ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Parameters Utilized in Screening for Salt Tolerance in Rice (*Oryza Sativa* L.).

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ABSTRACT

Rice is a salt sensitive crop and suffers yield losses due to increased salt concentrations in the cultivated soil or irrigated water. One of the best cost effective methods to overcome salt stress can be to combat it by growing salt tolerant rice genotypes. Screening for tolerance is an essential step to identify genotypes. Salt injury symptoms that can be observed in the yield contributing traits are utilized to analyze the level of tolerance under increasing concentrations of salt. *In vivo* studies under field conditions in salt affected environments and *in vitro* studies under laboratory experimental conditions at various growth stages of rice plant have been conducted by rice researchers throughout the rice growing nations. The article is an attempt to represent the work being carried out towards screening for salt tolerance in rice.

Keywords: Rice, salt, stress, germination, Na^+, K^+.

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INTRODUCTION

Screening is an essential part of a breeding program. High yielding rice varieties may fail to perform well in salt affected soils. The performance of a genotype and its reduction in yield when subjected to stress can be used to screen and evaluate it. Parameters and indices have been used to study various stages of growth by analyzing specific traits both under *in vivo* and *in vitro* conditions. Symptoms related to stress have been assessed by researchers using scoring patterns to evaluate tolerance. Usually rice plants are studied for the traits like days to 50 per cent flowering, plant height, productive tillers per plant, panicle length, grains per panicle, spikelet fertility, 100 grain weight, single plant yield and Na^+:K^+ ratio under normal and salt stress conditions to compare and evaluate the tolerance capacity of a genotype.

Salt tolerance indicators

Stunted growth, rolling and withering of leaves, occurrence of white blotches, browning and drying of older leaves are some of the symptoms observed as a result of salt injury [1,2,3]. Osmotic imbalance and increase in Cl^- can cause salt injury [2]. Spikelet sterility, Na^+/K^+ ratio can be used for assessing salt tolerance. Lower spikelet sterility and Na^+/K^+ ratio have a positive correlation with salt tolerance according to Mishra et al. (1997) [4]. The salt specific effect causes increased uptake of Na^+ and Cl^- and decreased uptake of particularly K^+ and Ca^{2+} ions. If the plant fails to partition the ions between different tissues or to transport the ions within the cells vacuole, they build up in the cytoplasm to toxic concentrations. Potassium is considered a major osmoticum in plant cells under high salt concentrations [5].

Growth stages

Rice is differentially affected by salinity at different stages. The effects of salinity on the growth of rice were found to be related to the stage of plant development, salt concentration, type of salt, duration of exposure to salt, soil pH, water regime, temperature, humidity and solar radiation [3]. Rice has been found to be tolerant during germination, vegetative growth and maturity but very sensitive at early seedling growth (2-3 leaf stage), pollination and fertilization stages [6,7]. Krishnamurthy et al. (1971) observed that tillering was not influenced by salinity [8]. Karim and Haque (1986) reported that plant height, root length, number of tillers per plant, straw yield and dry weight of root was affected by salinity during vegetative stage [9].

Germination and seedling survival

Rice crop is relatively tolerant to salinity and alkalinity during germination stage. Rice plants are much more sensitive during the early seedling stage (2-3 leaves) than at later stages [10]. The germination though affected by salinity can be appreciably overcome in the later stages [11]. Germination of seeds involves the activation of enzyme systems as well as mobilization of reserve foods and these processes are adversely affected by NaCl [12]. Tolerant lines exhibit higher enzymatic activity than the sensitive ones [13]. Tolerance at emergence is based on survival, whereas tolerance after emergence is based on decrease in growth or yield [14].

Powar and Mehta (1995) reported that the percentage germination, seedling height and root length decreased significantly with increasing salinity in an experiment conducted using rice seeds of Cv. Panvel 1, Panvel 2, Kala Rata 1-24, Bhura Rata 4-10, Palghar 1 and Jaya with saline water of different concentrations (1.5, 3.0 and 6.0 ds/m ; distilled water control) [15]. Bong et al. (1996) reported that, the salt calcium chloride had the most severe effect on the growth of rice seedlings and the salt sodium sulphate had the least effect [16]. Seed germination and seedling growth rates of salt susceptible rice Cv. Jaya and salt resistant Cv. Damodar were studied by Misra et al. (1997). They found that the effect of salinity on seed germination, shoot and root length, seedling vigour index (SVI) and increase in the root:shoot length ratio in the laboratory was relatively greater in Jaya than in Damodar [17]. According to Lee and Senadhira (1999) rice cultivars with high salinity tolerance at seedling stage can perform well under direct sown conditions. Two lines derived from one cross (HR 15258) had good tolerance due to root and shoot characteristics that lead to high shoot water content, thus diluting the toxic effects of the salt [18].

The effect of salinity stress as induced by NaCl and $MgCl_2$ at 0.3, 0.6 and 0.95 on the shoot and root characters of seedlings of seven rice cultivars were studied by Roy et al. (2002). They reported that the length and dry weight of shoots and roots, as well as number of roots per plant decreased with increasing levels of salinity [19]. Genotype IR63731-1-1-4-3-2 was found to show salt tolerance at early seedling stage [20]. Janaguiraman et al. (2003) reported that tolerant genotypes of rice record a higher germination percentage, root length, shoot length, vigour index and amylase and dehydrogenase activity with less accumulation of anthocyanin in their roots [21]. Older seedlings (8 weeks old) can help to improve survival rates under salt stress [22]. For transplanted rice, survival during transplanting decides its performance and is expected to produce better grain yield though spikelet sterility. In a study performed by Ansari et al. (2003) cultivars Ganga White, Nona Bokra, IR 6 and Shua-92 had better seedling survival, whereas IR 28 and Basmati had the lowest seedling survival [23].

Sankar et al. (2006) have ranked salt tolerant rice lines based on germination and seedling growth under salt stress conditions. The salt tolerant parents along with checks were assessed for their salt tolerance for the traits germination per cent, total biomass to utilized seed ratio and vigour index under controlled condition in various simulated salt concentrations. Increasing salt levels had detrimental effects on all the traits studied. Under the salt combinations (0-1M) of $MgCl_2$: $NaCl$:$CaCl_2$:Na_2SO_4 (1:4:5:10) the genotypes CSR 23 and CSR 10 ranked higher for the traits germination per cent and total biomass to utilized seed ratio. CSSRI 60 and Nona Bokra ranked better for the traits total biomass to utilized seed ratio and vigour index. Under varied NaCl concentrations (0-1.6%) the genotype CSSRI 13 performed well for the traits germination per cent and vigour index while CSSRI 60 performed well for total biomass to utilized seed ratio and vigour index. Under both, the combination of salts and NaCl the genotype CSR 23 and CSR 10 performed well for germination per cent, CO 43 and Nona Bokra performed well for total biomass to utilized seed ratio and CSSRI 60 for vigour index. The inclusion of these genotypes in salt tolerance breeding programmes may yield resistant varieties [24].

Effect of salt stress on germination and early seedling growth of rice was studied by Hakim et al. (2010). Rice varieties MR211, IR20, BR40 and MR232 were found to show greater salt tolerance during germination when germinated at 12 dS/m electrical conductivity. Varieties MR211, MR232 and IR20 performed better based on dry matter yield reduction [25]. Chutipaijit et al. (2011) have reported that increase in proline and anthocyanin content can be good traits indicating protective response to salinity in rice. Seedlings of eight indica rice genotypes were subjected to hierarchical cluster analysis based on which Sangyod, Khao Dang, Kulab Dang and TD49 were found to be salt-tolerant. Under salt stress conditions (100 mM NaCl), the tolerant genotypes showed the higher percentages of proline and anthocyanin. Photosynthetic pigment stabilizations were positively correlated to the proline and anthocyanin accumulations. Higher percentages of total antioxidant activity were observed in salt-tolerant genotypes [26]. Scented non-basmati type indica rice cultivars, Indrayani and Ambemohar were observed for germination, growth and biochemical parameters by Danai-Tambhale et al. (2011). Salt stress-induced proline accumulation and protein content was higher in Ambemohar cultivar when compared to Indrayani at 200 mM NaCl concentration [27].

Eight indica rice varieties at germination and seedling stage were tested for salt tolerance by Anbumalarmathi and Mehta (2013) up to a high salt concentration of 20 dS/m. Final germination percentage, speed of germination, germination energy percentage, plumule and radicle length and plumule and radicle dry weight were the traits analysed. Six genotypes failed to germinate at 20 dS/m salt concentration. Rice varieties ADT43, IR50 and MDU5 germinated at 12 dS/m salt concentration and hence might be useful for further studies [28]. Rice varieties NERICA 12, IR 20, IWA 11 and NERICA 19 were found to be showing greater salt tolerance during germination at 10 ds/m electrical conductivity by Ologundudu et al. (2014). NERICA 1, IR 29, and IR 20 were found to perform better based on dry matter yield reduction [29].

Spikelet sterility

Reduction in grain yield by salinity may be pronounced by the reduction in panicle length, number of primary branches per panicle, number of spikelets per panicle, seed setting percentage and panicle weight. Complete sterility was reported by Kapp (1947) due to the effect of salinity during reproductive stage [30]. Yield reduction at maximum tillering stage due to salinity was observed by Ahmed et al. (1989) [31]. Decreasing grain yield with increase in salinity was reported by Powar and Mehta (1997) [32]. Lehman et al. (1984) and Thirumeni (1998) reported that percentage of under developed spikelets increased with salinity and similar reports were given by Narayanan (2000) [33,34,35]. Zeng et al. (2000) reported that the reduction in spikelet number per panicle was a major cause of yield loss under salinity [36].

Rice genotypes were evaluated under coastal salinity for heterosis for nine yield related traits by Thirumeni and Subramanian (2000). The hybrid SSRC 920761 x TRY 1 was found to be superior for productive tillers per plant, spikelet sterility, Na^+:K^+ ratio and grain yield per plant and hence was recommended for commercial exploitation [37]. Ranking of rice genotypes for salt tolerance was carried out for twelve genotypes using NaCl and $CaCl_2$ in the ratio of 5:1 molar concentration at 4.4 and 8.2 dS/m electrical conductivity by Zeng et al. (2002). They identified wide differences in comparative salt tolerance in genotypes based on spikelet and tiller numbers and hence were considered good agronomic traits which can be used for screening salt tolerance in rice. Genotype IR63731-1-1-4-3-2 was found to show salt tolerance at seed maturity stage [20]. Sankar et al. (2008) utilized spikelet fertility as a trait to observe salt tolerance in rice genotypes. CSR 23, CSSRI 60 and CO 43 among parents and GD 98029/CSSRI 60 and GD 98021/CSR 10 among hybrids were observed to be the best based on mean, combining ability and heterosis. Spikelet fertility was found to be controlled by dominance gene action [38].

Osmotic imbalance and accumulation of metabolites

Plants initially adjust to saline conditions by decreasing tissue water content through osmotic adjustment [39]. Although differences in salt tolerance are not common among cultivars, significant differences have been observed for rice, barley, wheat and soybean. Salt tolerance may be due to varietal differences or variation in adaptation to climatic or nutritional conditions in which the experimentation was undertaken [14]. Bal *et al*. (1986) reported that salt tolerant rice varieties accumulate lesser Na^+ and higher K^+ than susceptible varieties [40].

Lutts et al. (1996) exposed the salt tolerant Cv. Nona Bokra and IR 4630 at the seedling stage for 1 or 2 weeks to 0, 20, 30, 40 and 50 mM NaCl. They found that the exposed cultivars accumulated lesser sodium, chlorine, zinc ions and proline and more potassium ions in roots and shoots than salt sensitive cultivar Cv. I Kang Pao and IR 31785. Accumulation of sodium and chlorine and decrease in potassium content in shoots were restricted to the oldest leaves in salt resistant cultivars [41]. Studies on salt tolerance in Korean rice cultivars by Ha (1996) revealed that the extent of potassium exclusion by sodium in the roots might be related to salt tolerance [42]. Asch et al. (1997) selected five lines I Kang Pao, IR 64, IR 4630-22-2, CSR 10 and Aiwu to be salt tolerant based on the traits grain yield decline, spikelet sterility and sodium and potassium distribution in top three leaves, stems, stem base and roots. They reported that potassium/sodium ratio in the three leaves from the top was high in these genotypes and this could be used as a reliable index for salt tolerance [43].

Shannon et al. (1998) observed that leaf tissues of plants grown at 16 ds/m had five times as much Na^+ and three times as much Cl^- as controls. Leaf concentration of K^+ was decreased by about 40% by salinity, but tissue levels of Ca^{2+} and Mg^{2+} were unaffected. Relative salt tolerance differences were found to be negligible among cultivars leading to the conclusion that genetic differences are limited [44]. Highest K^+/Na^+ ratio in the upper leaves and the highest content of Na^+, K^+, Ca^{2+} and Mg^{2+} in the lower leaves in the tolerant genotype T-45 was reported by Shareef et al. (2002). Reduction in K^+/Na^+ ratio was observed with increasing soil salinity [45]. Babu (2002) studied salt tolerant rice hybrids over saline environments. He observed that the rice hybrid TS 29 x BTS recorded high standard heterosis value than BTS 24 and CORH 2 in all environments and pooled condition for productive tillers per plant, leaf proline content, Na^+:K^+ ratio besides single plant yield [46].

Ansari et al. (2003) have reported that sodium uptake increased and potassium decreased with increasing salinity in a study performed using 2 week old seedlings of Shua-92 and IR 8C. Sodium seemed to have an unrestricted flow in the sensitive IR 8C, whereas the tolerant Shua-92 managed to control this buildup inside its tissue. The Na^+/K^+ ratio increased with time but decreased in the more tolerant cultivar Shua-92 [23]. Sankar et al. (2008) have reported that the genotypes CSR 10 and Vytilla 3 had recorded good mean and *gca* effects for Na^+: K^+ ratio in salt affected environments. In salt affected environments the hybrids GD 98028/CSR 23 and GD 98021/CO 43 had recorded high heterosis for both Na^+:K^+ ratio and grain yield per plant. Hybrids GD 98028/CSR 23, GD 98028/CO 43 and GD 98021/CO 43 can be exploited for improving salt tolerance and yield by heterosis breeding. Na^+:K^+ ratio was found to be governed by dominance gene action [38].

Enhancement of salinity tolerance

Increase in Na^+ concentration in the cytosol can lead to denaturation and loss of function of macromolecules and affect carbon fixation [47,48]. Salinity reduces net photosynthesis in the older leaves which accumulate sodium [49]. Under salt stress conditions growth of young leaves are affected in plants and senescence of mature leaves is accelerated. Salt tolerance in plants is due to the production of osmolytes, Na^+ or Cl^- exclusion into the vacuoles, and the tolerance of tissue to accumulated Na^+ or Cl^- [50]. Enhancement of salinity tolerance under salt stress was effected by transferring the bacterial *mtlD* coding gene for mannitol 1-phosphate dehydrogenase into *Arabidopsis thaliana* by Thomas et al. (1995). Transgenic seeds expressing mannitol was found to germinate in medium supplemented with up to 400 mol m^{-3} NaCl, while control seeds ceased germination at 100 mol m^{-3} NaCl. The ability to generate might have been due to the osmotic effect exerted by elevated levels of mannitol [51]. Overexpression of an endogenous vacuolar Na^+/H^+ antiport by Apse et al. (1999) in Arabidopsis promoted growth in soil treated with 200 µM NaCl [52]. NHX, SOS1, and HKT transporters have been transgenically engineered into plants to enhance salt tolerance [53].

Xu et al. (2011) have observed that seed presoaking by bovine hemoglobin increased the heme oxygenase-1 gene expression and also differentially induced catalase, ascorbate peroxidase, and superoxide dismutase activities or transcripts and thus decreased the lipid peroxidation in germinating rice seeds subjected to salt stress [54]. Ascorbic acid application can help in improving salt tolerance in rice as reported by Barus et al., 2015. Ascorbic acid was applied at the age of 15, 35, 55 and 75 days after sowing. At 1500 ppm ascorbic acid concentration, the variety Banyuasin exhibited positive effects on morphological characters [55].

CONCLUSION

Salinity is one of the major abiotic stresses faced by crop plants. Understanding the physiology of salt tolerance in plants and designing of plants suitable to be grown under salt affected environments are better solutions to meet the persistent demand for food. Salt stress indicators have helped breeders in designing tolerant genotypes through conventional breeding and genetic engineering. Hence the research towards selecting genotypes possessing salt tolerance using indices and parameters is pursued by researchers striving towards the goal of attaining food security.

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