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Seed Priming Effect on Banyuasin and IR 64 Rice Varieties Growth Under Salinity Stress

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Abstract. Rice classified as susceptible to salinity, to improve the salinity tolerance, one of the method use is through seed priming. This study aims to analyse the tolerance of rice seeds of IR 64 and Banyuasin varieties resulted from seed priming towards salinity in terms of seed level.. 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 experiment. The results revealed that priming with NaCl 0f 150 mM was able to increase the tolerance of the varieties towards salinity. Banyuasin variety showed better response towards halopriming, compared with IR 64 variety. In saline media, Banyuasin variety with priming showed the best results for the variables of the average of shoot biomass, root biomass, plant biomass, and the number of tiller (0.81g, 0.18g, 0.99g, and 4.33 shoots). This treatment, compared with the other treatments, also had the lowest Na content in the shoot, which only reached 2.33 mg $^{g-1}$.

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 (Harjadi & Yahya, 1988).

Rice is classified as a plant that is sensitive to salinity (Shannon et al., 1998). This sensitivity differs according to the phase of growth. Grattan (2002) in Sitorus (2012), found that rice plants have a smaller number of tillers if they grow on high-fertility soils. His research further showed that if the soil salinity of more than 6 ds / m of rice yields will decrease dramatically, up to more than 50% of the normal yield.

One of the varieties developed to adapt to saline fields is the Banyuasin variety. This variety is recommended for tidal land with an 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 on 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 activation of various defense responses that are faster and stronger against repetitive abiotic stresses (Conrath et al., 2006). Research conducted by Khajeh-Hosseini et al (2003), showed that priming effects were determined by media priming, priming substance concentration, and priming duration. While Bakht et al (2011), and Afzal et al (2006), reported that the response of different varieties to different priming concentrations was influenced by the genetic background of each variety. With different genetic backgrounds, Banyuasin and IR 64 varieties are expected to show a different response to the application of "seed priming." This study aims to: test the tolerance of Banyuasin varieties of rice seeds and IR 64 seed priming results to salinity stress at the seedling level.

2. Methodology

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 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) (Anonim, 2012). 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 adjusment *also* serves to give aeration to hydroponic solutions.

The salinity treatment was applied after 1 week of 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 reach 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) (Anonim, 2012) used 1.0 S/m^1 as a standard in salinity tolerance testing, while research conducted by Sjahril et al (2012) 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 standart, to ensure that the effect of salinity can be observed. This level of salinity is also used by Suriya-arunroj, et al (2005) 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 number of tillers. three sample plants were measured per treatment. For determination of dry weight, canopy and root were dried in an oven at 70ºC 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

Number of Stock	Chemical Compound	Amount (g or ml/)/5l
1	NH_4NO_3	457
2	$NaH_2PO_4H_2O$	201,5
3	K_2SO_4	357
4	$CaCl_2$	443
5	$MgSO_4$ $7H_2O$	1.620
6	$MnCl_2$ $4H_2O$	7,500
	$(NH_4)_6$ Mo_7O_{24} $4H_2O$	0,370
	H_3BO_3	4,670
	$ZnSO_4$ $7H_2O$	0,175
	$CuSO_4$ $5H_2O$	0,155
	$FeCl_3$ $6H_2O$	38,500
	$C_6H_8O_7$ H_2O	59,500
	1M H_2SO_4	250 ml

Sumber: Gregorio *et al*., 1997.

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. Canopy biomass (g). The leaves of plant sample is inserted into a paper envelope, then dried in a 70˚C oven for 48 hours. After drying, sample were weighing.
2.5.4. Root biomass (g). The root of plant sample is inserted into a paper envelope, then dried in an oven at 70˚C for 48 hours. After drying, sample were weighing.
2.5.5. Macro nutrient content. Macro nutrient Na, K, Mg, and Ca (mg g -1) in the canopy and root were measured using Atomic Absorption Photometer (Z-2000, Hitachi).
2.5.6. Chlorophyll. Chlorophyll is measured using chlorofilmeter. 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 was not statistically different with the V1H0M0, V1H1M0, and V2H1M0 (all treatment were the plant growth in non saline media). Significant different in this parameter only occurs when it compare with the V1H0M1, V1H1M1, V2H0M1, V2H1M1, were all the plant grown in saline media. The results showed that salinity significantly suppresses plant growth. The stress of plant growth due to salinity stress has been reported in various studies (Ashraf and Harris, 2004; Bakht et al., 2006; Munns et al., 2006; Ashraf et al., 2008; Mehmood et al., 2009; Achakzai et al. , 2010; Akram et al., 2010). High Na concentration suppresses plant growth by damaging the osmotic and ionic balance, as well as the accumulation of Na ions which are toxic to plants.

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

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

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). This results was significantly different with 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 grown a longer root in saline condition, this probably one of the strategy to overcome the salinity stress. In IR64 varieties, this research showed that salinity did not significantly reduced the root length. This results contrary with many others reports. Vibhuti et al reported that the higher salinity degree, the more root growth suppressed. Significant decrease of root growth in rice under salinity also reported by Alam et al (2004) Effect of Salinity on Growth of Some Modern Rice Cultivars. However the lowest root growth decrease of IR 64 varieties under salinity also reported by Safitri et al (2017). The inconsistency of root length under salinity condition indicate that this is not a reliable parameters for salinity tolerance screening in rice.

Tabel 2 showed that the best results on canopy biomass (4,63 g) was found in V2HIM0 (Banyuasin varieties, Primed seeds, Non saline media), and this result was significantly different compare 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 results shows the positive effects of seed priming on plant early growth. Seed priming lead to uniform germination, more vigorously seedling, and establish better plant growth as result of pre germination metabolism activation during the priming duration. Seed priming has shown benefits in germination and early growth of several species (Bradford, 1986), this technique can also reduce germination time so that it can be shorter and increase uniform growth and crop yield (Rao and Sing, 1997; Park et al., 1999; and Arif, 2005).

Even though not statistically significant, in this study, primed seeds also showed a better vegetative growth compare to unprimed seeds under saline condition. This results indicate the beneficial effect of seed priming with NaCl. A better vegetative growth of plants on seeds that are primed with NaCl in saline conditions is also reported by Bakh, et al., (2011) and Farahbakhsh and Saiid (2009) in corn, Omami also reported the same positive effect. , EN (2005) at Amaranthus. According to Shafi (2009) in Abraha (2013), priming increases free radical deterrence enzymes such as peroxidase, catalase and superoxide dismutase, to improve plant survival and strength under salinity stress. Meanwhile according to Okcu (2005) in Abraha (2013), seed priming allows membrane and protein hydration, and initiation of various metabolic systems in the face of stress.

Table 3 showed that the accumulation of Na in plant canopy are significantly different between plant that growth in saline and non saline media. The lowest Na accumulation in canopy 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 canopy 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})	Chlorophyll
V1H0M0	0.07^{c}	4.42^{ab}	0.02^{a}	0.43^{ab}	0.06^{c}	39.03^{ab}
V1H0M1	4.96^{a}	2.31^{c}	2.17^{c}	0.31^{c}	0.16^{a}	35.97^{b}
V1H1M0	0.07^{c}	4.60^{a}	0.02^{a}	0.46^{a}	0.09^{bc}	41.07^{a}
V1H1M1	4.51^{a}	2.34^{c}	2.55^{c}	0.20^{d}	0.13^{ab}	36.47^{b}
V2H0M0	0.10^{c}	4.20^{b}	0.02^{b}	0.54^{a}	0.10^{bc}	39.07^{ab}
V2H0M1	4.23^{a}	2.38^{c}	1.77^{c}	0.35^{bc}	0.13^{ab}	36.03^{b}
V2H1M0	0.10^{c}	4.36^{ab}	0.02^{b}	0.53^{a}	0.09^{bc}	40.53^{a}
V2H1M1	2.33^{b}	2.64^{c}	0.88^{c}	0.34^{bc}	0.09^{bc}	40.23^{a}

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 canopy (2.33), and this results are significantly different compare to other treatment in. Lower Na accumulation in this treatment suspected as the results of seed priming and correlate with better growth that indicate by a 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. According to Horie et al., (2012) the ability to regulate osmotic pressure in these cells is an important mechanism in overcoming salinity stress in tolerant plants. According to Cayuela et al., (1966) in Bakh et al., (2011) better growth in seeded seed, is a result of better osmotic regulation capacity. The results of Omami's study, E.N (2005) which conducted seed priming research on spinach seeds also showed that there was a decrease in Na + accumulation and an increase in Ca2 +, K +, and the ratio of Ca: Na and K: Na to the spinach spinach leaves.

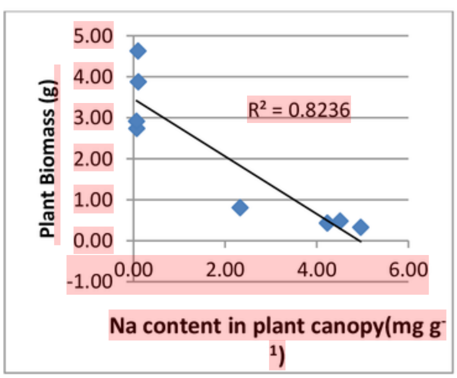

Figure 1. Correlation between plant biomass and Na content in plant canopy

Table 4 showed that even though it accumulates the lowest Na ion in the canopy, the Banyuasin 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 canopy, so that the damaging effects of excess salt in the canopy can be reduced. This also seems to affect the chlorophyll content of plants. In all varieties, the seeded seed showed chlorophyll content compared with no priming in the same medium. In Banyuasin varieties, plants that are bred and 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 seeded seeds have higher chlorophyll content compared to plants without priming, the value is still lower than the chlorophyll content in plants without priming on nonsaline 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^{b}	2.352^{a}	0.22^{a}	0.254^{a}	0.034^{bcd}
V1H0M1	1.478^{a}	1.253^{d}	1.20^{c}	0.148^{c}	0.051^{ab}
V1H1M0	0.539^{b}	2.188^{ab}	0.25^{a}	0.248^{a}	0.033^{bcd}
V1H1M1	1.657^{a}	1.503^{cd}	1.10^{c}	0.161^{c}	0.047^{abc}
V2H0M0	0.778^{b}	1.776^{bc}	0.45^{b}	0.233^{ab}	0.029^{cd}
V2H0M1	1.669^{a}	1.450^{cd}	1.23^{c}	0.140^{c}	0.062^{a}
V2H1M0	0.763^{b}	1.924^{bc}	0.40^{b}	0.228^{b}	0.024^{d}
V2H1M1	0.745^{a}	1.163^{d}	0.64^{bc}	0.146^{c}	0.032^{cd}

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 less Na in the canopy can show better tolerance to salinity stress. Yeo et al. (1990) reported that the ability of plants to reduce accumulation of Na in the canopy correlated with the ability of rice plants to survive in salinity stress. A similar relationship was reported by Garthwaite et al. (2005) in wheat and barley. 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.

Conclusion

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