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Variation in photosynthetic efficiency in response to salinity in rice genotypes targeted for Konkan coast

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Abstract

Rice grown in coast is highly prone to salinity. High salt in plant cell damages the membrane system and thylakoids in chloroplasts and hence affect photosynthetic performance. This can be quantified by assessing Photosystem-II (PS-II) efficiency. However, this technique has not been employed so far to assess salinity tolerance in rice genotypes adapted to salinity in Konkan Coast. Hence, PS-II efficiency in response to salinity, as a potential trait, was considered for optimising screening protocol that can accelerate breeding varieties for salt-affected areas. Experiment was conducted with 20 rice genotypes collected from Regional Agricultural Research Station, Karjat and Khar Land Research Station, Panvel of coastal area including checks as well-known salt tolerant and salt sensitive varieties. Plants were exposed to medium (MSS) and high (HSS) salt stress with electrical conductivity 6 and 9 dSm⁻¹ respectively with control (3 dSm⁻¹) (Coastal saline soil with no additional salts) 21 days after emergence. The PS-II efficiency (Fv/Fm) was measured at 6, 24 and 36 hours after imposing salt stress. There was a large variation in salinity tolerance among the rice germplasms as revealed by Fv/Fm. Among 20 rice genotypes CST 7-1, Kala rata, SR 3-9 and Damodar retained high Fv/Fm in medium salt stress condition (6 dSm⁻¹) and high salt stress condition (9 dSm⁻¹) as compared to check such as FL478 (salt tolerant) and Karjat 4 (salt sensitive) at different time intervals. This work reveals the potential of PS-II efficiency as a trait for differentiating the responses of rice genotypes to coastal salinity. This can help identify relevant genes essential to develop salt-tolerant varieties.

Keywords: Rice, salt, stress, PS-II efficiency

Introduction

Oryza sativa L. (2n=24), known as rice, is a popular self-pollinated and a model species for monocots and cereals belonging to the family Gramineae or Poaceae ("real grass"), which has 22 wild species and just two cultivated varieties (Vaughan *et al.*, 2003) [25]. Rice is grown throughout Asia, Africa, Australia, and Central and South America in humid tropical and subtropical climates. Rice is also categorized as a natural inbred crop, which can benefit plant breeding programmes significantly (Blair *et al.*, 2002) [6]. India ranks second in rice production, followed by China. In India, rice occupies an area of 45.76 million hectares with an annual production of 124.36 million tonnes, and productivity is 2.72 tons/ha (Anonymous, 2021) [4-5]. Konkan has a rice-dominating area and occupies about 0.387 million hectares with an annual production of around 1.031 million tonnes, and the average productivity of the Konkan is about 2.66 t/ha milled rice (Anonymous, 2021) [4-5].

In India, there are 6.73 million hectares of land affected by salt, and by 2050, that number is anticipated to rise to 16.2 million hectares (CSSRI Vision-2050, 2019; Singh, 2018) [8, 20]. More than 127000 ha of saline soil have been found in Maharashtra state. Out of these, 70,000-hectare land is classified as coastal salinity and 57000 ha land is classified as inland salinity. The coastal saline soils are fertile, but their productivity is limited due to the inundation of tidal brackish water and submergence during the rainy season (Sawardekar *et al.*, 2003) [19].

Natural disasters caused by climate change and environmental (biotic and abiotic) stresses represent a severe threat to the world's 60 per cent population's food security and economic development. It was estimated that the demand for rice in 2025 will be 140 million tonnes (Singh *et al.*, 2004) [21]. Contrarily, the abiotic stress of soil salinity is the subject of this study since it contributes significantly to the decline in global rice output. Notwithstanding, the improvement of salt tolerance of rice at the seedling stage is an important breeding goal in many Asian countries, where seedlings must often establish in soils already contaminated by

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salt. Screening at the field level proved difficult due to soil heterogeneity, climatic factors and other environmental factors that may influence the physiological processes. Hence, screening under laboratory conditions is considered advantageous over field screening. Screening of genotypes for salt tolerance at early stages may be necessary for screening salt tolerance as there is a considerable saving in time (Ali *et al.*, 2014) [2] and to develop salt-tolerant high-yielding cultivars. This work reveals the potential of PS-II efficiency as a trait for differentiating the responses of rice genotypes to coastal salinity. This can help identify relevant genes essential to develop salt-tolerant varieties.

Materials and Methods

Plant growth environment and plant materials

The present replicated experiment was conducted with 20 rice genotypes collected from the Regional Agricultural Research Station, Karjat and Khar Land Research Station, Panvel of the coastal area, including checks as well-known salt-tolerant and salt-sensitive varieties undertaken in collaboration with the school of water stress management, ICAR-National Institute of Abiotic Stress Management (NIASM), Malegaon, Baramati, 413115 during 2021-2022. The soil used in the experiment was coastal saline soil from the Konkan coast (Khar land research station, Panvel, Dr B.S.K.K.V., Dapoli). For the establishment of seedlings, pre-germinated seeds were transferred to plastic pots filled with normal soil medium for the seedling establishment for 15 days outside a greenhouse in open-air (natural) conditions. Only three uniform seedlings were transferred and kept in each pot containing 3.5 kg coastal saline soil from the Konkan coastal area with EC 3 dSm⁻¹ as a growth media. Plants were exposed to medium (MSS) and high (HSS) salt stress with electrical conductivity of 6 and 9 dS/m, respectively, with control (C) (Coastal saline soil with no additional salts) 21 days after emergence. The EC

was measured using a portable EC meter in a day interval. The experimental design was set up with saline and non-saline conditions in a Factorial Completely Randomized Design (FCRD) with three replicates. Temperatures in the greenhouse were maintained at 32/24 °C (day/night), 50–65 per cent relative air humidity, and 450–750 molm⁻²s⁻¹ PAR.

Method

At four different time intervals (0, 6, 24 and 36 Hrs), fully formed third leaves were sampled at the top of each replication. Samples were then brought to a dark room and acclimated and stabilized for one hour in the dark were collected, and chlorophyll fluorescence was measured on each piece using an imaging fluorometer (Handy FluorCam, P.S.I., Brno, Czech Republic), as reported in (Nedbal *et al.*, 2000) [15]. Fluorescence was detected using a high-sensitivity charge-coupled device camera operated by the FluorCam software (FluorCam version 1.2.5.3) (Plate 1). Variable fluorescence (Fv), which represents the distinction between Fm and Fo, was used to calculate the maximum photochemical efficiency as

$$PSII (Fv/Fm) = (Fm - F0)/Fm$$

Results

There was a significant variation in salinity tolerance among the rice germplasms, as revealed by Fv/Fm. The PS-II efficiency of all the genotypes was measured for different time intervals (0, 6, 24, 36 Hrs) after the imposition of salt stress. There was a gradual decrease in PS-II efficiency with the increase in NaCl up to 6 dSm⁻¹; however, up to 9 dSm⁻¹, there was a drastic decline in PS-II efficiency in all the genotypes. A significant difference was observed with increasing salinity stress over a distinct time and also noted a substantial difference in PS-II efficiency between treatments (Table 1, Fig. 1).

Table 1: Effect of salt stress on Fv/Fm of rice genotypes

Genotypes	PS II Efficiency (Fv/Fm)																
	0 Hrs. Before Treatment				6 Hrs. After Treatment				24 Hrs. After Treatment				36 Hrs. After Treatment				
	Interaction effect			Mean (Gen)	Interaction effect			Mean (Gen)	Interaction effect			Mean (Gen)	Interaction effect		Mean (Gen)		
	Salinity levels				Salinity levels				Salinity levels				Salinity levels				
3 EC	6 EC	9 EC	3 EC	6 EC	9 EC	3 EC	6 EC	9 EC	3 EC	6 EC	9 EC	3 EC	6 EC	9 EC			
Bhura rata	0.77	0.77	0.78	0.77	0.78	0.72	0.68	0.72	0.78	0.63	0.53	0.65	0.78	0.56	0.42	0.58	
CSR 27	0.76	0.77	0.77	0.76	0.77	0.71	0.66	0.71	0.77	0.63	0.53	0.64	0.77	0.53	0.32	0.54	
CSR 36	0.80	0.79	0.79	0.79	0.79	0.77	0.77	0.78	0.80	0.72	0.72	0.75	0.79	0.65	0.65	0.70	
CST 7-1	0.81	0.80	0.82	0.81	0.81	0.79	0.79	0.80	0.80	0.75	0.75	0.77	0.81	0.71	0.71	0.75	
Damodar	0.81	0.81	0.80	0.81	0.81	0.79	0.79	0.79	0.80	0.74	0.74	0.76	0.81	0.68	0.67	0.72	
FL 478	0.81	0.82	0.81	0.81	0.80	0.78	0.78	0.79	0.80	0.73	0.74	0.76	0.81	0.68	0.68	0.72	
Kala rata	0.82	0.81	0.82	0.82	0.82	0.80	0.80	0.80	0.82	0.74	0.75	0.77	0.81	0.69	0.69	0.73	
Karjat 184	0.79	0.80	0.80	0.80	0.80	0.74	0.74	0.76	0.79	0.68	0.65	0.71	0.80	0.63	0.53	0.65	
Karjat 3	0.74	0.74	0.73	0.73	0.72	0.65	0.57	0.65	0.73	0.53	0.38	0.54	0.72	0.43	0.21	0.45	
Karjat 4	0.76	0.77	0.75	0.76	0.76	0.68	0.62	0.69	0.75	0.53	0.42	0.57	0.76	0.44	0.30	0.50	
Karjat 6	0.71	0.71	0.71	0.71	0.71	0.65	0.53	0.63	0.71	0.41	0.32	0.48	0.70	0.37	0.14	0.40	
Karjat 8	0.72	0.72	0.72	0.72	0.73	0.65	0.57	0.65	0.72	0.44	0.43	0.53	0.72	0.37	0.21	0.43	
Khara muga	0.77	0.77	0.78	0.77	0.77	0.72	0.67	0.72	0.77	0.62	0.53	0.64	0.77	0.55	0.33	0.55	
Panvel 1	0.79	0.81	0.80	0.80	0.80	0.75	0.73	0.76	0.80	0.71	0.67	0.73	0.80	0.65	0.61	0.69	
Panvel 2	0.78	0.79	0.79	0.79	0.78	0.73	0.68	0.73	0.78	0.65	0.53	0.66	0.78	0.57	0.43	0.59	
Panvel 3	0.79	0.79	0.79	0.79	0.79	0.73	0.68	0.73	0.79	0.68	0.58	0.68	0.78	0.59	0.53	0.63	
Panvel 61	0.79	0.79	0.79	0.79	0.79	0.73	0.71	0.74	0.79	0.65	0.57	0.67	0.78	0.58	0.44	0.60	
Ratnagiri 5	0.76	0.76	0.76	0.76	0.75	0.66	0.64	0.68	0.75	0.57	0.50	0.61	0.76	0.46	0.22	0.48	
Ratnagiri 6	0.76	0.77	0.76	0.76	0.76	0.68	0.65	0.70	0.76	0.58	0.51	0.62	0.77	0.53	0.31	0.54	
SR 3-9	0.80	0.80	0.81	0.80	0.80	0.80	0.80	0.80	0.81	0.74	0.74	0.77	0.81	0.70	0.70	0.74	
Mean (Sal)	0.78	0.78	0.78		0.78	0.73	0.69		0.78	0.64	0.58		0.78	0.57	0.46		
		S.E (m)±	C.D at 5%	C.D at 1%		S.E (m)±	C.D at 5%	C.D at 1%		S.E (m)±	C.D at 5%	C.D at 1%		S.E (m)±	C.D at 5%	C.D at 1%	
Factor A: Salinity levels	0.001	0.002	NS	0.003	NS	Fac. A	0.001	0.003	0.004	Fac. A	0.001	0.003	0.004	Fac. A	0.001	0.003	0.005

Factor B: Genotypes	0.002	0.006	0.007	Fac. B	0.002	0.007	0.009	Fac. B	0.003	0.008	0.011	Fac. B	0.003	0.009	0.012
Interaction effect (A x B)	0.003	0.010	0.013	A x B	0.004	0.012	0.016	A x B	0.005	0.015	0.019	A x B	0.005	0.015	0.020

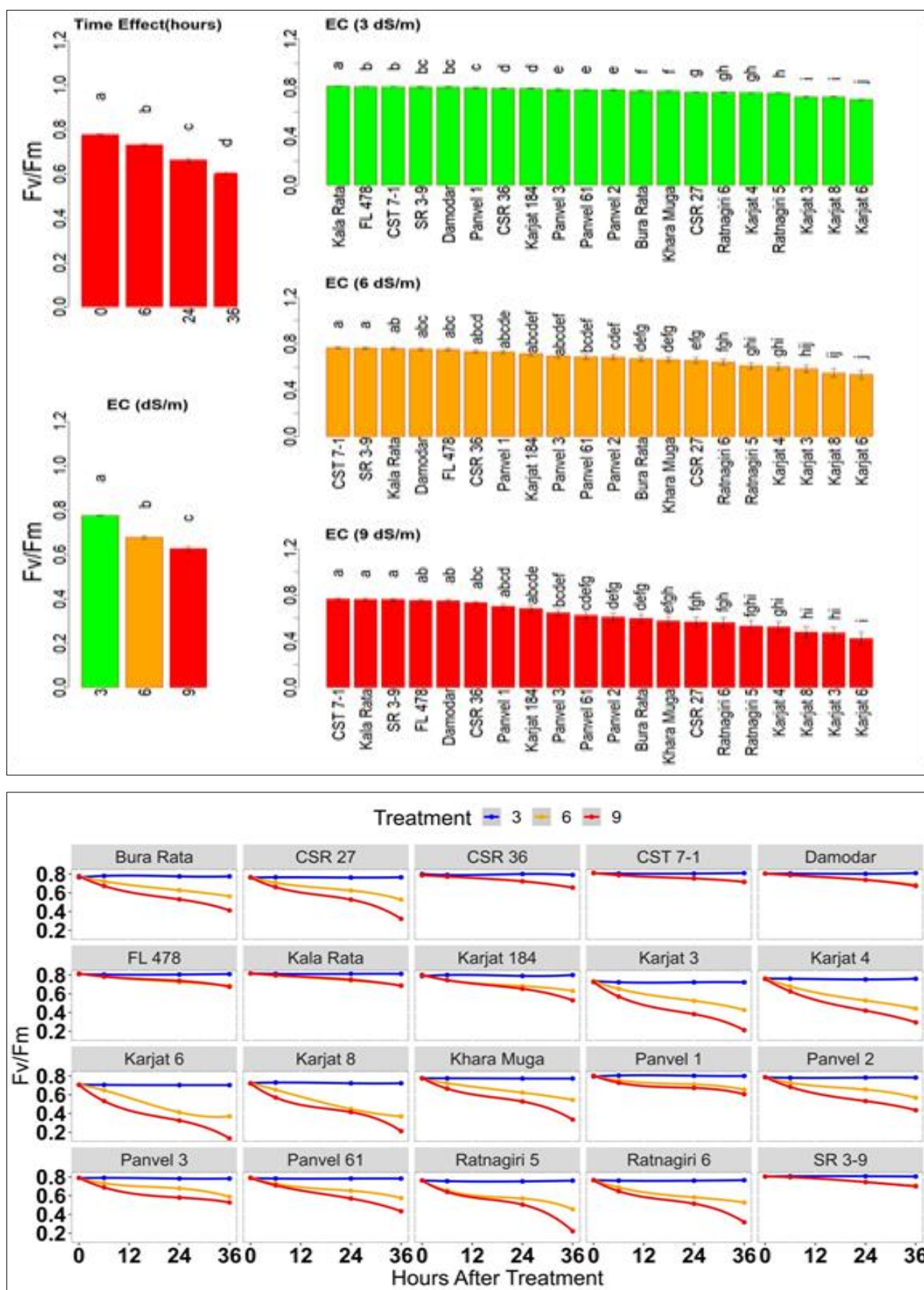


Fig 1: Effect of salt stress on PS II efficiency (Fv/Fm) of rice genotypes

On average, the PS-II efficiency of all the genotypes started to decline after the imposition of salt treatments. Among the treatments, 9 dSm-1 severely impacted the PS-II efficiency of all the genotypes (Fig. 1).

Before the imposition of salt stress, i.e. at zero Hrs. No significant difference was found in PS-II efficiency, with a mean of 0.78. There was a significant genotypic variation observed between all the genotypes. Among the genotypes, Kala rata, CST 7-1, and Damodar had better PS-II efficiency, including salt tolerant check FL 478 ranging to 0.81-0.82 compared to salt-sensitive check Karjat 4 (0.76). A significant treatment x genotype interaction for PS-II efficiency was observed across all genotypes. At 3 dSm-1, Kala rata, CST 7-1, Damodar and FL 478 had a significant maximum PS-II efficiency of approximately 0.81-0.82 than the rest of the genotypes; however, there was no significant difference observed between these genotypes. Among the genotypes, Karjat 6 (0.71) had significantly lower PS-II efficiency than the check FL 478 (0.81) and Karjat 4 (0.76). At 6 dSm-1 and 9 dSm-1, a similar treatment x genotype interaction trend was observed as in 3 dSm-1 (Table 1, Fig. 1).

At 6 Hrs after imposition of salt treatment, plants maintained their PS-II efficiency significantly at 3 dSm-1, 6 dSm-1 and 9 dSm-1, which exhibited 0.78, 0.73 and 0.69, respectively. There was a significant genotypic variation observed between all the genotypes. Among the genotypes Kala rata, CST 7-1 and SR 3-9 had significantly higher PS-II efficiency of approximately 0.80, followed by Damodar and the minimum PS-II efficiency was observed in Karjat 6 (0.63) to that of check FL 478 (0.79) and Karjat 4 (0.69). A significant treatment x genotype interaction for the PS-II efficiency was observed across all genotypes. At 3 dSm-1, a similar trend of genotypes was observed at zero Hrs before the imposition of salt treatment with the same PS-II efficiency range. At 6 dSm-1, Kala rata and SR 3-9 had a significantly higher PS-II efficiency of approximately 0.80, followed by CST 7-1 and Damodar. Among the genotypes, Karjat 3, Karjat 6 and Karjat 8 showed minimum PS-II efficiency of around 0.65 compared to check FL 478 (0.78) and Karjat 4 (0.68). At 9 dSm-1, a similar trend of genotypes was observed as in 6 dSm-1 for maximum PS-II efficiency, where, Karjat 6 (0.53) showed minimum PS-II efficiency (Table 1, Fig. 1).

The PS-II efficiency of plants was gradually impacted 24 hours after the application of salt treatment and considerably varied at 3 dSm-1, 6 dSm-1, and 9 dSm-1, exhibiting 0.78, 0.64, and 0.58, respectively. Between each genotype, a significant genotypic variance was found. In comparison to check FL 478 (0.76) and Karjat 4 (0.57), Kala rata, CST 7-1, and SR 3-9 had much greater PS-II efficiency of around 0.77, followed by Damodar, and Karjat 6 had the lowest PS-II efficiency of 0.48. For the PS-II efficiency, a significant treatment x genotype interaction was found across all genotypes. At 3 dSm-1, maximum PS-II efficiency was observed in Kala rata (0.82) and SR 3-9 (0.81), followed by CST 7-1 and Damodar, where minimum PS-II efficiency was observed in Karjat 6 (0.71). At 6 dSm-1, CST 7-1 had a significantly highest PS-II efficiency of approximately 0.75, followed by Kala rata, SR 3-9 and Damodar. However, Karjat 6 showed minimum PS-II efficiency of around 0.41 compared to check FL 478 (0.73) and Karjat 4 (0.53). At 9 dSm-1, Kala rata and CST 7-1 had maximum PS-II efficiency of approximately 0.75, followed by Damodar and SR 3-9 to that of check FL 478 (0.74). However, Karjat 6 (0.32) showed minimum PS-II efficiency compared to check Karjat 4 (0.42)

(Table 1, Fig. 1).

The PS-II efficiency of plants steadily dropped and considerably varied at 3 dSm-1, 6 dSm-1, and 9 dSm-1, exhibiting mean 0.78, 0.57 and 0.46, respectively, 36 hours after salt treatment imposition. A significant genotypic variation was observed between the genotypes. Kala rata (0.73), CST 7-1 (0.75) and SR 3-9 (0.74) had significantly higher PS-II efficiency, followed by Damodar, and the minimum PS-II efficiency was observed in Karjat 6 (0.40) to that of check FL 478 (0.72) and Karjat 4 (0.50). A significant treatment x genotype interaction was observed across all genotypes for the PS-II efficiency. At 3 dSm-1, Kala rata, SR 3-9, CST 7-1, and Damodar showed maximum PS-II efficiency of about 0.81, whereas Karjat 6 showed the lowest PS-II efficiency of 0.70. At 6 dSm-1, CST 7-1 (0.71), SR 3-9 (0.70), and Kala rata (0.69) all had PS-II efficiency that was noticeably greater than check FL 478 (0.68), followed by Damodar. However, Karjat 6 and Karjat 8 had lower PS-II efficiency of 0.37 compared to check Karjat 4 (0.44). At 9 dSm-1, CST 7-1 (0.71), SR 3-9 (0.70) and Kala rata (0.69) had maximum PS-II efficiency, and Karjat 6 showed minimum PS-II efficiency as compared to check FL 478 and Karjat 4 (Table 1, Fig. 1).

Discussion

Photosynthesis, the most important physiological process of plants, is highly susceptible to salinity stress, and it can be significantly studied by measuring the PSII characteristics via analyzing the Chlorophyll fluorescence parameters. The effects of salinity stress on target sites, such as components of photosynthetic electron transport, can be detected and analyzed. Concerning the effect of NaCl stress on the photosynthetic electron transport and photosynthetic efficiency, the variation in Chlorophyll fluorescence parameters may be due to the inhibition of electron transport or damage to the donor side or acceptor side of PSII. However, the fundamental question remains about how well Fv/Fm describes the health status of a plant. Generally, values of Fv/Fm between 0.78 and 0.83 are a sign of healthiness, yet sometimes it cannot be guaranteed that values of Fv/Fm between these two limits would give a perfect assumption of a plant's health. It is one of the most influential and comprehensive parameters related to plant vitality, and it was drastically reduced when rice seedlings were subjected to salinity stress due to inactive reaction centres and a reduction in the electron transfer from QA-. These parameters are used to understand the general health of a plant in regular as well as stressful environments (Stirbet and Govindjee 2011) [23]. Our results showed a significant decrease in PSII efficiency on an absorption basis in rice leaves after exposure to salt stress. The data obtained from Chlorophyll fluorescence analysis revealed that some fluorescence parameters could be selectively used for identifying and evaluating the effect of high NaCl stress in rice seedlings. Fv/Fm (1-F0/Fm) is a ratio of F0 and Fm, and any alteration of these two parameters in the same direction is not likely to change the balance, though there may be significant PSII damage (Singh and Sarkar 2014) [22], as occurred in the present investigation. The results from the current study strongly indicate that these chosen Chlorophyll fluorescence parameters could be used as a tool for identifying a particular genotype's tolerance to salt stress even in the earlier growth stages of rice seedlings. It has been observed that the tolerant cultivars maintain a high Fv/Fm ratio than the susceptible ones. Many authors have reported

that it is the best parameter to investigate the photosynthetic efficiency and the effects of salinity stress on PSII efficiency in different plants, e.g., NaCl stress in rice Moradi and Ismail (2007) [13]; in wheat by Abdeslahian *et al.* (2010) [1]; Kanwal *et al.* (2011) [10]; Oyiga *et al.* (2016) [16]. Similar findings were also reported by Amirjani, M. R. (2012) [18]; Morales *et al.* (2012) [14]; Lee *et al.* (2013) [12]; Sarkar *et al.* and Wankhade *et al.* (2013) [18, 26]; Kordrostami *et al.* (2017) [11]; Pradhan *et al.* (2018) [17]; Tsai *et al.* (2019) [24]; Chakraborty *et al.* (2020) [7] in rice.

Conclusion

Understanding the salt tolerance mechanisms and the assessment of salt tolerance is expected to be much more well-defined by screening the genotypes with different salinity levels at the physiological (PS II efficiency) basis at the seedling stage, as undertaken in the present research. Among 20 rice genotypes, CST 7-1, Kala rata, SR 3-9, and Damodar retained high Fv/Fm in medium salt stress condition (6 dSm⁻¹) and high salt stress condition (9 dSm⁻¹) as compared to checks such as FL478 (salt tolerant) and Karjat 4 (salt sensitive). This work reveals the potential of PS-II efficiency as a trait for differentiating the responses of rice genotypes to coastal salinity. This can help identify relevant genes essential to develop salt-tolerant varieties.

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