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Consequences of salt stress on chlorophyll pigments of rice genotypes

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Abstract
Soil salinity seriously threatens rice production worldwide, particularly in coastal regions. Improving screening methodologies and identification of tolerant traits for improved breeding for salt-tolerant rice is of enduring importance. Rice genotypes of the Konkan region varying in salt tolerance, such as tolerant Panvel-1 and sensitive Karjat-4 were grown in coastal saline soil of EC 3 dS/m and additional NaCl was imposed to achieve 6 and 9 dS/m under a controlled environment. The study aimed to examine the impact of salt stress on chlorophyll pigments during the reproductive stage. It was found that Chlorophyll a, and chlorophyll b significantly diminished due to salinity in both genotypes. However, pigments were more drastically reduced in sensitive Karjat-4. The study concluded that salt stress has a severe impact on photosynthetic pigments. Identifying such traits that can be proven as tolerant against salinity is crucial for the physiological phenotyping of coastal rice genotypes and ultimately for the crop improvement programs.

Keywords: Rice, coastal salinity, chlorophyll content, salt stress, phenotyping

1. Introduction
Salinity is a major abiotic stress that severely hampers the growth, development, and productivity of rice crops. Saline soils are categorized by an excess of sodium ions with higher electrical conductivity (>4 dS/m) (Ali *et al.*, 2013) [1]. Over half of the world's population is sustained by rice, which is mostly produced in Asia, Africa, and South America (Joseph *et al.*, 2010) [5]. However, rice productivity in coastal areas is affected by the intrusion of seawater into nearby areas. In general, salinity stress initially induces osmotic stress and subsequently toxicity of ions into tissues. Additionally, excessive reactive oxygen species (ROS), which are frequently generated at a high rate and accumulate in plant tissues as a result of ionic imbalances and hyperosmotic stressors, can cause damage. An imbalance of ion homeostasis, i.e. high Na+/K+ or low K+/Na+ ratios has a negative effect on cellular metabolism and physiology, thus adversely ruining the photosynthetic apparatus including the chlorophyll pigments. Adaptation of various physiological, biochemical, and molecular mechanisms to exclude excess Na+ ions are the basic strategies of crop plants to withstand salt stress.
Rice is generally characterized as salt salt-sensitive but the extent of its sensitivity varies during different growth and developmental stages. It is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early vegetative and reproductive stages (Zhu *et al.*, 2001) [11]. Evaluation and comparison of growth and physiological traits of rice genotypes such as photosynthetic pigments are considered useful way to better understand physiological changes during the development of rice varieties grown on salt-affected coastal soils. The present study was conducted to investigate changes in photosynthetic pigments during panicle initiation subjected to salinity stress in two well-known Konkan rice varieties that differ in their level of salt tolerance. The information generated might be brought to use to aid the relative field performance of various rice genotypes and characterize physiological features that could be utilized as reliable indicators for breeding and selection for salt tolerance.

2. Material and Methods
2.1 Location
The present investigation was conducted at ICAR – National Institute of Abiotic Stress Management, Malegaon. Geographically, Institute is situated at 18° 09' 30.62''N; 74° 30' 03.08''E; MSL 570 m at Malegaon Khurd, Baramati in Pune district of Maharashtra state.

2.2 Experimental details

Two rice genotypes were used for the pot experiment i.e. salt tolerant - Panvel-1 and salt-intolerant - Karjat-4. The experiment was carried out under controlled conditions in pots (coastal saline soil) in Factorial Randomized Block Design with two factors and four replications, Factor A – Genotypes and Factor B- Salinity treatments.

2.3 Imposition of salt stress

Three salt treatments were used for the experiments, 3 dS/m (Actual EC of soil, without salt stress), 6 dS/m (moderate), and 9 dS/m (high). Salt stress was imposed at panicle initiation stage by adding pure NaCl into the soil through watering. For comparison, control plants were also grown at 3 dS/m (in the absence of NaCl application).

2.4 Estimation of chlorophyll content (mg/g fresh weight)

Chlorophyll content from leaves was estimated by using Dimethyl sulphoxide (DMSO). 25 mg of leaf sample was dipped in 5 ml DMSO overnight and then absorbance was recorded at 649 and a 665 nm wavelength in a spectrophotometer and running DMSO as blank. Chlorophyll pigments were calculated using the following formulae.

Chlorophyll a= 14.85 (A_{665}) x 5.14 (A_{649})
Chlorophyll b= 25.48 (A_{649}) x 5.14 (A_{665})

2.5 Statistical analysis

Statistical analysis of the data obtained during the course of the investigation was carried out by using the standard statistical analysis method of analysis of variance by Panse and Sukhatme 1987 [9].

3. Results

Measurements of the chlorophyll content of two rice genotypes at the panicle initiation stage were started a day before imposing salt stress and made at 5-day intervals. Almost all of the plants at 9 dS/m died after ten days of treatment. After that, up to 20 DAT, data was acquired only for two treatments. Hence, significant results reported for chlorophyll content up to 10 DAT are included in the data that is presented below.

3.1 Chlorophyll ‘a’ (mg/g FW)

Chlorophyll ‘a’ has been employed to evaluate the impacts of salinity stress on photosynthetic efficiency. After five days of salt stress, a significant impact of salt stress on Chl ‘a’ was seen (Table 1). It was lesser in the salt-treated condition than in the control (Fig. 1a). Up until the final day of observation, Chl ‘a’ under the control condition was 1.91 mg/g FW. At moderate stress, it was drop down to 1.63 mg/g FW (by 8.33%), whereas at high salt stress, Chl ‘a’ content was 0.440 mg/g FW, falling by 76.93%.

At 10 DAT, a significant variation between the two genotypes was seen in relation to Chl ‘a’ content (Table 1). It was higher in Panvel-1 (1.46 mg/g FW) than in Karjat-4 (1.19 mg/g FW) (Fig. 1b).

The salt stress x genotype interaction was non-significant for the Chlorophyll ‘a’ content.

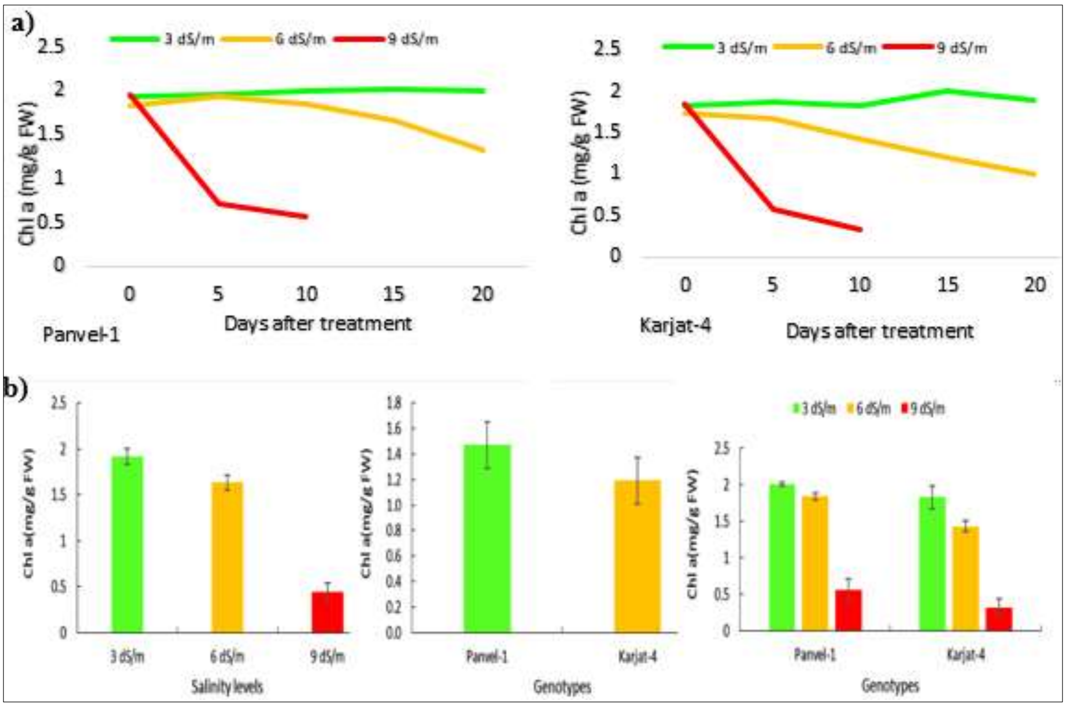

Fig 1: Effect of salt stress on Chl ‘a’ content of rice genotypes a) over a time period and b) at 10 DAT

3.2 Chlorophyll ‘b’ (mg/g FW)

Results exhibited a lesser amount of Chl ‘b’ in the salt-treated condition than in the control (Fig. 2a). Comparing the percentage decrease in salt-treated plants, it was found that after 10 days of treatment, high salt stress reduced the more Chl ‘b’ (by 57.83%) than moderate salt stress. While at moderate stress, 1.23 mg/g FW of Chl ‘b’ was noted prior to treatment which declined to 0.972 mg/g FW, which accounted for a 20% reduction (Fig. 2b, Table 1).

With regard to Chl ‘b’ content, no significant difference between the two genotypes was obtained during the experiment (Table 1).

The salt stress x genotype interaction was non-significant for the Chlorophyll ‘b’ content.

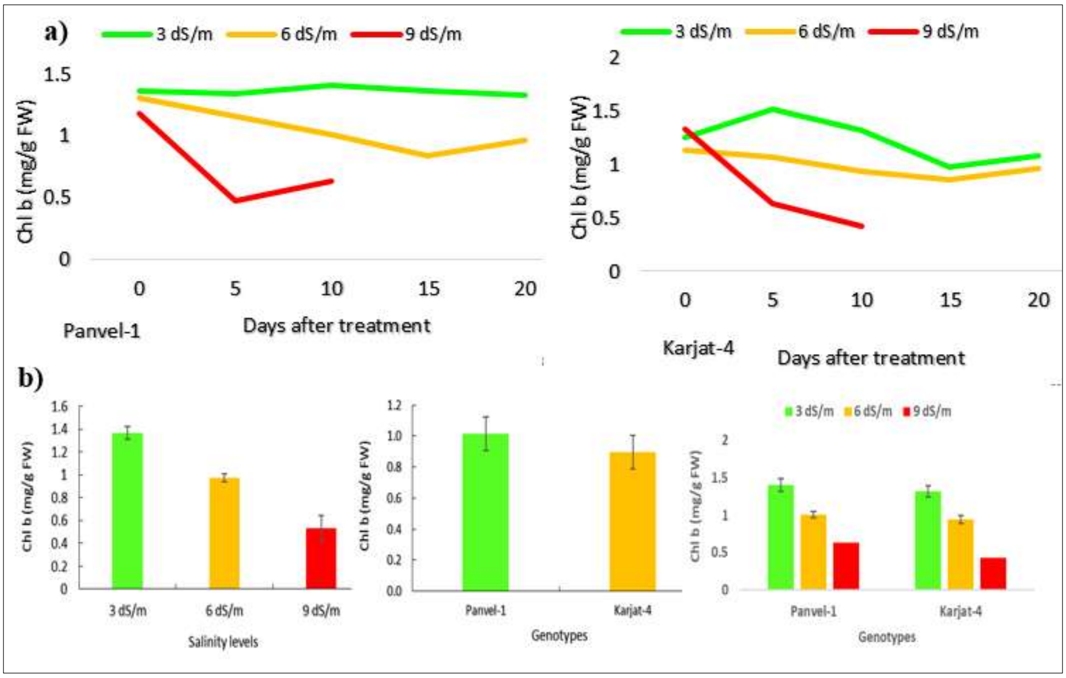

Fig 2: Effect of salt stress on Chl 'b' content of rice genotypes a) over a time period and b) at 10 DAT

Table 1: Effect of salt stress on chlorophyll content of rice genotypes at panicle initiation stage

		Chlorophyll 'a'			Chlorophyll 'b'		
Salinity levels		**00DAT**	**05DAT**	**10DAT**	**00DAT**	**05DAT**	**10DAT**
S_0	3 EC	1.881	1.918	1.916	1.303	1.429	1.362
S_1	6 EC	1.785	1.799	1.636	1.223	1.107	0.972
S_2	9 EC	1.907	0.647	0.440	1.256	0.554	0.530
S.E (m)±		0.049	0.078	0.077	0.093	0.050	0.085
C.D at 5%		0.14 NS	0.229	0.226	0.274 NS	0.147	0.250
C.D at 1%		0.19 NS	0.312	0.309	0.374 NS	0.201	0.341
Genotypes							
G-01	Panvel-1	1.912	1.538	1.469	1.284	0.992	1.015
G-02	Karjat-4	1.803	1.371	1.192	1.237	1.068	0.894
S.E (m)±		0.040	0.063	0.063	0.076	0.041	0.069
C.D at 5%		0.11 NS	0.18 NS	0.185	0.224 NS	0.120 NS	0.20 NS
C.D at 1%		0.16 NS	0.25 NS	0.252	0.305 NS	0.163 NS	0.27 NS
Interaction effect							
S_0: 3EC	Panvel-1	1.941	1.964	2.006	1.363	1.346	1.407
S_0: 3EC	Karjat-4	1.821	1.872	1.826	1.244	1.512	1.318
S_1: 6EC	Panvel-1	1.833	1.937	1.842	1.312	1.154	1.006
S_1: 6EC	Karjat-4	1.737	1.662	1.430	1.134	1.060	0.939
S_2: 9EC	Panvel-1	1.963	0.714	0.559	1.178	0.475	0.633
S_2: 9EC	Karjat-4	1.851	0.581	0.320	1.334	0.633	0.427
S.E (m)±		0.069	0.110	0.108	0.132	0.071	0.120
C.D at 5%		0.20 NS	0.32 NS	0.32 NS	0.388 NS	0.208 NS	0.353 NS
C.D at 1%		0.27 NS	0.44 NS	0.43 NS	0.529 NS	0.283 NS	0.48 S

4. Discussion

Leaf chlorosis is one of the impacts of salt stress, resulting from the reduction in photosynthetic pigments (Moradi and Ismail, 2007) [8]. Salt stress causes chlorophyll breakdown, which alters electron transport during photosynthesis, carbon metabolism, and photophosphorylation. Salt-sensitive plants revealed substantial chlorophyll degradation and growth inhibition during salt stress (Moradi and Ismail, 2007) [8].

In this experiment, photosynthetic pigments, i.e. chlorophyll a, and chlorophyll b content were significantly reduced in response to salt stress imposed at panicle emergence. The salt stress of 6 dS/m had comparatively less reduction in chlorophyll a and chlorophyll b content than high salt stress (9 dS/m). The degradation of photosynthetic pigments was more severe in Karjat-4 than the Panvel-1 (with the exception of chlorophyll b which was non-significantly less in Karjat-4), suggesting the ability of this trait to differentiate the response of salt-tolerant and salt-sensitive genotypes. In the present study, salt stress had more impact on chlorophyll a than chlorophyll b, similar to the findings of Jamil *et al*. (2012) [4]. It is evident from the previous study that the retention of chlorophyll contents in the leaves of tolerant rice varieties was better than that of the sensitive varieties (Hakim *et al*., 2014; Kibria *et al*., 2017; Ma *et al*., 2018; Pongprayoon *et al*., 2019; Chakraborty *et al*., 2020) [3, 6, 7, 10, 2].

5. Conclusion

The investigation came to the conclusion that photosynthetic pigments are severely impacted by salt stress. Chlorophyll content was significantly reduced when exposed to moderate and high salt stress. The reduction was more severe on exposure to high salt stress (9 dS/m); however, the degradation of photosynthetic pigments was more severe in Karjat-4 than the Panvel-1. For the physiological phenotyping of coastal rice genotypes and, eventually, for crop development programmes, it is critical to identify such features that can be demonstrated to be tolerant against salinity.

6. Abbreviations

Chl-Chlorophyll, DAT- Days after treatment, EC- Electrical conductivity, FW- Fresh weight

7. References

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