Physiological traits as the primary tool for screening salt tolerance in rice

Abstract

Salt stress is a common abiotic stress that significantly restricts crop development and productivity. Physiological alterations in response to salt stress were assessed for fourteen rice varieties during the panicle initiation stage at 120mM NaCl. Statistical analysis program (SPSS 15.0) was used to carry out the study. Under salt stress, all the rice varieties were assessed manifested a reduction in chlorophyll accumulation, stable chlorophylls, membranes and hydration status. On the other hand, all the varieties showed an increase in proline, hydrogen peroxide, and superoxide dismutase activity. It is noteworthy that the rice varieties *surakuruvai, kaivarasamba, mallam punchai,* and *mappillai samba* had better levels of salt tolerance than the salt-sensitive ones due to increased SOD activity, proline accumulation, relative water content, chlorophyll, and membrane stability index. The ability to tolerate salt during the reproductive stage under field conditions will be further investigated using these varieties.

Key Words: CSI Proline, RWC, SOD, Salt Tolerance Rice

Introduction

Rice is the most important global food crops that providing food for more than half of the world's population. However, rice productivity in several areas is affected by salinity stress due to the buildup of underground salt and is exacerbated by salt mining, deforestation, and irrigation. Nearly 1 billion hectares of land on Earth are affected by salinity, which damages 900 million ha of land, or almost 20% of all land on Earth. Additionally, about half of all irrigated arable land on Earth is affected by salinity (Velmurugan et al., 2020). The most pervasive issue with soil toxicity in nations that grow rice is soil salinity. Because appropriate agricultural land is scarce, boosting rice's salinity resistance is essential for further expanding the rice-growing region.

However, damage can also ensue with the results of excessive reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) which are produced at a fast rate accumulated in a plant tissue as a result of ion imbalance and hyperosmotic stresses. As a result, ROS accumulation causes lipid oxidation which has a detrimental effect on cellular metabolism and physiology thus adversely destructs the membrane integrity (Munns et al., 2006). Several plants have developed mechanisms to regulate the synthesis and accumulation of compatible solutes like proline and glycine betaine that serve as osmoprotectants and are essential for plants to adapt to osmotic stress by stabilizing the tertiary structure of proteins, in addition to ion homeostasis strategies (Munns and Tester, 2008).

Salinity-induced yield reduction of rice is alarming for the food security of the ever-growing population of the world, especially in Asia, because 90% of the world's rice is produced and consumed in Asia and more than 3 billion Asian intakes their 50-80 % daily calorie from rice (Khush, 2005). Keeping this in view, the present study was conducted to explore growth and physiological changes in rice varieties subjected to salinity stress differing in their level of salt tolerance.

2. Materials and methods

2.1. Experimental site, Plant material and salt stress

This study was conducted in pot culture at the glasshouse of Tamil Nadu Rice Research Institute, Aduthurai, India. Fourteen rice genotypes (local types) were collected from farmer’s fields over Tamil Nadu and screened for their salt tolerance levels at the early reproductive stage which is the panicle initiation stage. Rice seeds were surface sterilized, and directly sewn into pots (15 cm in height and 30 cm diameter). Ten kg of soil was collected from paddy fields and mixed with river sand and FYM in a 4:1:1 ratio. This experiment was laid out in a complete randomized block design with four replications. Best genotypes from the previous hydroponic study were used for pot culture (Preliminary study already completed). Pokkali, a well-known salt-tolerant genotype was used as a standard tolerant check and IR64 as a susceptible check. Salt solutions were prepared by dissolving NaCl salt in water with a concentration of 6 EC (60 mM NaCl) and subsequently rose to 12 dS/m (120 mM NaCl). Then pots were irrigated with saltwater and salinity levels were closely monitored for each treatment. Seedlings of each rice variety were subjected to salinity stress at 120 mM NaCl for 15 days during panicle initiation (50 to 65 DAS).

Sampling was performed at the end of the experiments and physiological changes were recorded. Chlorophyll content was measured by the method of Arnon (1949). Relative water content (RWC) was calculated using the formula proposed by Weatherley (1950). Proline content was estimated by the modified procedure of Bates *et al.* (1973) and expressed as µg g^{-1} tissue FW. Chlorophyll Stability Index (CSI) in the leaf was estimated using the method of Koleyoreas (1958). Membrane Stability Index (MSI) was determined by the estimation of electrolyte leakage in leaf samples by using the method proposed by Pinhero and Fletcher (1994). The content of H_2O_2 was measured by the method of Velikova *et al.* (2000). The SOD activity was assayed by the method of Beauchamp and Fridovich (1971).

The pot culture experiments were arranged in a completely randomized design. The data were subjected to one-way analysis of variance (ANOVA) as suggested by Gomez and Gomez, 1984, and to mean separation with the Fisher's Least Significant Differences (LSD) test with $P<0.05$, using the statistical analysis program (SPSS 15.0).

3. Results & Discussion

Salinity can cause negative effects on plant growth and development. The adaptive behaviour of rice varieties under a salt stress environment is discussed hereunder through various physiological and biochemical aspects. Chlorophyll pigments play a vital role in crop productivity because these pigments are highly responsible for photosynthesis in plants. In our study, the chlorophyll a, b, and total contents generally declined under salt stress which is in line with Wang et al. (1997). Accordingly, tolerant genotypes had higher chlorophyll content than susceptible ones. The genotypes namely *mallam punchai* and *surakuruvai* recorded higher chlorophyll a, b and total content under salt stress conditions. This might be due to better protection of salt-induced chlorophyll loss in rice cultivars with higher salt tolerance was also observed in earlier reports by Khan and Abdallah (2003). The lowest was recorded in *chinnapunchai*, *uppumilagai* and *IR64* under salt stress conditions than the rest of the genotypes tested. Salt stress reduced the amount of chlorophyll in the leaves by degrading or inhibiting the synthesis of chlorophyll (Ashraf and Harris 2013). The increased rate of chlorophyllase enzyme activity (enzymes degrading chlorophyll) is favoured under high salt conditions. This might be one of the important factors for the reduction of photosynthesis under salt stress. Hence, variation in the chlorophyll content can be used as a stress indicator (Naumann et al. 2008), because chlorophyll content decreased in sensitive crop plants under salt stress conditions (Ashraf and Harris 2013).

RWC is the indicator of the water status of the plant. Salt stress significantly affects the water status of the plants. All the genotypes maintained good water status under well-watered conditions. A higher leaf RWC value of 83.5, 82.5, 81.5 and 81.4 percent were evident with the genotypes namely *kattaikar*, *kaivarasamba*, *kallundaikar* and *surakuruvai* respectively which was on par with the tolerant check Pokkali, which were also significantly superior to the rest of the genotypes under salt stress condition. It seems that these genotypes were able to maintain the relatively high turgidity required for leaf function. Since sensitive genotypes usually transfer larger amounts of Na from roots to shoots, this could result in higher osmotic potential in their roots and less water uptake from the saline soil solution. Sensitive genotypes are also known to have a less stomatal function when subjected to salt stress, resulting in higher transpiration and greater water loss, both of which could be reflected in lower values of leaf RWC and consequent cellular dehydration (Qin et al., 2010).

The CSI is an indication of the stress tolerance capacity of plants. In all the investigated genotypes, the CSI and MSI percentage decreased in genotypes under salt stress conditions. But there were no significant differences between the genotypes under control conditions (Table 2). Among the genotypes, *kaivarasamba*, *mappillai samba*, *mallam punchai*, *surakuruvai* and *kattai kar* had significantly higher CSI percentages (79.36, 78.65, 79.94, 78.61 and 78.36% respectively) under salt stress conditions along with the tolerant check (80.50%). A high CSI value means that the stress did not have much effect on the chlorophyll content of plants and also helps the plants to withstand stress conditions through better availability of chlorophyll. This leads to an increased photosynthetic rate, more dry matter production and higher productivity. We also observed significant positive correlations of RWC with CSI ($r = 0.842$, $P<0.01$), MSI ($r= 0.660$, $P<0.05$), H_2O_2 ($r = 0.709$, $P < 0.01$), proline ($r = 0.774$, $P<0.01$), chlorophyll a ($r = 0.819$, $P<0.01$), chlorophyll b ($r = 0.558$, $P<0.05$) and total chlorophyll ($r = 0.714$, $P<0.01$) furnished in Table 1.

MSI is an indicative of salt tolerance as it measures the extent of cell membrane injury under stress, as observed previously for salt stress (Bhattacharjee and Mukherjee 1996). Like RWC, MSI was also significantly higher in tolerant genotypes viz., *kaivarasamba* (79.60%), *mappillai samba* (76.23%), *mallam punchai* (77.45%) and *surakuruvai* (78.60%). We also observed significant positive correlations of MSI with SOD ($r = 0.56$, $P<0.05$), proline ($r= 0.778$, $P<0.01$), H_2O_2 ($r = 0.789$, $P < 0.01$), chlorophyll a ($r = 0.897$, $P<0.01$), chlorophyll b ($r = 0.830$, $P<0.01$) and total chlorophyll ($r = 0.893$, $P<0.01$) (Table 1).

The result of the present study showed that the amount of H_2O_2 varied among different genotypes under salt stress conditions. Salt stress increases ROS production which automatically activates the antioxidant enzymes in the tolerant plants. Higher amount of H_2O_2 accumulated in the susceptible genotypes namely *china punchai* (61.14 µmol g^{-1}), *uppu milagai* (60.86 µmol g^{-1}) and *IR64* (63.21 µmol g^{-1}). It seems that NaCl-induced H_2O_2 accumulation reduces plant growth, development and productivity (Vaidyanathan et al., 2003). The removal of the free oxygen radicals is an important mechanism of salt tolerance in plants (Motohashi et al., 2010). In this present study, the salinity stress affected the activity of the antioxidant system which is in line with Wi et al. (2006). Since the H_2O_2 content varied significantly among the varieties, the activity of major H_2O_2 scavenging enzyme SOD was variable in these genotypes under the salt stress conditions. Interestingly, salt stress increased the SOD activity in all the genotypes tested than control. Among the genotypes, *kaivarasamba, mappillai samba, mallam punchai, surakuruvai, kadi kannan* and *sivapuchithirai kar* recorded significantly higher SOD activity under salt stress conditions. Thus, it seems that the genotypes had an efficient enzymatic detoxification system for H_2O_2 scavenging. The higher activity of SOD was also observed in other salt-tolerant plants (Sekmen et al. 2007; Sairam et al. 2002).

Proline acts as a compatible solute which seems to have diverse adaptive roles including stabilization of proteins and stabilization of membrane and sub-cellular structures (Van Rensburg et al., 1993), protecting cellular functions by scavenging reactive oxygen species (Smirnoff and Cumbes 1989), the storage form of carbon to provide the energy needed for recovery (Hare and Cress 1997) and acting as a signal molecule controlling reproductive development (Mattioli et al., 2008). Igarashi et al. (1997) suggested that proline accumulation was related to the degree of salt tolerance. The accumulation of high proline content in the rice cultivars under salt stress was able to maintain a higher green leaf area (Kordrostami et al., 2017). Accordingly, in the present study, the proline content was increased in all the rice genotypes under salt stress conditions than in the control.

Conclusion

The results of this study showed that the rice genotypes namely *kaivarasamba, mappillai samba, mallam punchai* and *surakuruvai* possessed higher degrees of salt tolerance by enhanced activity of physiological traits such as RWC, CSI, MSI, Proline and SOD activity. These genotypes could be further investigated at the reproductive stage salt tolerance ability under salt affected soil conditions.

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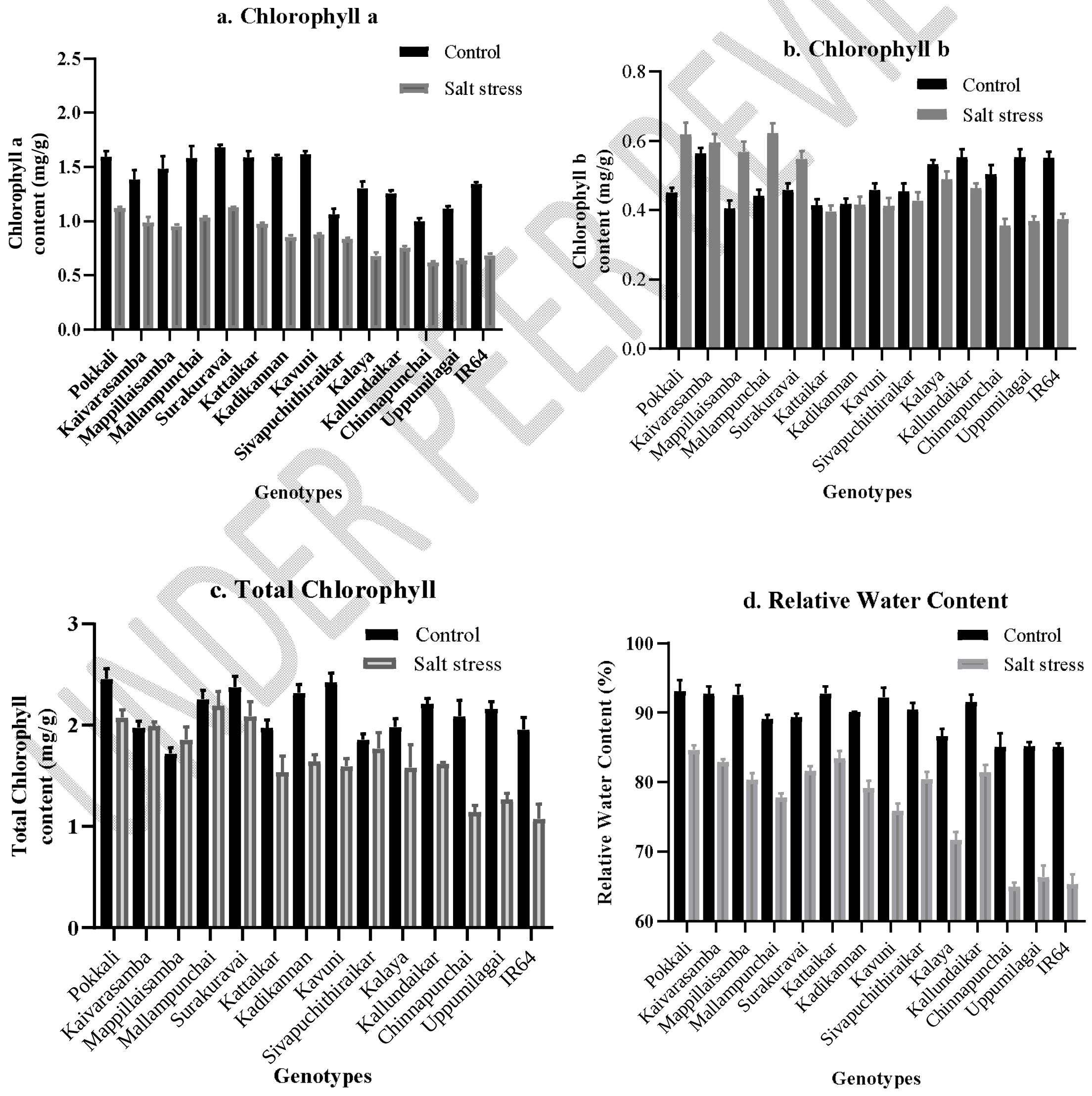
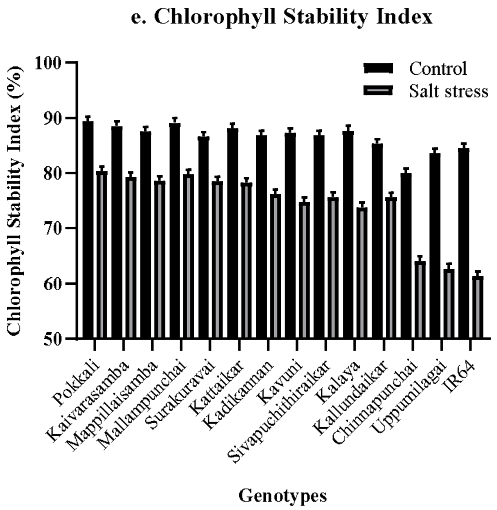
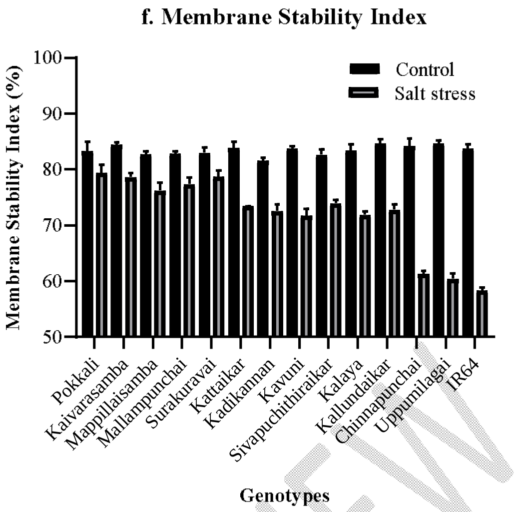
31.

Figure 1. Comparison of physiological parameters in rice genotypes exposed to salt stress condition.

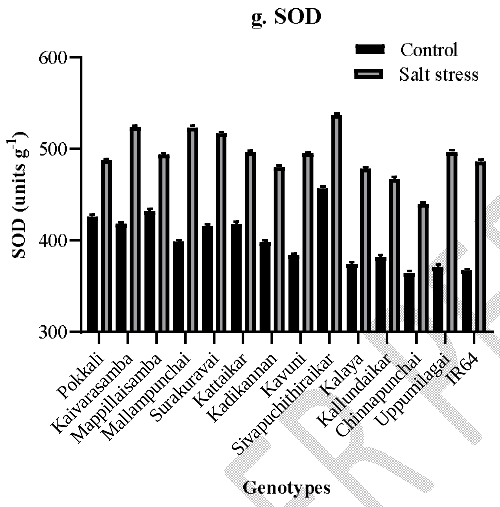




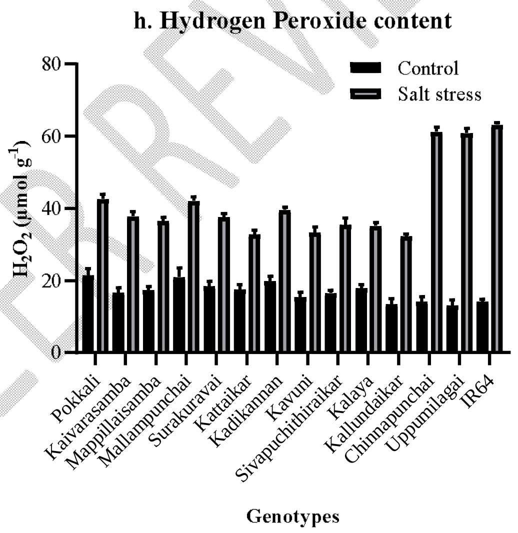
e. Chlorophyll Stability Index



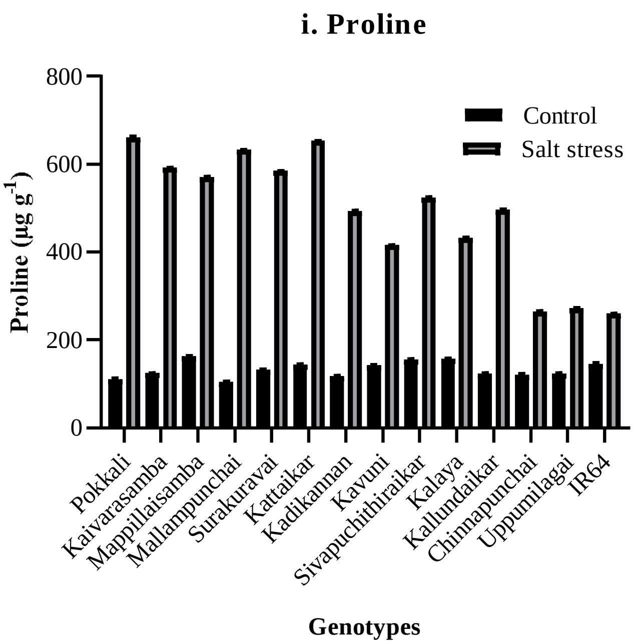
f. Membrane Stability Index



g. SOD



h. Hydrogen Peroxide content



i. Proline

1a. Chlorophyll a content (mg g^{-1}), 1b. Chlorophyll b content (mg g^{-1}), 1c. Total Chlorophyll content (mg g^{-1}), 1d. Relative Water Content (%), 1e. Chlorophyll Stability Index (%), 1f. Membrane Stability Index (%), 1g. Super Oxide Dismutase (SOD: units g^{-1}), 1h. H_2O_2 content (µmol g^{-1}), 1i. Proline content (µg g^{-1}).

Table 1. Salt tolerance index of physiological parameters in rice genotypes under salt stress

Genotypes	RWC	CSI	MSI	SOD	H_2O_2	Proline	Chl a	Chl b	TC
Pokkali	0.91	0.89	0.92	1.14	2.00	5.08	0.70	1.36	0.83
Kaivarasamba	0.89	0.91	0.88	1.25	2.27	3.95	0.75	1.05	0.99
Mappillai samba	0.87	0.90	0.91	1.14	2.10	2.88	0.64	1.41	1.08
Mallam Punchai	0.87	0.90	0.93	1.31	2.01	6.05	0.65	1.41	0.98
Surakuravai	0.91	0.91	0.94	1.25	2.05	4.41	0.67	1.20	0.89
Kattai kar	0.90	0.89	0.88	1.19	1.88	4.53	0.61	0.94	0.79
Kadi kannan	0.87	0.88	0.89	1.21	1.98	4.19	0.53	0.98	0.71
Kavuni	0.83	0.86	0.86	1.29	2.15	2.93	0.54	0.89	0.66
Sivapuchithiraikar	0.89	0.87	0.89	1.18	2.16	3.36	0.79	0.94	0.94
Kalaya	0.83	0.84	0.86	1.28	1.41	2.76	0.52	0.91	0.79
Kallundai kar	0.89	0.89	0.85	1.22	1.64	4.02	0.60	0.85	0.73
Chinna punchai	0.84	0.85	0.85	1.21	1.50	2.21	0.61	0.71	0.54
Uppumilagai	0.83	0.85	0.84	1.34	1.60	2.22	0.57	0.67	0.59
IR64	0.85	0.81	0.85	1.32	1.64	1.78	0.51	0.68	0.54

[a] Salt tolerance index was defined as the observations under salt stress divided by the means of the controls.

Table 2. Pearson correlation coefficients among physiological parameters from rice genotypes under salt stress

Parameters	**RWC**	**CSI**	**MSI**	**SOD**	**H_2O_2**	**Proline**	**Chl a**	**Chl b**	**Total Chl**
RWC	1								
CSI	0.842**	1							
MSI	0.660*	0.786**	1						
SOD	0.424	0.604*	0.560*	1					
H_2O_2	0.709**	0.842**	0.789**	0.599*	1				
Proline	0.825**	0.889**	0.758**	0.521*	0.756**	1			
Chl a	0.819**	0.888**	0.897**	0.557*	0.895**	0.890**	1		
Chl b	0.558*	0.796**	0.830**	0.441	0.697**	0.509	0.756**	1	
TC	0.714**	0.928**	0.893**	0.626*	0.850**	0.754**	0.873**	0.887**	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).