Seed priming effect on Banyuasin and Ir 64 rice varieties growth at the seedling stage under salinity stress

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Abstract. Rice classified as susceptible to salinity, especially at the seedling stage. To improve the salinity tolerance, one of the methods used is through seed priming. This study aims to figure out the effect of seed priming with NaCl on the seedling of Banyuasin and IR 64 varieties growth under salinity stress (1.2 m/s). The research was conducted in the Laboratory of Plant Science, Faculty of Agriculture, Ehime University, Japan using an experiment with clustered randomized design pattern. There were eight package of treatment, including V1H0M0, V1H0M1, V1H1M0, V1H1M1, V2H0M0, V2H0M1, V2H1M0, and V2H1M1 (V1: IR 64 variety, V2: Banyuasin variety, H0: Seeds without priming, H1: Seeds with priming, M0: non-saline media. M1: saline media of 1.2 m/s). Each treatment was replicated three times resulting in 24 units of the experiment. The results showed that salinity suppressed seedling growth. Even though primed Banyuasin seeds showed the best results for the variables mean of plant height, root length, shoot biomass, root biomass, and plant biomass, (34.27 cm, 19.03 cm, 0.81 g, 0.18g, and 0.99 g) under salinity stress, these results were not statistically different from any other treatment under the same growth condition. We suspected that the salinity level of the media was too high so that even primed seeds are unable to overcome the stress. This study also showed that primed Banyuasin seeds growth under salinity stress can maintain the lowest Na⁺ ions accumulation in the shoot (2.33 mg g⁻¹), and these results were significantly different with any other treatment under the same condition. This data indicated that seed priming enhances plant ability to maintain lower Na⁺ accumulation in a shoot, that is crucial to overcome salinity and to improve tolerance to salinity stress.

1. Introduction
The conversion of productive land for non-agricultural purposes has led to agricultural cultivation, especially food, now shifting to marginal lands with various abiotic stresses, one of which is salinity stress. Salinity is the presence of mineral salts in excessive concentration so that it suppresses plant growth, by damaging the growing cells so that cell growth does not take place and limits the supply of essential metabolic products to cells [1].

Rice is classified as a plant that is sensitive to salinity [2]. This sensitivity differs according to the phase of growth. Research [3] found that salinity effect all the vegetative and generative growth parameters on rice plants. The effect was more severe with the increase of salinity.
One of the varieties developed to adapt to saline fields is the Banyuasin variety. This variety is recommended for tidal land with average productivity of 5 tons ha$^{-1}$. Meanwhile, other rice varieties that are quite popular in the community are IR 64 varieties. Increased tolerance of the two varieties will support efforts to increase rice productivity in saline fields.

To increase rice seed tolerance to salinity, one method that can be used is "seed priming". Priming in plants is described as one of the activations of various defense responses that are faster and stronger against repetitive abiotic stresses [4]. Research conducted [5], showed that priming effects were determined by media priming, priming substance concentration, and priming duration [6,7] reported that the response of different varieties to different priming concentrations was influenced by the genetic background of each variety. This study aims to study the seed priming effect on Banyuasin and IR 64 varieties at the seedling stage, under salinity stress.

2. Methods

2.1. Experimental Design
The research method was carried out in Randomized Block Design patterns, with 8 treatment packages, namely: V1H0M0 (IR 64 varieties, without priming, on non-saline media); V1H0M1 (IR 64 varieties, without priming, on saline media); V1H1M0 (IR 64 varieties, with priming, on non-saline media); V1H1M1 (IR 64 varieties, with priming, on saline media); V2H0M0 (Banyuasin variety, without priming, on non-saline media); V2H0M1 (Banyuasin variety, without priming, on saline media); V2H1M0 (Banyuasin variety, by priming, on non-saline media); V2H1M1 (Banyuasin variety, with priming, on saline media). Each treatment was repeated three times, so there were 24 experimental units. Data was analyzed by Analysis of Variance (ANOVA). Further tests are carried out if the F test shows significant or very significant.

2.2. Seeds Source
The material used is rice varieties IR 64 (susceptible to salinity stress) and Banyuasin (salinity tolerant). The seeds obtained from the Indonesian Center for Rice Research (ICCR) in Sukamandi, West Java.

2.3. Seeds Priming and Germination
Seeds of the two varieties soaked in a glass containing 1 liter of 150 mM NaCl solution. The IR 64 variety seeds were soaked for 36 hours, while Banyuasin was for 39 hours. The length of immersion time was determined from preliminary experiments conducted to determine how long it took the seeds of each variety to begin to germinate when immersed in priming solution. During the priming, the glass container is placed on top of the shaker so that the aeration runs well. After the priming time is complete, the seeds are rinsed under running tap water for about two minutes and dried in the room temperature.

The seeds are germinated on moist paper until the radicle emerges from the seed. After that, the seeds are sown on the planting media in the form of sand and maintained for 14 days. Then the seed is transferred to a hydroponic medium containing 50 l Yoshida's solution. The composition of the solution is shown in table 1.

2.4. Salinity Treatment
The seeds are grown hydroponically based on the IAEA protocol "Salt tolerance screening in rice using hydroponics" (International Atomic Energy Association) [8]. Every two days, the volume of the solution will be increased until it reaches the initial volume, to avoid pH change. The measurement and adjustment of pH to ideal condition (pH 5) is carried out every day by adding 1N NaOH or 1N HCl. This pH adjustment also serves to give aeration to hydroponic solutions.

The salinity treatment was applied after 1 week of a seedling growing in Yoshida's solution. Salinization of the nutrient solution was carried out in large volumes by adding dried NaCl to the tank containing Yoshida's solution. NaCl is added up until the electronic conductivity reaches 1.2 S/m. Electronic conductivity is measured using an EC meter. In "Salt tolerance screening in rice using
hydroponics" IAEA (International Atomic Energy Association) [8] used 1.0 S/m as a standard in salinity tolerance testing, while research conducted [9] showed that some rice varieties can continue to grow at 1.5 S/m, in this study, we used 1.2 S/m as a standard, to ensure that the effect of salinity can be observed. This level of salinity is also used by other researchers [10] to filter salinity tolerant rice genotypes, Plant samples were taken from each treatment, before stress, and 16 days after NaCl added. Harvested plant roots are washed with water and dried with tissue paper before recording fresh weight, plant height and a number of tillers. three sample plants were measured per treatment. For determination of dry weight, leaves and root were dried in an oven at 70°C for four days and then weighed. Macro elements were analyzed using Atomic Absorption Photometer (Z-2000, Hitachi).

**Table 1. The Content of Yoshida’s Solution** [11]

| Number of Stock | Chemical Compound | Amount (g or ml)/5l |
|-----------------|-------------------|---------------------|
| 1               | NH₄NO₃           | 457                 |
| 2               | NaH₂PO₄.H₂O      | 201.5               |
| 3               | K₂SO₄            | 357                 |
| 4               | CaCl₂            | 443                 |
| 5               | MgSO₄ 7H₂O       | 1.620               |
| 6               | MnCl₂ 4H₂O       | 7.500               |
|                 | (NH₄)₆ Mo₄O₂₄ 4H₂O| 0.370              |
|                 | H₃BO₃           | 4.670               |
|                 | ZnSO₄ 7H₂O       | 0.175               |
|                 | CuSO₄ 5H₂O       | 0.155               |
|                 | FeCl₃ 6H₂O       | 38.500              |
|                 | C₆H₈O₇ H₂O       | 59.500              |
|                 | 1M H₂SO₄        | 250 ml              |

2.5. **Parameters**

2.5.1. *Plant height (cm)*. Plant height measured from the base of the stem to the highest leaf tip observed. Sample for observation taken before salinity stress treatment, and 16 days after seedling growth under saline condition.

2.5.2. *Root length (cm)*. The root length measured from the root base to the longest root tip. Sample for observation taken before salinity stress treatment, and 16 days after seedling growth under saline condition.

2.5.3. *Leaves biomass (g)*. The leaves of the plant sample are inserted into a paper envelope, then dried in a 70°C oven for 48 hours. After drying, sample was weighing.

2.5.4. *Root biomass (g)*. The root of the plant sample is inserted into a paper envelope, then dried in an oven at 70°C for 48 hours. After drying, sample was weighing.

2.5.5. * Macronutrient content*. Macronutrient Na, K, Mg, and Ca (mg g⁻¹) in the leaves and root were measured using Atomic Absorption Photometer (Z-2000, Hitachi).

2.5.6. *Chlorophyll*. Chlorophyll is measured using chlorophyll meter. The samples are leaves that have been fully developed, namely the third leaf blade, measurements are made on the widest part of the leaf.
3. Results and discussion

Table 2 showed that V2HIM0 (Banyuasin varieties, Primed seeds, Non-saline media) have the highest results in plant height (63.27 cm). However, this results were not statistically different from the V1H0M0, V1H1M0, and V2H1M0 (all treatment were the plant growth in non-saline media). Significant different in this parameter only occurs when it compares with the V1H0M1, V1H1M1, V2H0M1, V2H1M1, were all the plant grown in saline media. The results showed that salinity significantly suppresses plant growth. The inhibit of plant growth due to salinity stress has been reported in various studies [6], [12]. High Na concentration suppresses plant growth by damaging the osmotic and ionic balance, as well as the accumulation of Na which are toxic to plants [13].

Table 2. Mean of plant height, root length, number of tillers, leaves biomass, root biomass, the ratio of leaves and root biomass, the ratio of leaves and root length at 16 days after salinity stress treatment.

| Treatment      | Plant height (cm) | Root length (cm) | Leaves Biomass (g) | Root Biomass (g) | Plant Biomass (g) |
|----------------|-------------------|------------------|-------------------|------------------|-------------------|
| V1H0M0         | 58.07             | 16.73            | 2.74              | 1.02             | 3.76              |
| V1H0M1         | 24.80             | 14.87            | 0.33              | 0.09             | 0.42              |
| V1H1M0         | 59.60             | 18.40            | 2.92              | 1.05             | 3.96              |
| V1H1M1         | 28.50             | 14.70            | 0.48              | 0.12             | 0.60              |
| V2H0M0         | 57.27             | 18.73            | 3.88              | 1.46             | 5.34              |
| V2H0M1         | 22.43             | 14.40            | 0.43              | 0.09             | 0.53              |
| V2H1M0         | 63.27             | 18.97            | 4.63              | 1.63             | 6.26              |
| V2H1M1         | 34.27             | 19.03            | 0.81              | 0.18             | 0.99              |

The numbers on the same column followed by different letters are significantly different at Duncan test 0.01.

The V2H1M1 (Banyuasin varieties, Primed seeds, Saline media) showed the best results in root length (19.03 cm). These results were significantly different from the V2H0M1 (Banyuasin varieties, Unprimed seeds, Saline media), and not different with V2H1M0, V2H0M0 (the treatment were the plant growth in non-saline media). The results indicate that in Banyuasin varieties, the primed seeds grew a longer root in saline condition. In IR64 varieties, this research showed that salinity did not significantly reduce the root length. This results contrary to many other reports. Vibhuti et al (2015) reported that the higher salinity degree, the more root growth suppressed [14]. Significant decrease of root growth in rice under salinity also reported [15]. However, the lowest root growth decrease of IR 64 varieties under salinity also reported [16]. The inconsistency of root length under salinity condition indicates that this is not a reliable parameter for salinity tolerance screening in rice.

Table 2 showed that the best results on leaves biomass (4.63 g) were found in V2HIM0 (Banyuasin varieties, Primed seeds, Non-saline media), and this result was significantly different compared to any other treatment. The V2H1M0 treatment also shows the best results for root biomass (1.63 g), and plant biomass (6.26 g). This result showed the positive effects of seed priming on plant early growth. Seed priming leads to uniform germination, more vigorously seedling, and establish better plant growth as a result of pre germination metabolism activation during the priming duration. Seed priming has shown benefits in germination and early growth of several species [17], this technique can also reduce germination time so that it can be shorter and increase uniform growth and crop yield [12,18].

Even though not statistically significant, in this study, primed seeds also showed a better vegetative growth compare to unprimed seeds under saline condition. These results indicate the beneficial effect of seed priming with NaCl. Better vegetative growth of plants on seeds that are primed with NaCl in saline conditions is also reported [6] and Farahbakhsh and Saiid [19] in maize, priming increases free radical deterrence enzymes such as peroxidase, catalase, and superoxide dismutase, to improve plant survival and strength under salinity stress. Seed priming allows membrane and protein hydration, and initiation of various metabolic systems in the face of stress [19,20].
Table 3 showed that the accumulation of Na⁺ ions in plant leaves are significantly different between plant that growth in saline and non saline media. The lowest Na⁺ accumulation in leaves showed in V1H0M0 (0.07) and V1H1M1 (0.07), while the highest accumulation showed in V1H0M1 (4.96). It was clear that the plant growth in saline media accumulate higher Na⁺ ions.

**Table 3.** Mean content of Sodium (Na), Potassium (K), Ratio of Na: K, Magnesium (Mg), Calcium (Ca) and Chlorophyll in the leaves at 16 days after salinity stress treatment.

| Treatment   | Na⁺ (mg g⁻¹) | K⁺ (mg g⁻¹) | Ratio Na⁺:K⁺ | Chlorophyll |
|-------------|--------------|-------------|--------------|-------------|
| V1H0M0      | 0.07⁻       | 4.42⁻       | 0.02⁻        | 39.03⁻      |
| V1H0M1      | 4.96⁺       | 2.31⁻       | 2.17⁻        | 35.97⁻      |
| V1H1M0      | 0.07⁻       | 4.60⁺       | 0.02⁻        | 41.07⁺      |
| V1H1M1      | 4.51⁺       | 2.34⁻       | 2.55⁻        | 36.47⁻      |
| V2H0M0      | 0.10⁻       | 4.20⁻       | 0.02⁻        | 39.07⁻      |
| V2H0M1      | 4.23⁺       | 2.38⁻       | 1.77⁻        | 36.03⁻      |
| V2H1M0      | 0.10⁻       | 4.36⁻       | 0.02⁻        | 40.53⁻      |
| V2H1M1      | 2.33⁻       | 2.64⁻       | 0.88⁻        | 40.23⁻      |

The numbers on the same column followed by different letters are significantly different at Duncan test 0.01.

The data in Table 3 also showed that in saline media, the V2H1M1 accumulate the lowest Na⁺ ions in leaves (2.33), and these results are significantly different compared to other treatment in. Lower Na⁺ accumulation in this treatment suspected as the result of seed priming and correlate with better growth that indicates by better plant biomass. This correlation showed in Figure 1. By accumulating less Na⁺ ions which are toxic, plants treated with halopriming have a better osmotic regulation capacity compared to plants without priming. The ability to regulate osmotic pressure in these cells is an important mechanism in overcoming salinity stress in tolerant plants. Better growth in seeded seed is a result of better osmotic regulation capacity. The results of Omami E.N which conducted seed priming research on spinach seeds also showed that there was a decrease in Na⁺ ions accumulation and an increase in Ca²⁺, K⁺, and the ratio of Ca²⁺: Na⁺ and K⁺: Na⁺ at the spinach leaves [21,22].

![Figure 1. Correlation between plant biomass and Na⁺ ions content in plant leaves](image)

Table 4 showed that even though it accumulates the lowest Na⁺ ion in the leaves, the Banyusasin variety which is primed actually shows the highest Na⁺ ion accumulation at the root compared to other treatments on saline media. It is suspected that priming induces the ability of plants to hold more salt in the roots and not channel it to the leaves so that the damaging effects of excess salt in the leaves can be reduced. Priming also seems to affect the chlorophyll content of plants. In all varieties, the primed seed
showed higher chlorophyll content compared with unprimed seeds in the same medium. In Banyuasin varieties, primed seeds that grown on saline media show a chlorophyll content of 40.23, this content is even higher compared to plants without priming grown on non-saline media which only reaches 39.07. Whereas in IR 64 varieties, even though the saline media of plants with primed seeds have higher chlorophyll content compared to plants without priming, the value is still lower than the chlorophyll content in plants without priming on non-saline media.

Table 4. Mean content of Sodium (Na), Potassium (K), Ratio of Na:K, Magnesium (Mg), Calcium (Ca) and Chlorophyll in the root at 16 days after salinity stress treatment.

| Treatment  | Na (mg g⁻¹) | K (mg g⁻¹) | Ratio Na:K | Mg (mg g⁻¹) | Ca (mg g⁻¹) |
|------------|-------------|------------|------------|-------------|-------------|
| V1H0M0     | 0.505       | 2.352      | 0.22       | 0.254       | 0.034       |
| V1H0M1     | 1.478       | 1.253      | 1.20       | 0.148       | 0.051       |
| V1H1M0     | 0.539       | 2.188      | 0.25       | 0.248       | 0.033       |
| V1H1M1     | 1.657       | 1.503      | 1.10       | 0.161       | 0.047       |
| V2H0M0     | 0.778       | 1.776      | 0.45       | 0.233       | 0.029       |
| V2H0M1     | 1.669       | 1.450      | 1.23       | 0.140       | 0.062       |
| V2H1M0     | 0.763       | 1.924      | 0.40       | 0.228       | 0.024       |
| V2H1M1     | 0.745       | 1.163      | 0.64       | 0.146       | 0.032       |

The numbers on the same column followed by different letters are significantly different at Duncan test 0.01

This research shows that the ability to accumulate fewer Na⁺ in the leaves indicate better tolerance to salinity stress. The ability of plants to reduce the accumulation of Na⁺ in the leaves correlated with the ability of rice plants to survive in salinity stress [21]. A similar relationship was reported by Garthwaite (2009) in wheat and barley [23]. The ability to reduce Na⁺ ions in leaves is vital because Na⁺ accumulation reduces the absorption of Potassium (K⁺) needed by the plants to activate at least 60 different enzymes involved in plants growth. Although K⁺ is not the constituents of chlorophyll, the characteristic of K⁺ deficiency is chlorophyll damage [24] led to reduce of photosynthesis rate and eventually affect the plant growth. Salinity also causes syntheses of abscisic acid which causes the closure of the stoma if it is transported to guard cells, causing the stomata to close, photosynthesis decreases and growth decreases. An interesting thing to study further is whether priming affects the accumulation of certain proteins such as aquaporin or influences certain morphological changes in roots to increase plant tolerance.

4. Conclusion
Salinity stress at 1.2 S/m suppressed the seedling growth of Banyuasin and IR 64. Primed Banyuasin at seedling stage able to maintain lower Na⁺ ions accumulation in the leaves, this indicates that priming using 150 mM NaCl help plant to reduce negative effect of salinity stress.

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