Salt-induced growth promotion in rice varieties during nursery

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Abstract. Climate change will increase the occurrence of salinity in agricultural land along with the coastal areas. One of the technologies to reduce salinity is NaCl pretreatment. This study aimed to evaluate salinity treatment's effect during nurseries on the growth of lowland rice seedlings. There were three separate experiments, and all the experiments used Randomized Complete Block Design. In the first experiment, local black rice seeds (var. Jelitheng) was used. The nursery was carried out at three salinity levels, i.e. 0.2, 3 and 5 dS/m. The second experiment was conducted using salt-resistant rice seeds (var. Dendang) and salt susceptible rice seeds (var. IR 64). The salinity levels applied were non-saline (0.2 dS/m) and saline (5 dS/m). The third experiment used rice seedling var. IR 64, with the first factor being the salinity level (0.2 and 5 dS/m) and the second factor was a wet nursery and dry nursery. In general, the results from the three experiments showed that giving salinity levels of 3-5 dS/m in several rice varieties improved seedling performance. Although salinity during nursery could increase the concentration of Na+ and decrease the concentration of K+ in leaves, salinity during nursery increased the seedlings fresh weight, and dry weight increased the number of seedlings leaves and increased the concentration of leaf chlorophyll. The better seedlings growth variable in the saline nursery will help the plants cope with salinity in the later growth stage in the field.

1. Introduction
The increase in soil salt concentration can be caused by several things, such as poor soil drainage and leaching, either due to inadequate rainfall or irrigation [1]. Salinity can also result from land-use changes from natural forest vegetation to cereal cultivation or changes in cropping patterns [1, 2, 3]. In coastal areas, salinity is generally caused by seawater intrusion through land aquifers [1, 2]. Salinity suppresses plants in two ways, i.e. by inhibiting roots from absorbing water (osmotic stress) and causing toxicity in the plant cells (ionic stress) [4, 5]. Osmotic stress is generally related to initial response, while ionic stress is the final response [6]. Osmotic stress has the same impact as drought stress, which causes water absorption inhibition, root growth, cell elongation, leaf development, reduced production of new leaves, and damage to plant cells [7, 8]. Therefore, rice plants that are exposed to salinity stress will also be exposed to drought stress.

In general, rice is tolerant to salinity during the germination process [9, 10]. However, it is sensitive at later growth phases. The degree of sensitivity varies during different growths. The tolerance of plants to salt is higher in the tiller formation stage but decreased in the flowering phase [11, 12]. Seedling and flowering phases are the most sensitive to salinity compared to the germination and tillering phase. However, rice plants' response to salinity levels is different for each rice genotype [11, 12]. The damage caused by salinity in rice seedlings includes excess transport of NaCl from the root system to the leaves.
Osmotic stress in rice plants will induce osmoprotectants' production, such as proline [13]. Salinity stress can also be followed by oxidative stress which can damage plants [14, 15]. Therefore, efforts to increase plant productivity need to be done through effective and efficient technological improvements.

Several cultivation technologies have been reported to cope with environmental stress in rice [16]. One promising cultivation technology in saline land is the pre-planting salinity treatment or NaCl pretreatment [17, 18]. This includes seed priming and salt treatment during nursery [19]. Pre-planting salinity treatment is a way to improve the quality of germination in dry or saline conditions by activating the antioxidant defence system and osmotic adjustment [20, 21]. According to [22], low concentrations of NaCl treatment are reported to increase rice tolerance. Seedlings that grow in unfavourable conditions will have different adaptations according to the seeds' tolerance level [23, 24].

The pre-planting salinity treatment on tomatoes resulted in a better plant adaptation to saline conditions. The adaptation ability of tomato plants largely depends on the salt concentration that is applied during the nursery. When the salt concentration used is below the stress threshold, the plants are unlikely to increase the salt tolerance level [25]. NaCl pretreatment increases growth and yield when plants are exposed to salinity [26, 27]. The initial salinity of 15 mM (~1.5 dS/m) can increase maize plants' tolerance to 45 mM (~4.5 dS/m) at the later growth stage. The same thing happened to Sorghum bicolor [28]. Tomato seedlings, exposed to the salinity of 7 dS/m during salt pretreatment, can increase fruit production by 29% in salinity, compared to those without salt pretreatment [25]. Also, mung bean exposed to salt stress of 5 dS/m during nursery can increase growth, chlorophyll content, increase proline accumulation and reduce H₂O₂ concentrations in seedlings [29].

There are two rice nursery methods, i.e. dry bed nursery and wet bed nursery [30]. Wet seeding is carried out on land that contains a lot of water, while dry seeding is done on land with little water content [31]. Instead of longer seeding time, more seeds, and less tolerant to salinity stress, dry seedbed is more water efficient, more comfortable to implement, faster germination, and shorter flowering age [30]. Meanwhile, [32] and [33] showed that dry seedbeds could reduce crop damage when they are inundated. Rice germinated in flooded saline conditions will have higher salt resistance than those germinated in dry conditions [34]. These three experiments were carried out to evaluate rice varieties' response to salt pretreatment, in different nursery conditions.

2. Methods

2.1. Salt pretreatment on two rice varieties

The research was carried out in the Baros coastal area, Tirtohargo Village, Kretek District, Bantul Regency, Yogyakarta Province. Rice varieties of IR 64 and Dendang were used in this experiment. Their germination rates were 96% and 95%. A single factor Randomized Complete Block Design was used to evaluate each variety of response to pre-planting salt treatment. The factor consisted of two treatment levels, i.e. the pre-planting salinity treatment and non-saline (without salt pre-planting) treatment. Each treatment was repeated three times. Land preparation includes plowing the soil so that 3 x 3 m plots were obtained. The basic fertilizer application consisted of manure as much as 216 kg is spread evenly over the land. The experiment was terminated at 28 days after sowing when all seedlings were transplanted to the rice field. Observations included plant height, leaf area, seed dry weight, chlorophyll content and nitrate reductase activity in leaf. Observation of leaf chlorophyll content was measured by spectronic 21D [35].

2.2. Salt pretreatment in wet and dry bed rice nursery

The research was conducted at the Greenhouse of the Department of Agricultural Cultivation, Faculty of Agriculture, Gadjah Mada University, Yogyakarta. The material used was IR64 cultivar rice seed, which was sown on sand media with Hoagland nutrient solution added with NaCl by the determined treatment. The nursery uses a split-plot design. The main plot was the nursery condition, i.e. wet and dry. Subplots were the salinity treatment, consisted of 0.7 dS/m and seven dS/m. The determination of the salinity concentration of 7 dS/m is based on preliminary research that has been done previously, referring to [36, 37]. Rice is sown in submerged conditions in the wet nursery. While in the dry nursery, the medium was left damp and not inundated. The two factors were combined and repeated three times.
Environmental observations include ambient temperature, light intensity, Relative Humidity and Electrical Conductivity (EC) of seeding media. Soil EC was measured every day for 14 days. The soil EC value was maintained at seven dS/m, with five dS/m and two dS/m were the contributions from NaCl and Hoagland's nutrient solution, respectively. The germinating rate was calculated for 14 days using the following formula:

\[
\text{Germination Rate} (\%) = \frac{\text{Number of germinated seed}}{\text{Total number of seed}} \times 100\%
\]

Observation of leaf Na\(^+\) and K\(^+\) concentrations were carried out at 8, 11, and 14 days after sowing using concentrated HNO\(_3\) extraction method [38].

2.3. Salt pretreatment in black rice
This experiment was conducted at a screenhouse Faculty of Agriculture and was arranged in a Completely Randomized Block Design. Black rice seeds var. Jeliteng was used. It has a fluffier rice texture with an amylose content of 19.6%. This variety also has a high phenolic content and antioxidant content [39, 40]. Black rice seeds are soaked using warm water for 24 hours, before being planted in a nursery tray. The growing medium was a mixture of soil and organic fertilizer. The seedlings were irrigated using saline water of 0.2; 3 and 5 dS/m. The levels of soil Electrical Conductivity (EC) were maintained throughout the experiment. At 21 days after planting, plant height, number of leaves, plumule fresh and dry weight were measured.

2.4. Data analysis
Data were analyzed using analysis of variance with a confidence level of 95% in SAS Portable 9.1.3 software.

3. Results and Discussion

3.1. Salt pretreatment on two rice varieties
According to [41], the IR64 rice variety is a national superior rice cultivar resistant to brown planthoppers, green leafhoppers, late blight bacteria, and grass dwarf viruses. At the same time, Dendang variety is known as a salt-tolerant variety. These two varieties showed a similar response when exposed to the salt pre-planting treatment. Despite the similar number in leaves and root area between those given the salt pre-planting treatment and the without the treatment, the total dry weight of the seedlings during transplanting showed that seedlings with pre-planting had greater biomass than seedlings without treatment. Meanwhile, IR64 variety, which is relatively sensitive to salinity, showed higher plant and larger leaf area than in salt-tolerant Dendang variety, when both were treated with salt pre-planting treatment (Tables 1 and 2).

Table 1. Plant height, leaf number, total fresh and dry weight, leaf area and root area of IR64 variety under different nursery conditions at 28 days after sowing.

| Pre-planting Treatment | Growth variables | Plant height (cm) | Leaf number | Total fresh weight (g) | Total dry weight (g) | Leaf area (mm\(^2\)) | Root area (mm\(^2\)) |
|------------------------|------------------|-------------------|-------------|------------------------|---------------------|---------------------|-------------------|
| Non-priming            |                  | 29.750 b          | 4.812 a     | 0.578 a                | 0.125 a             | 17,911 b            | 2,943 a           |
| priming                |                  | 41.927 a          | 4.958 a     | 1.456 a                | 0.250 b             | 40,715 a            | 3,019 a           |
| CV (%)                 |                  | 2.836             | 3.930       | 29.183                 | 6.679               | 25.287              | 26,002            |

The value followed by the same letter in the same column shows no significant difference based on the T-test on \(\alpha\) 5%.
Table 2. Plant height, leaf number, total fresh and dry weight, leaf area and root area of Dendang variety under different nursery conditions at 28 days after sowing.

| Pre-planting Treatment | Growth variables | | |
|------------------------|------------------|------------------|------------------|------------------|
|                        | Plant height (cm) | Leaf number | Total fresh weight (g) | Total dry weight (g) | Leaf area (mm²) | Root area (mm²) |
| Non-priming            | 29.711 a          | 4.979 a       | 0.450 a                | 0.106 b            | 21,811 a        | 2,915 a         |
| priming                | 31.859 a          | 4.830 a       | 1.190 a                | 0.254 a            | 33,821 a        | 2,953 a         |
| CV (%)                 | 4.106             | 12.017        | 9.709                  | 10.600             | 32.524          | 7.514           |

The value followed by the same letter in the same column shows no significant difference based on the T-test on α 5%.

Table 3 shows that salt pre-planting treatment did not affect both the chlorophyll a or the total chlorophyll content in IR 64 variety, but increased the chlorophyll content. Whereas in the Dendang variety, salinity increased the total chlorophyll in seedlings with pre-planting treatment [17]. As reported by [42], salinity affects the chlorophyll content of rice. In salt-sensitive rice varieties, salinity reduces the chlorophyll content. In contrast, in salt-resistant rice, mild saltiness increases chlorophyll. To a certain extent, an increase in salinity level will be followed by a decrease in chlorophyll content.

Several biochemical compounds are affected by salinity stress, one of which is nitrate assimilation. Table 3 shows no difference in the Nitrate Reductase Activity (NRA) value between the salt-treated and untreated IR 64 rice plants. This also happened to Dendang variety (Table 4). Nitrate assimilation is catalyzed by nitrate reductase (NR), an enzyme that affects nitrogen absorption and assimilation in the plant body [43]. Several studies reported that nitrate uptake and nitrate reductase activity decreased in plants under salinity [44, 45, 46, 47]. However, other studies showed that salinity stress increased nitrate reductase activity, as one mechanism in rice plants to cope with salinity [48, 49].

Table 3. Chlorophyll and nitrate reductase activity of IR 64 variety under different nursery conditions.

| Pre-planting Treatment | Chlorophyll a | Chlorophyll b | Total chlorophyll | NRA |
|------------------------|---------------|---------------|-------------------|-----|
| Non-priming            | 27.154 a      | 30.525 b      | 57.661 a          | 1.914 a |
| priming                | 28.671 a      | 31.061 a      | 59.713 a          | 3.133 a |
| CV (%)                 | 2.226         | 0.153         | 1.036             | 20.286 |

The value followed by the same letter in the same column shows no significant difference based on the T-test on α 5%.

Table 4. Chlorophyll and nitrate reductase activity of Dendang variety under different nursery conditions.

| Pre-planting Treatment | Chlorophyll a | Chlorophyll b | Total Chlorophyll | NRA |
|------------------------|---------------|---------------|-------------------|-----|
| Non-priming            | 22.467 a      | 30.609 a      | 53.058 b          | 3.878 a |
| priming                | 28.481 a      | 31.132 a      | 59.593 a          | 4.415 a |
| CV (%)                 | 5.505         | 3.447         | 0.602             | 31.643 |

The value followed by the same letter in the same column shows no significant difference based on the T-test on α 5%.

3.2. Salt pretreatment in wet and dry bed nursery

The concentration of Na⁺ and K⁺ ions in leaves can be good indicators of salt toxicity experienced by plant cells. Table 5 shows that the salt pre-planting treatment increased the concentration of leaf Na⁺ that accompanied by a decrease in leaf K⁺ concentration. As reported by [47], K⁺ ions play an essential role as a catalyst for various enzymes. Salinity stress causes Na⁺ ion toxicity and K⁺ ions deficiency.
Large Na⁺ concentration inhibits the absorption of K⁺. In increasing cell turgor, the deficiency of K⁺ can be replaced by Na⁺.

**Table 5.** Concentrations of Na⁺ and K⁺ (mmol/FW) in wet and dry nurseries under saline and non-saline conditions at 14 days after seeding.

| Nursery condition | Na⁺ (mmol/FW) | K⁺ (mmol/FW) |
|-------------------|--------------|-------------|
| Wet nursery       | 3.82 a       | 1.03 a      |
| Dry nursery       | 3.78 a       | 0.73 b      |

| Salinity          | Na⁺ (mmol/FW) | K⁺ (mmol/FW) |
|-------------------|--------------|-------------|
| Non-saline        | 0.90 q       | 1.06 q      |
| saline            | 6.72 p       | 0.72 p      |

| Interaction       | (-)          | (-)         |
| CV Nursery (%)    | 20.44        | 14.57       |
| CV Salinity (%)   | 19.64        | 10.06       |

The value followed by the same letter in the same column shows no significant difference based on Tukey's test at the 95% confidence level. The sign (-) indicates that there is no interaction between the treatment levels tested.

Table 6 shows that salinity reduces the germination rate of rice seedlings. Compared to dry nurseries, wet nurseries will increase the germination rate when the seeds are exposed to salinity [18]. It is suspected that cell homeostasis during inundation is more balanced than in moist soil conditions. Inundation conditions are reported to reduce the impact of salinity in rice seedlings. Ion homeostasis in submergence can reduce the Na⁺ concentration in rice seedlings in high salinity [48]. High salinity concentrations in moist soil conditions could increase the water absorption rate in rice. If not balanced with the soil's adequate water availability, the increase in water absorption rate will cause cell plasmolysis.

**Table 6.** The germination rate of seedlings in wet and dry nurseries in saline and non-saline conditions at 14 days after sowing.

| Nursery condition | Germination rate (%) |
|-------------------|----------------------|
|                   | Non-saline | Saline     |
| Wet nursery       | 66.67 a     | 73.33 a    |
| Dry nursery       | 53.67 a     | 30.67 b    |

| Interaction       | (+)         |              |
| CV Nursery (%)    | 18.84       |              |
| CV Salinity (%)   | 11.97       |              |

The value followed by the same letter in the same column shows no significant difference based on Tukey's test at the 95% confidence level. The sign (+) indicates that there is an interaction between the treatment levels being tested.

3.3. Salt pretreatment in black rice variety

Many studies reported that salinity reduced various growth variables of rice plants. The inhibition of water absorption reduced water availability in plants, thus inhibiting the photosynthesis process and reducing plant biomass accumulation. In some cases, light salinity could stimulate growth, which may be related to Na⁺ and Cl⁺ ions' role in contributing to the regulation of osmotic potential in plants.
Figure 1 - 3 shows that giving a salinity of 3 dS/m increased seedling height and plumule biomass. However, increasing the salinity concentration to 5 dS/m lower plant height and plumule dry weight did not differ from those in non-saline treatment. This is following the study by [12] who showed that soil salinity above four dS/m decrease root distribution, plant height [10], and the formation of tillers [19, 49].

![Graph showing plant height and leaf numbers](image1)

**Figure 1.** Leaf number (a) and plant height (b) of rice var. Jeliteng at 14 days in the nursery under three different salinity treatments; N0 (Non-saline); N3 (3 dS/m) and N5 (5 dS/m)

![Graph showing plumule fresh weight](image2)

**Figure 2.** Plumule Fresh Weight of rice var. Jeliteng at 14 days in the nursery under three different salinity treatments; N0 (Non-saline); N3 (3 dS/m) and N5 (5 dS/m)

4. Conclusion

The salinity pre-planting treatment increased the growth of seeds of various rice varieties, both white and black rice. Salt-sensitive rice varieties showed a better response to the salinity pre-planting treatment than salt-resistant varieties. Pre-planting salinity treatment was more effective in the wet nursery than in the dry nursery.

Giving salinity levels of 3-5 dS/m in several rice varieties improved seedling performance. Despite the increase in leaf Na⁺ concentrations and decrease the leaf K⁺ concentrations, salinity during nursery could increase seedlings fresh weight and dry weight and increase leaf chlorophyll concentration. Good seedling growth during the nursery will support better future plant growth in the field.

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