, namely long harvest age and low production [1]. To overcome this problem, one of the modern methods is mutation of plants. Gamma rays have often been used to induce plants to mutate because they have the ability to penetrate deep into the plant tissue, hence it can enable the occurrence of mutation induction that is directed to change one or several important characters that benefit the plant while maintaining most of the original characters [2].

Several studies have been carried out with the aim of obtaining genetic diversity as a source of availability of red rice lines resulting from mutations from broodstock with early maturing plants. However, research has not been carried out related to the response of the red rice mutant strain to be a salinity tolerant variety. The process of developing rice production in marginal land, such as saline land, crops, especially rice will experience abiotic stress that greatly affects the productivity and quality of plants such as root growth, stem and leaf area. This is due to metabolic imbalance due to ion poisoning, osmotic stress and nutrient deficiency. Rice plants are very sensitive to salinity stress, especially in the germination phase. Salinity stress in rice can result in a decrease in yield [3].
The selection method for selecting salinity tolerant varieties can be conducted in the field or in the laboratory. In vitro techniques are an effective and efficient method for propagation of plants in aseptic and controlled environmental conditions. By in-vitro method, it is expected to provide a variety of solutions that are resistant or sensitive to salinity. Research conducted by Zannaty [3] showed a validation step to determine the consistency of tolerance and sensitivity and identify responses to salinity stress in brown rice mutant strains. Mass screening consistently has tolerant properties when validated in the germination phase until the germination is carried out by germinating rice seeds in culture bottles in Yoshida Solution with a selection factor using NaCl solution. NaCl is used as a selection factor, because NaCl is a type of salt that greatly affects the salinity of seawater.

2. Materials and methods
The materials used in this research were local red rice mutant seeds from Sinjai 7th generation (M7), NaCl as a selection agent, Citrid acid (monohydrate), NH₄NO₃, K₂SO₄, NaH₂PO₄, H₂O, H₃BO₃, H₂SO₄, CaCl₂, FeCl₃, 6H₂O, MgSO₄, 7H₂O, (NH₄) 6.MO₇O₂₄, 4H₂O, MnCl₂.4H₂O, ZnSO₄.7H₂O, CuSO₄.5H₂O, aquades, filter paper, Dithane M45, rockwool, styrofoam. Tools which were utilized in this study, namely: magnetic stirrer, hotplate, pH meter, plastic tray, oven, dropper, tweezers, camera, analytical balance, hand sprayer, 1 L measuring cup.

The study was arranged based on a two-factor factorial design with a randomized block design as an environmental design with 3 replications. The first factor was 8 rice mutant lines and 1 local Sinjai red rice parent plant. The second factor was NaCl concentration consisting of 4 levels namely 0 dS/m (n0), 4 dS/m² (n1), 8 dS/m² (n2), 12 dS/m² (n3) where (1 dS/m² = 640 ppm = 640 mg/kg = 0.64 g NaCl/L).

This research activity consisted of planting media preparation namely Yoshida Solution stock, seed preparation and screening process research. The implementation of this research began by germinating rice seeds for seven days on filter paper in two layers in a germination container, each day the seeds were sprayed with distilled water to keep the germination media in a moist condition. After seven days the germinated rice seedlings were transferred into Yoshida Solution for seven days to stabilize the seeds before treatment. When the seeds were 2 weeks after planting, the seeds were transferred into the Yoshida Solution that had been added with NaCl.

Each concentration was formulated by weighing the NaCl according to the concentration used and then dissolved into 4 liters of water for 14 days with the media solution changed every 4 days. Evaluation of visual symptoms of salt poisoning was performed to determine sensitive and tolerant mutants. Observation and evaluation of salinity tolerance was carried out on the 14th day after salinization.

3. Results and discussion
Environmental stress is an environmental condition that puts pressure on plants and results in plant responses to certain environmental factors being lower than their optimum response under normal conditions [4]. Plant height parameters in table 1 shows that g2 mutant lines at 4 dS/m and 8 dS/m NaCl concentrations had the highest increase in plant height and g4 was highest at the concentration of 12 dS/m NaCl. Mutant lines g2 and g4 have decreasing plant height accumulation for each concentration of NaCl, yet it was not too significant or quite constant when compared to other mutant lines. Decrease in plant height accumulation experienced by mutant g2 and g4 in each NaCl concentration was a result of the concentration of NaCl or the salinity stress caused. The higher the concentration, the more it will inhibit the growth of a plant. Salinity stress in crops can cause disruption in growth and production. Rice is vulnerable to salinity which could result in the plants are unable to grow.

Table 1 shows that the mutant lines are able to maintain plant height under conditions of salinity stress by implementing certain mechanisms. Growth retardation in treatments given high concentrations of NaCl is due to high levels of salt ions such as Na⁺, Cl⁻, Mg²⁺. This situation results in inhibition of the process of cell division, cell elongation, or both due to stress which is the
accumulation of salinity in plants [5]. This results in a bottleneck in the absorption of water by the roots and the effect is seen in the impeded development of plant height, number of leaves, and number of tillers, as shown in tables 2 and 3.

Table 1. Average increase in plant height (cm) of various local brown rice mutant lines with various NaCl concentrations from a week after treatment (21 DAP) to 2 weeks after treatment (DAP)

| Lines | NaCl | Average | CV  |
|-------|------|---------|-----|
|       | n0   | n1      | n2  | n3  |       |
| g1    | 2.72$_{de}$ | 2.71$_{abc}$ | 2.07$_{ab}$ | 1.29$_b$ | 2.20  | 0.506 |
| g2    | 2.77$_{edef}$ | 2.31$_{xy}$ | 2.15$_y$ | 1.40$_q$ | 2.16  | 0.533 |
| g3    | 2.62$_e$ | 2.80$_{ab}$ | 2.06$_{ab}$ | 1.43$_b$ | 2.23  | 0.551 |
| g4    | 2.47$_{xy}$ | 2.53$_{abe}$ | 2.17$_y$ | 1.43$_q$ | 2.15  | 0.562 |
| g5    | 2.11$_{y}$ | 2.95$_a$ | 2.19$_y$ | 1.28$_b$ | 2.13  | 0.573 |
| g6    | 2.67$_e$ | 2.65$_{ab}$ | 2.11$_y$ | 1.35$_b$ | 2.19  | 0.580 |
| g7    | 2.75$_{xy}$ | 2.77$_{ab}$ | 2.27$_y$ | 1.42$_b$ | 2.30  | 0.587 |
| g8    | 3.34$_a$ | 2.78$_y$ | 1.88$_y$ | 1.47$_b$ | 2.37  | 0.592 |
| g9    | 3.27$_{xy}$ | 2.13$_a$ | 2.49$_y$ | 2.15$_a$ | 2.51  | 0.511 |

CV Duncan 0.506 0.533 0.551

Notes: The average value followed by unequal letters in the columns (a, b, c, d, e, f) and row (q, r, x, y) means significantly different in the Duncan Multiple Range Test (DMRT) at the 0.05 level. DAP = Days after planting.

Table 2. Average number of leaves (strands) of various red rice mutant lines with various concentrations of NaCl on the 14th day after treatment (28 DAP)

| Lines | NaCl | Average | CV  |
|-------|------|---------|-----|
|       | n0   | n1      | n2  | n3  |       |
| g1    | 8.20 | 6.27    | 5.73 | 4.27 | 6.12$_b$ | 0.653 |
| g2    | 8.20 | 6.00    | 5.67 | 4.47 | 6.08$_b$ | 0.688 |
| g3    | 8.67 | 5.47    | 5.20 | 4.53 | 5.97$_b$ | 0.711 |
| g4    | 9.23 | 6.87    | 6.67 | 4.40 | 6.79$_a$ | 0.725 |
| g5    | 8.73 | 5.60    | 5.40 | 4.65 | 6.10$_b$ | 0.739 |
| g6    | 8.63 | 5.35    | 5.13 | 4.27 | 5.85$_b$ | 0.749 |
| g7    | 8.47 | 5.13    | 4.87 | 4.07 | 5.63$_{bc}$ | 0.758 |
| g8    | 8.87 | 5.60    | 5.20 | 3.80 | 5.87$_b$ | 0.765 |
| g9    | 7.40 | 4.67    | 4.47 | 3.93 | 5.12$_c$ | 0.749 |

Average 8.49$_a$ 5.66$_q$ 5.37$_q$ 4.26$_r$

CV Duncan 0.435 0.459 0.474

Note: Average values followed by unequal letters in columns (a, b, c) and row (p, q, r) mean significantly different in the Duncan Multiple Range Test (DMRT) at the 0.05 level. DAP = days after planting.

Parameters of number of leaves and number of tillers showed that the g4 mutant strain had the highest number of plant leaves and number of tillers at each NaCl interval compared to other mutant lines. This shows that at these conditions the strain enabled the efficient use of water in rice cultivation and also shows that rice is a plant that is tolerant to low groundwater content. Salinity stress causes nutrient absorption and retrieval of water is blocked, if this event occurs the plant will experience osmotic stress, low photosynthesis rate due to H$_2$O deficiency, unbalanced transpiration and water absorption. Consequently, it becomes barrier as it can inhibit photosynthesis and photosynthesize translocation. According to Maulana [6] the number of tillers and number of leaves is influenced by
The higher the concentration of NaCl, the lower the decrease in dry weight of plants. The duration of NaCl availability affects the wet weight and dry weight in concentration compared to other mutant lines. Therefore, the mutant lines have a greater number of tillers than the number of tillers in the control.

The mutant g2 strain had the highest average in the plant dry weight parameters at each given NaCl concentration of NaCl compared to other mutant lines. The dry weight of some tested lines showed decreased in dry weight.

**Table 3.** Average number of tillers (stems) of various red rice mutant lines with various concentrations of NaCl on the 14th day after treatment (28 DAP)

| Lines | NaCl | Average | CV |
|-------|------|---------|----|
|       | n0   | n1      | n2 | n3 |      |    |
| g1    | 1.00 | 0.73    | 0.47 | 0.07 | 0.57 | 0.123 |
| g2    | 1.22 | 0.73    | 0.60  | 0.53 | 0.80 | 0.130 |
| g3    | 1.27 | 0.27    | 0.33 | 0.20 | 0.52 | 0.134 |
| g4    | 1.65 | 0.93    | 0.67 | 0.47 | 0.93 | 0.137 |
| g5    | 1.13 | 0.53    | 0.60 | 0.33 | 0.62 | 0.140 |
| g6    | 1.00 | 0.42    | 0.33 | 0.33 | 0.52 | 0.141 |
| g7    | 1.73 | 0.40    | 0.40 | 0.40 | 0.72 | 0.143 |
| g8    | 1.47 | 0.40    | 0.67 | 0.67 | 0.67 | 0.144 |
| g9    | 0.80 | 0.27    | 0.40 | 0.40 | 0.45 | 0.45  |
| Average | 1.26 | 0.52 | 0.50 | 0.30 |       |
| CV Duncan | 0.082 | 0.087 | 0.090 |       |

Note: Average values followed by unequal letters in columns (a, b, c, d, e) and rows (p, q, r, s) mean significantly different in the Duncan Multiple Range Test (DMRT) at the 0.05 level. DAP=days after planting.

**Table 4.** Average plant dry weight (g) of various red rice mutant lines with various concentrations of NaCl on the 14th day after treatment (28 DAP)

| Lines | NaCl | Average | CV |
|-------|------|---------|----|
|       | N0   | N1      | N2 | N3 |      |    |
| G1    | 0.70 | 0.55    | 0.46 | 0.35 | 0.52 | 0.746 |
| G2    | 0.85 | 0.72    | 0.54 | 0.40 | 0.63 | 0.786 |
| G3    | 0.78 | 0.60    | 0.49 | 0.39 | 0.57 | 0.812 |
| G4    | 0.78 | 0.74    | 0.48 | 0.39 | 0.60 | 0.829 |
| G5    | 0.75 | 0.60    | 0.50 | 0.37 | 0.56 | 0.845 |
| G6    | 0.69 | 0.62    | 0.49 | 0.35 | 0.54 | 0.855 |
| G7    | 0.77 | 0.64    | 0.46 | 0.38 | 0.56 | 0.866 |
| G8    | 0.75 | 0.58    | 0.49 | 0.35 | 0.54 | 0.874 |
| G9    | 0.66 | 0.62    | 0.53 | 0.41 | 0.55 | 0.875 |
| Average | 0.74 | 0.62 | 0.49 | 0.38 | 0.56 |
| CV Duncan | 0.079 | 0.085 | 0.088 |       |

Note: Average values followed by unequal letters in columns (a, b, c, d, e) and rows (q, r, x, y) mean significantly different in the Duncan Multiple Range Test (DMRT) at the 0.05 level. DAP=days after planting.

This result indicates the occurrence of salinity stress that causes abnormal growth and directly affects the wet weight and dry weight [7]. According to Sobrizal [2] under salt stress conditions, water availability is also reduced but the rate of plant respiration tends to increase. This is what causes the decrease in dry weight of plants. The duration of NaCl administration also affects the rate of rice growth. The higher the concentration of NaCl, the lower the wet weight and dry weight of the plant.
The increasing concentration of NaCl causes an increase in Na$^+$ and Cl$^-$ absorbed into the tissue which will then inhibit metabolism in plants.

Root growth, namely root length, root volume, and high root dry weight are indications that plants avoid salinity stress and drought. The g2 mutant strain has better root growth in each given NaCl concentration (4 dS/m, 8dS/m, and 12 dS/m) than other mutant lines.

Table 5. Average root length increments (cm) of various red rice mutant lines with various concentrations of NaCl on the 14th day after treatment (28 DAP).

| Lines | NaCl | Average |
|-------|------|---------|
|       | n0   | n1     | n2     | n3     |         |
| g1    | 10.47| 7.24   | 6.17   | 4.28   | 7.04    |
| g2    | 9.88 | 6.35   | 5.69   | 4.99   | 6.73    |
| g3    | 9.26 | 6.60   | 6.13   | 4.11   | 6.53    |
| g4    | 10.47| 6.64   | 5.77   | 4.43   | 6.83    |
| g5    | 10.51| 6.13   | 5.92   | 3.75   | 6.58    |
| g6    | 8.30 | 7.76   | 6.50   | 4.30   | 6.71    |
| g7    | 10.72| 6.62   | 5.71   | 4.76   | 1.27    |
| g8    | 11.06| 7.88   | 6.03   | 4.77   | 7.44    |
| g9    | 12.25| 5.57   | 4.05   | 1.93   | 5.95    |
| Average| 10.33| 6.76   | 5.77   | 4.15   |

Note: The average value followed by unequal letters (p, q, r, s) means significantly different in the Duncan Multiple Range Test (DMRT) at the 0.05 level. DAP=days after planting.

Table 6. Average root volume of plants (ml) of various red rice mutant lines with various concentrations of NaCl on the 14th day after treatment (28 DAP).

| Lines | NaCl | Average | CV |
|-------|------|---------|----|
|       | n0   | n1     | n2  | n3  |         |    |
| g1    | 2.93 | 2.50   | 1.53| 1.40| 2.09d   | 0.332|
| g2    | 3.80 | 3.23   | 2.53| 1.90| 2.87a   | 0.349|
| g3    | 3.47 | 2.73   | 2.17| 1.87| 2.56ab  | 0.361|
| g4    | 4.01 | 3.17   | 2.47| 1.80| 2.86ab  | 0.368|
| g5    | 3.40 | 2.70   | 2.50| 1.63| 2.56ab  | 0.375|
| g6    | 3.67 | 2.80   | 2.43| 1.70| 2.65ab  | 0.380|
| g7    | 3.37 | 2.33   | 1.73| 1.20| 2.16a   | 0.385|
| g8    | 3.13 | 2.83   | 2.33| 1.97| 2.56ab  | 0.388|
| g9    | 3.07 | 2.60   | 2.10| 1.50| 2.32abcd | 0.241|
| Average| 3.43| 2.77   | 2.20| 1.66|         |
| CV    | 0.221| 0.233  | 0.241|

Note: Average values followed by unequal letters in columns (a, b, c, d) and row (p, q, r, s) mean significantly different in the Duncan Multiple Range Test (DMRT) at the level 0.05. DAP=days after planting.

The growth of g2 root has decreased with increasing concentration of NaCl. Plant seeds will use a tolerant strategy in dealing with stress conditions through a tolerance mechanism with low tissue water potential, so that the osmotic adjustment will produce and accumulate certain compounds in the form
of free amino acids in plant tissues to maintain their turgor by adjusting the osmotic potential or by tissue elasticity during conditions of stress [8]. Stress reactions to drought and salinity stress differ depending on the intensity and duration of the stresses themselves and the plant species, the stage of growth and the nature of plant tolerance.

| Lines | NaCl | Average | CV  |
|-------|------|---------|-----|
|       | n0   | n1     | n2  | n3  |       |       |
| g1    | 0.22 | 0.20   | 0.15| 0.13| 0.17c | 0.023 |
| g2    | 0.38 | 0.26   | 0.20| 0.17| 0.25a | 0.025 |
| g3    | 0.33 | 0.23   | 0.16| 0.14| 0.22b | 0.025 |
| g4    | 0.36 | 0.27   | 0.18| 0.14| 0.24ab| 0.026 |
| g5    | 0.33 | 0.23   | 0.18| 0.13| 0.22b | 0.026 |
| g6    | 0.33 | 0.23   | 0.17| 0.14| 0.22b | 0.027 |
| g7    | 0.34 | 0.23   | 0.15| 0.12| 0.21b | 0.027 |
| g8    | 0.31 | 0.24   | 0.16| 0.14| 0.21b | 0.027 |
| g9    | 0.27 | 0.18   | 0.17| 0.12| 0.19c | 0.027 |

Average dry weight of plant roots (g) of various red rice mutant lines with various concentrations of NaCl on the 14th day after treatment (28 DAP).

| Lines | NaCl | Average | CV  |
|-------|------|---------|-----|
|       | n0   | n1     | n2  | n3  |       |       |
| g1    | 0.22 | 0.20   | 0.15| 0.13| 0.17c | 0.023 |
| g2    | 0.38 | 0.26   | 0.20| 0.17| 0.25a | 0.025 |
| g3    | 0.33 | 0.23   | 0.16| 0.14| 0.22b | 0.025 |
| g4    | 0.36 | 0.27   | 0.18| 0.14| 0.24ab| 0.026 |
| g5    | 0.33 | 0.23   | 0.18| 0.13| 0.22b | 0.026 |
| g6    | 0.33 | 0.23   | 0.17| 0.14| 0.22b | 0.027 |
| g7    | 0.34 | 0.23   | 0.15| 0.12| 0.21b | 0.027 |
| g8    | 0.31 | 0.24   | 0.16| 0.14| 0.21b | 0.027 |
| g9    | 0.27 | 0.18   | 0.17| 0.12| 0.19c | 0.027 |

Average 0.32p, 0.23q, 0.17r, 0.14s
CV Duncan 0.016, 0.016, 0.017

Note: Average values followed by unequal letters in columns (a, b, c, d) and row (p, q, r, s) mean significantly different in the Duncan Multiple Range Test (DMRT) at the level 0.05. DAP=days after planting.

4. Conclusions
Mutant strain of g2 has the highest average value of all observable parameter and shows the results classified as mutant lines that are tolerant of salinity stress in the vegetative growth phase. There is an interaction between mutant lines and NaCl concentrations, namely the increase in plant height at 21 days after planting and 28 days after planting.

References
[1] Makarim A K and Ikhwani 2013 System of Rice Intensification (SRI) dan Peluang Peningkatan Produktivitas Padi Nasional (Bogor)
[2] Sobrizal 2007 Potensi Pemuliaan Mutasi untuk Perbaikan Padi Lokal Indonesia J. Ilm. Apl. Isot. dan Radiasio 12 1-14.
[3] Zannaty A, Widyastuti U and Nugroho S 2015 Skrining Salinitas Padi Mutan Insersi Pembawa Activation - Tagging pada Fase Perkecambahan (Germination Phase Screening of Insert Mutant Rice Carrying Activation-Tagging).
[4] Rafiuddin 2014 Seleksi Genotipe Jagung Hasil Irradiasi Sinar Gamma Terhadap Toleransi Kekeringan dan Salinitas (Makassar)
[5] Situmorang A, Zannati A, Widyajayatie D and Nugroho S 2010 Seleksi Padi Mutan Insersi Toleran Cekaman Salinitas Berdasarkan Karakter Pertumbuhan dan Biokimia (Bogor)
[6] Maulana 2011 Karakter Vegetatif dan reproduktif Tanaman Mutan Pad Sensitif Alumunium (IPB, Bogor)
[7] Adriani W A 2012 Pertumbuhan Beberapa Varietas Jagung Hasil Irradiasi Pada Berbagai Konsentrasi PEG dan NaCl (Universitas Hasanuddin, Makassar)
[8] Taiz L, Zeiger E, Møller I M and Murphy A 2015 Plant physiology and development.