

RESEARCH ARTICLE

## Novel Rice (*Oryza sativa* L.) Genotypes Tolerant to Combined Effect of Submergence and Salt Stress

**Nibedita Prusty<sup>#</sup>, Bhubaneswar Pradhan<sup>#</sup>, Deepa, Krishnendu Chattopadhyay, Bhaskar Chandra Patra and Ramani Kumar Sarkar<sup>\*</sup>**

ICAR-National Rice Research Institute, Cuttack–753 006, Odisha, India.

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The implications of both complete and partial submergence with saline water on growth and survival of different rice genotypes were investigated. The study showed that among the 199 genotypes, only five genotypes namely IC-459744, IC-469712, IC-464746, IC-459649 and IC-17024 were tolerant to both complete (plants under water) and partial submergence (water depth 45±5 cm) with saline water (12 dS m<sup>-1</sup>). The association of Chlorophyll a, Chlorophyll b, total Chlorophyll, normalized difference vegetative index (NDVI) and photochemical reflectance index (PRI) with score under partial submergence with salt stress were highly negatively significant ( $p < 0.001$ \*\*\*). Biplot derived from principal component analysis showed that accessions such as IC-459744 and IC-459649, tolerant to both complete and partial submergence with salty water were in the same quarter where PRI was located with long vertex. The genotypes identified in this investigation have great potential as unique genetic resources for utilization in crop improvement programmes.

**Key Words:** Coastal area, Genetic resource, *Oryza sativa*, Submergence-salt stress combination, Tolerant genotypes

### Introduction

Rice is adapted to various agro-climatic zones starting from favourable irrigated condition to highly unfavourable harsh environments. It is grown at altitudes ranging from below sea level (Kuttanad, Kerala, India) to 2761 m above sea level (Jumulla valley, Nepal), thus making its presence across environments (Chang, 2000). This adaptation to extremely variable conditions in rice offers a hope to mitigate the current challenges posed by variable abiotic stresses, as-well-as means to cope with the adverse effects of climate change, to secure food and livelihood (Das *et al.*, 2004; Wassman *et al.*, 2009; Mackill *et al.*, 2010; Waziri *et al.*, 2016; Ray *et al.*, 2017). Rice genetic resources are vast, yet a fraction of it has been utilized for crop improvement programme, it is mainly due to the lack of knowledge on beneficial attributes of landraces (Glaszmann *et al.*, 2010; Sarkar and Bhattacharjee, 2011; Singh and Sarkar, 2014; Kuanar *et al.*, 2017). By tapping the genetic resources, development of climate resilient rice varieties is possible. This is because genes associated with tolerance of various abiotic stresses are available within the cultivated rice gene pool (Ismail *et al.*, 2007; Sarkar *et al.*, 2009; Reddy *et al.*, 2009; Thomson *et al.*, 2010).

Farmers of coastal areas of eastern India still grow landraces that are photoperiod-sensitive, less-responsive to fertilisers and prone to lodging with limited yield potential and poor grain quality (Sarkar and Bhattacharjee, 2011). These landraces are frequently encountered by complete and partial submergence with or without saline water (Mahata *et al.*, 2010; Yan *et al.*, 2013). Therefore, the main aim of this investigation was to identify rice germplasm tolerant to complete and partial submergence along with salt stress. The germplasm lines showing tolerance to submergence under saline environment were tested further under partial submergence with saline water. The rice germplasm accessions thus identified through this study will be useful in developing high yielding rice varieties, which can tolerate both complete and partial submergence under saline environment. Due to complexity in evaluation of partial submergence with salt stress, additional physiological parameters were taken in categorizing the tolerance level. The association studies of some physiological parameters with plant performance under partial submergence with saline water were useful in identifying physiological indicator.

<sup>\*</sup>Author for Correspondence: Email- rksarkarcrri@gmail.com,

## Materials and Methods

### ***Evaluation of Rice Germplasm for Submergence Tolerance under Saline Environment***

One hundred and ninety nine cultivars were tested for submergence tolerance under saline water. Two cultivars namely Swarna (susceptible to submergence) and FR13A (tolerant to submergence both under saline and non-saline water) were used as checks (Sarkar and Ray, 2016). Seeds were sown directly in earthenware pots containing 2 Kg of farm soil and farmyard manure in a 3:1 ratio. Each pot was supplied with 192 mg single super phosphate ( $P_2O_5$ ) and 80 mg murate of potash ( $K_2O$ ). Ten days after germination, seedlings were thinned and ten plants per pot were maintained. Urea @ 100 mg was applied in each pot after thinning. The pots were placed inside the cemented tanks (2 m × 1 m × 1.2 m) after 20 days of growth. Complete submergence was provided with salty water (12 dS  $m^{-1}$ ) for 15 days. The depth of water was 100 cm. Plant height was taken before and after submergence. Survival count was taken after 10 days of de-submergence. The following formulas were used to calculate elongation and survival percent.

Elongation (%) =  $\{(Plant\ height\ after\ submergence - Plant\ height\ before\ submergence) / (Plant\ height\ before\ submergence)\} * 100$ .

Survival (%) =  $\{(Plant\ number\ before\ submergence - Plant\ number\ after\ 10\ days\ of\ de-submergence) / (Plant\ number\ before\ submergence)\} * 100$ .

### ***Evaluation of Submergence Tolerant Rice Germplasm for Medium Depth (~50 cm) Partial Submergence under Saline Environment***

The experiment was conducted with sixty six rice cultivars. Three cultivars such as FR13A (susceptible to salinity but tolerant to both complete and partial submergence), Varshadhan (susceptible to salinity but tolerant to partial submergence) and Rashpanjor (tolerant to both salinity and partial submergence) were used as checks (Sarkar *et al.*, 2013; Sarkar and Ray, 2016). Complete submergence is more detrimental than partial submergence (personal observation). Therefore, greater emphasis was given in selection of accessions for further testing under partial submergence with salt stress. All the accessions which showed  $\geq 60\%$  survival were selected for the study leaving behind all the accessions which showed 100% mortality under complete submergence with salt stress. A set of 63 accessions was prepared

with mostly tolerant to highly submergence tolerant type materials to test for partial submergence with salt stress. Plants were grown as described above; however, after 30 days of growth another dose of urea @ 100 mg  $pot^{-1}$  was applied. Partial submergence (depth of water 45 cm) with salinity ( $12.0 \pm 0.2$  dS  $m^{-1}$ ) was imposed on forty five days old seedlings (Sarkar and Ray, 2016). Concentration of salt was checked in alternate days. The depth of water and salt concentration were maintained also in alternate days. Scoring was done after 10 and 15 days of treatment following 1-9 scale as follows:

Score 1: No apparent damage – highly tolerant, Score 3: Little damage of leaf, healthy stem – tolerant, Score 5: Greater damage of leaf, less damage of stem – medium tolerant, Score 7: Greater damage of both leaf and stem – susceptible, and Score 9: Almost dead – highly susceptible.

### ***Measurement of Normalized Difference Vegetation Index (NDVI) and Photochemical Reflectance Index (PRI)***

The NDVI and PRI readings were taken from 2<sup>nd</sup> leaf from top using Plant Pen NDVI 300 and PRI 200 (Photon System Instruments, Czech Republic), respectively according to the following formula after 10 days of partial submergence with saline water.

NDVI (Normalized Difference Vegetative Index) =  $(NIR760 - VIS635) / (NIR760 + VIS635)$

PRI (Photochemical Reflectance Index) =  $(R525 - R592) / (R525 + R592)$

### ***Chlorophyll Estimation***

After taking PRI and NDVI reading, same leaf was used for the measurement of total chlorophyll (Chl) content, which comprised both Chl *a* and Chl *b*. Three leaves were pooled together, finely chopped and mixed thoroughly. One hundred mg chopped leaf was placed in a capped measuring tube containing 10 mL of pre-chilled 80% acetone, and placed inside a refrigerator (4°C) for 48 h. The chlorophyll was measured spectrophotometrically at 646.6 and 663.6 nm following Sarkar and Ray (2016).

Chl *a* and Chl *b* are calculated using the following equations:

Chl *a* (mg  $g^{-1}$  fresh weight) =  $\{12.25 \times (A_{663.6}) - 2.25 \times (A_{646.6})\} \times 10 / Sample\ weight\ (g) \times 1/1000$

Chl *b* (mg g<sup>-1</sup> fresh weight) = {20.31 × (A<sub>646.6</sub>) – 4.91 × (A<sub>663.6</sub>)} × 10 / Sample weight (g) × 1/1000

### Statistical Analysis

Statistical analysis for different parameters was done using the *CropStat* software (International Rice Research Institute, Philippines). Mean values were compared by the least significant difference (LSD<sub>p</sub>\* < 0.05), wherever the *F*-test was significant. Associations among different parameters were examined by simple correlation analysis using the same package.

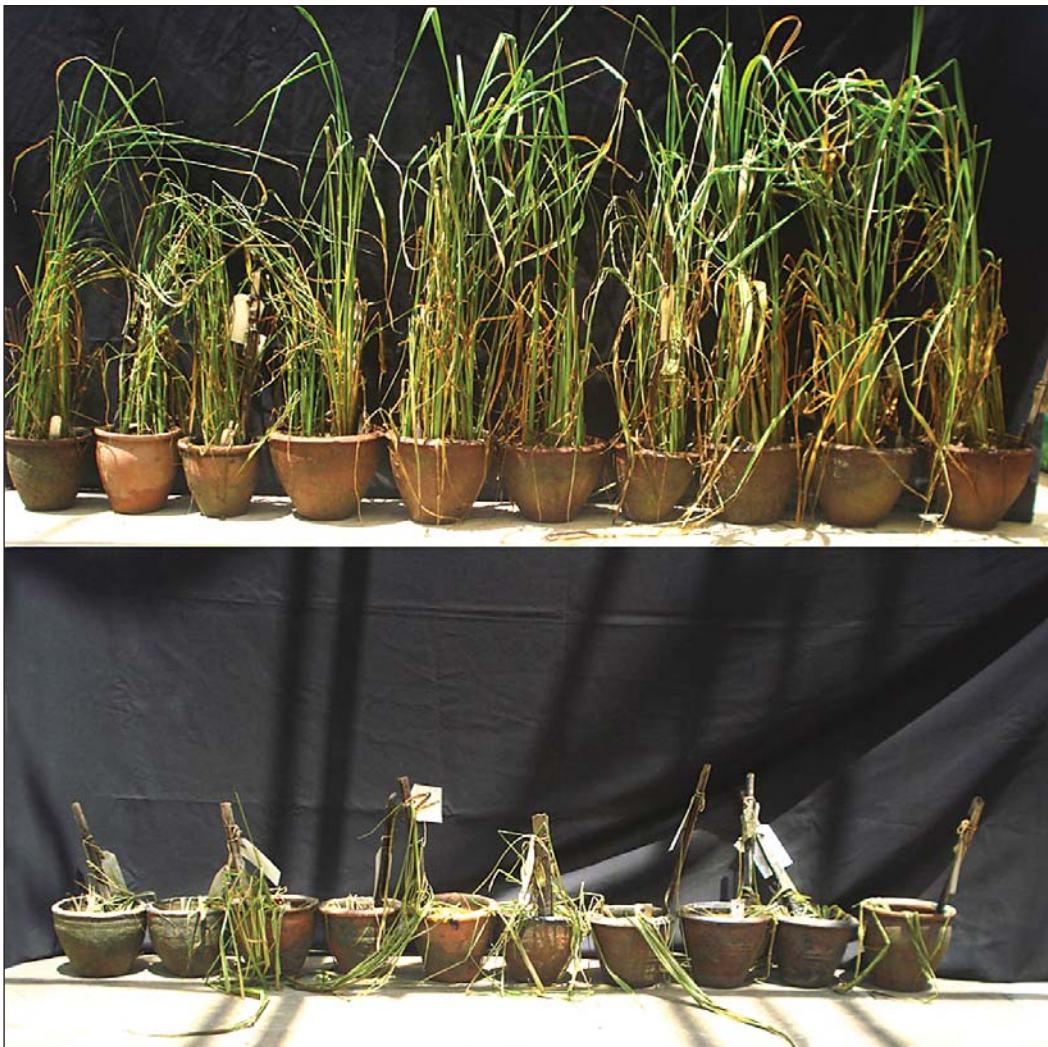
Principal component analysis (PCA) was performed to understand the pattern of data matrix, identifying tolerant germplasm in response to important contributing factors under partial submergence with salt stress. Eigen vectors and principal components were calculated and a 2-D biplot was generated between PC-I and PC-II

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### Results

#### *Genotypes Tolerant to Combined Effect of Submergence and Salt Stress*

Among the one hundred ninety nine accessions, only three genotypes such as IC-449848, IC-580261 and IC-43351 showed more than 90% survival under submergence with saline water (Table 1, Fig. 1). Elongation % varied greatly even among highly submergence tolerant accessions (Table 1). The genotypes were divided into five groups based on survival % under submergence with saline water (Fig. 2). Group 1 consisted of 91 highly susceptible genotypes where survival percentage was below 20%. Twenty three genotypes were in the sensitive Group 2 where survival % was more than 20%



**Fig. 1.** Phenotypic characteristics of different rice genotypes under the impacts of submergence with salty water (12 dS m<sup>-1</sup>).

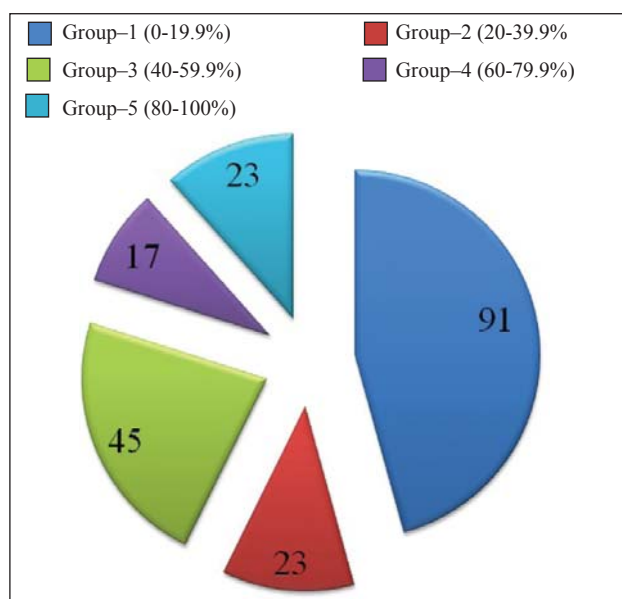


**Table 1. Elongation and survival percentage under submergence with saline water (12 dS m<sup>-1</sup>) of highly tolerant accessions**

Name	Height (BS) in cm	Height (AS) in cm	Elongation (cm)	Elongation (%)	Survival (%)
IC - 450262	31.6	36.7	5.1	16.2	80
IC-574839	34.4	57.6	23.2	67.4	80
IC-463054	36.9	49.3	12.3	33.4	80
IC-461222	36.4	56.6	20.2	55.7	80
IC-450092	43.2	54.5	11.3	26.3	82
IC-451031	35.8	53.8	18.0	50.3	82
AC-43409	38.9	43.7	4.8	12.2	84
IC-450639	34.6	46.3	11.8	34.1	84
AC-43365	39.7	48.3	8.7	21.9	85
IC - 459649	29.6	37.0	7.4	24.9	85
IC-419465	32.3	45.2	12.9	39.9	85
IC - 17024	37.3	47.7	10.3	27.7	86
IC - 449745	29.8	41.0	11.2	37.6	86
AC-43391	30.5	36.7	6.2	20.4	86
IC-449750	34.3	49.7	15.5	45.2	86
IC-145357	35.5	46.3	10.9	30.7	87
IC - 459362	37.7	43.7	6.0	15.8	88
IC-451469	37.4	44.3	6.9	18.4	88
IC-450292	33.3	44.4	11.2	33.6	90
FR 13A	31.4	34.7	3.3	10.4	90
IC-449848	30.2	45.5	15.3	50.7	92
IC-580261	26.8	33.8	7.0	26.0	92
AC-43351	31.7	35.7	4.0	12.7	95
LSD* <sub>&lt;0.05</sub>	3.6	4.9	2.4	9.8	6

BS-plant height before submergence; AS- plant height after 15 days of submergence.

but below 40%. Highly tolerant genotypes (Group 5), which showed more than 80% survival consisted of twenty three genotypes. Group 4 designated as tolerant



**Fig. 2. Plant mortality differs greatly under the impacts of submergence with salty water (12 dS m<sup>-1</sup>). Group was made base on survival %. Range of survival % is given in parenthesis.**

group consisted of 17 genotypes (survival % > 60% but below 80%), whereas, intermediate tolerant Group 3 where survival % was above 40% but below 60% was comprised of 45 genotypes. Average elongation % was greater in Group 2 and Group 1, followed by Group 3, Group 4 and Group 5 (Fig. 3). It was 54.6%, 58.3%, 49.8%, 40.9% and 30.9%, respectively.

**Genotypes Tolerant to Combined Effect of Partial Submergence and Salt Stress**

FR13A, a highly submergence tolerant cultivar showed score of 9 even after 7 days of partial submergence treatment with saline water (data not shown). After 10 days of treatment the cultivar FR13A was completely died out. Among the rest sixty five accessions two exhibited score of 9; twenty five showed score of 7, twenty five showed score of 5 and thirteen showed score of 3 (Table 2). None of the tested accessions showed score of 1. After 15 days of the treatment, the scoring increased greatly and none of the accessions even showed a score of 3. Among the thirteen accessions, which showed score of 3 after 10 days of partial submergence with salty water; ten showed score of 5 and three showed score of 7 after 15 days of the imposition of the treatment. The rest of

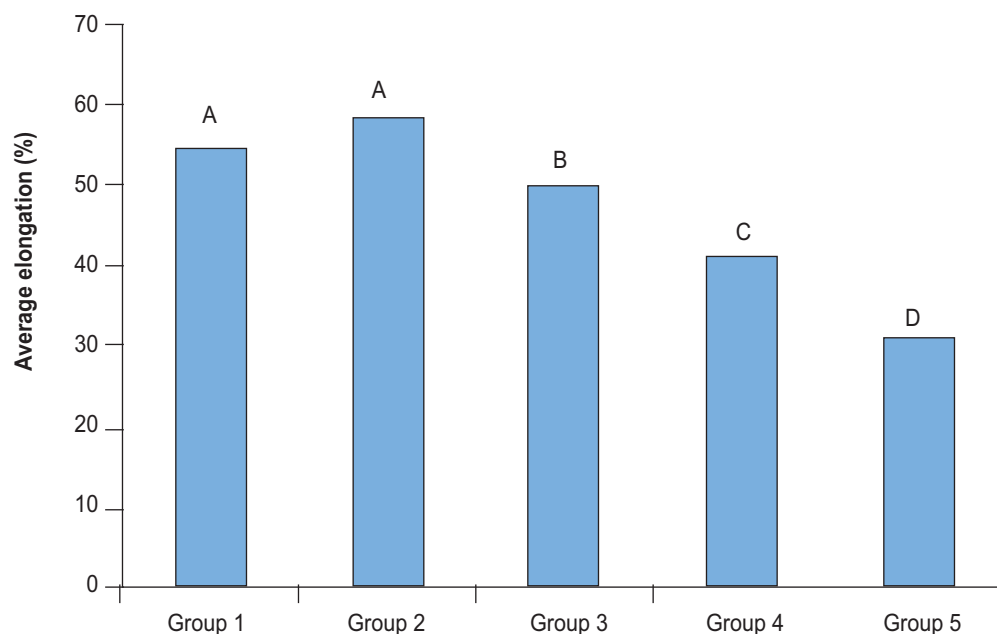
**Table 2. Combined effect of stagnant flooding and salinity on plant pigment and survival score**

Sl. No.	Genotypes	Chlorophyll content (mg g <sup>-1</sup> fresh weight)			NDVI	PRI	Score	
		Chl <i>a</i>	Chl <i>b</i>	Chl T			10d	15d
1	IC-574839	0.74	0.25	0.99	0.324	0.008	9	9
2	IC-451031	0.66	0.20	0.82	0.355	0.009	9	9
3	Varshadhan	0.82	0.28	1.10	0.397	0.014	7	9
4	IC-545261	1.02	0.35	1.37	0.392	0.015	7	9
5	IC-459958	0.82	0.30	1.11	0.320	0.007	7	7
6	IC-450369	0.90	0.34	1.23	0.378	0.007	7	7
7	EC-496903	0.92	0.35	1.27	0.395	0.015	7	9
8	AC-41620	1.17	0.38	1.55	0.399	0.017	7	7
9	IC-381330	0.92	0.32	1.24	0.420	0.016	7	9
10	AC-1764	1.76	0.57	2.33	0.322	0.006	7	9
11	AC-43314	1.17	0.37	1.55	0.429	0.017	7	9
12	IC-461253	1.70	0.56	2.25	0.503	0.032	7	7
13	IC-450067	0.58	0.20	0.79	0.354	0.011	7	9
14	IC-578006	0.63	0.21	0.85	0.394	0.012	7	9
15	IC-450135	1.05	0.34	1.40	0.485	0.023	7	7
16	IC-459931	1.70	0.55	2.26	0.537	0.026	7	9
17	IC-463954	1.24	0.38	1.62	0.448	0.019	7	9
18	IC-463054	1.66	0.52	2.19	0.478	0.030	7	9
19	IC-461222	0.72	0.26	0.98	0.339	0.018	7	7
20	IC-450092	0.65	0.25	0.90	0.409	0.018	7	9
21	AC-43409	1.48	0.48	1.95	0.488	0.021	7	7
22	AC-43391	1.08	0.33	1.41	0.502	0.018	7	9
23	IC-145357	1.00	0.29	1.28	0.531	0.020	7	9
24	IC-459362	1.13	0.35	1.47	0.487	0.017	7	9
25	IC-451469	1.25	0.36	1.62	0.470	0.025	7	9
26	IC-450292	1.13	0.36	1.49	0.493	0.022	7	9
27	IC-580261	0.77	0.26	1.03	0.401	0.012	7	9
28	AC-43358	1.66	0.51	2.17	0.339	0.052	5	7
29	IC-98834	1.53	0.48	2.01	0.561	0.032	5	7
30	IC-459884	1.20	0.40	1.59	0.501	0.026	5	7
31	IC-459889	1.63	0.55	2.18	0.498	0.029	5	7
32	IC-449831	1.34	0.42	1.76	0.533	0.028	5	7
33	IC-381834	2.00	0.66	2.66	0.658	0.048	5	7
34	IC-461224	1.32	0.47	1.78	0.612	0.048	5	7
35	IC-261125	1.54	0.53	2.07	0.654	0.047	5	7
36	AC-43359	1.37	0.48	1.85	0.559	0.042	5	7
37	EC-516602	1.45	0.53	1.98	0.476	0.039	5	7
38	IC-330793	1.67	0.55	2.22	0.63	0.042	5	7
39	IC-451378	1.28	0.39	1.67	0.587	0.039	5	7
40	IC-399488	1.51	0.57	2.08	0.554	0.041	5	7
41	IC-450443	1.17	0.40	1.57	0.515	0.030	5	7
42	IC-459794	0.94	0.31	1.26	0.537	0.029	5	9
43	IC-450262	0.76	0.26	1.02	0.55	0.023	5	7
44	IC-450639	0.94	0.32	1.26	0.521	0.029	5	7
45	AC-43365	1.68	0.60	2.28	0.501	0.032	5	7
46	IC-419465	1.69	0.52	2.21	0.617	0.035	5	9
47	IC-449745	1.56	0.51	2.07	0.584	0.026	5	7
48	IC-449750	1.67	0.50	2.17	0.589	0.037	5	9
49	IC-449848	1.38	0.44	1.82	0.549	0.022	5	9
50	AC-43351	1.91	0.58	2.49	0.480	0.019	5	9

Contd.

Sl. No.	Genotypes	Chlorophyll content (mg g <sup>-1</sup> fresh weight)			NDVI	PRI	Score	
		Chl <i>a</i>	Chl <i>b</i>	Chl T			10d	15d
51	IC-491212	2.08	0.79	2.87	0.552	0.031	5	9
52	AC-43353	1.57	0.54	2.11	0.543	0.034	5	9
53	Rashpanjor	2.10	0.61	2.72	0.560	0.046	3	5
54	IC-545518	1.23	0.43	1.65	0.517	0.036	3	5
55	IC-459733	1.87	0.58	2.45	0.659	0.057	3	5
56	IC-499780	2.30	0.75	3.05	0.631	0.054	3	5
57	IC-450250	2.60	0.80	3.41	0.596	0.047	3	7
58	IC-115617	2.11	0.72	2.83	0.557	0.049	3	5
59	AC-39460	1.34	0.54	1.88	0.547	0.033	3	7
60	AC-39293	2.59	0.82	3.41	0.660	0.053	3	5
61	IC-459744	1.21	0.42	1.63	0.518	0.032	3	5
62	IC-469712	2.48	0.85	3.33	0.672	0.057	3	5
63	IC-464746	1.00	0.41	1.42	0.590	0.043	3	7
64	IC-459649	1.84	0.61	2.45	0.646	0.058	3	5
65	IC-17024	1.37	0.46	1.83	0.614	0.041	3	7
	LSD <sub>p&lt;0.05</sub>	0.23	0.18	0.35	0.061	0.006	–	–

NDVI, Normalized Difference Vegetative Index; PRI, Photochemical Reflectance Index; Score: No apparent damage (Score 1), Little damage of leaf, healthy stems (Score 3), Greater damage of leaf, less damage of stem (Score 5), Greater damage of both leaf and stem (Score 7), and Almost dead (Score 9)



**Fig. 3.** Average elongation (%) in different survival groups under submergence with 12 dS m<sup>-1</sup> saline water. Different letters across the groups indicate the significant differences whereas the same letter shows the non-significant difference.

the accessions either showed score of 7 or 9 after 15 days of the partial submergence with salty water.

#### ***Variation in Pigment Level, NDVI and PRI under Partial Submergence with Salinity***

Levels of pigment content among the different accessions were significantly different (Table 2). Four accessions

such as IC-491212, IC-499780, AC-39293 and IC-469712 maintained greater chlorophyll level compared to other. Total chlorophyll as well as both Chl *a* and Chl *b* contents were more than 3 mg g<sup>-1</sup> fresh weight of leaf in these genotypes. NDVI reading ranged from 0.320 to 0.672 among the different accessions. NDVI reading

**Table 3. Average value of different pigment content, light radiance (NDVI) and light reflectance (PRI) under different scoring group. Data represent as mean  $\pm$  standard error**

Score Group	Chl <i>a</i> (mg g <sup>-1</sup> FW)	Chl <i>b</i> (mg g <sup>-1</sup> FW)	Chl T (mg g <sup>-1</sup> FW)	NDVI	PRI
Score 9	0.70 $\pm$ 0.04	0.22 $\pm$ 0.03	0.92 $\pm$ 0.06	0.339 $\pm$ 0.015	0.009 $\pm$ 0.0001
Score 7	1.08 $\pm$ 0.07	0.36 $\pm$ 0.02	1.44 $\pm$ 0.09	0.439 $\pm$ 0.015	0.019 $\pm$ 0.001
Score 5	1.44 $\pm$ 0.06	0.48 $\pm$ 0.02	1.92 $\pm$ 0.08	0.548 $\pm$ 0.014	0.035 $\pm$ 0.002
Score 3	1.92 $\pm$ 0.15	0.63 $\pm$ 0.04	2.55 $\pm$ 0.19	0.601 $\pm$ 0.014	0.048 $\pm$ 0.002

Chl, chlorophyll; T, total; NDVI, Normalized Difference Vegetative Index; PRI, Photochemical Reflectance Index

more than 0.600 was observed in twelve accessions such as IC-381834, IC-461224, IC-261125, IC-330793, IC-419465, IC-491212, IC-459733, IC499780, AC-39293, IC-469712, IC-459649 and IC-17024. Similarly, the reading of PRI also varied among the different accessions. It ranged from 0.006 to 0.061. The PRI value was greater in IC-491212 (0.061), followed by IC-459649 (0.058), IC-469712 and IC-459733 (0.057), IC-499780 (0.054) and AC-39293 (0.053).

Table 3 depicts the average value of different pigment content, light radiance (NDVI) and light reflectance (PRI) under different scoring groups. The average values of Chl *a*, Chl *b*, Chl T, NDVI and PRI was greater in the group which showed score of 3 compared to score of Group 5, 7 and 9. The value was in the order of Score 3 > Score 5 > Score 7 > Score 9. The values of Chl *a*, Chl *b*, Chl T, NDVI and PRI under score 3 were 174%, 186%, 177%, 77% and 433% greater respectively, compared to score of 9 whereas in comparison to score 7 the values were 54%, 64%, 56%, 29% and 111% greater.

### Correlation and Regression Analysis

Correlation coefficients were determined among different physiological parameters taken after 10 days of partial submergence with salinity and scoring (Table 4). Pigment content, light radiance and reflectance parameters showed highly significant ( $p < 0.001$ \*\*\*) association. The 'r' values of Chl *a*, Chl *b*, Chl T, NDVI and PRI with the

score taken after 10 days of treatment were -0.666\*\*\*, -0.695\*\*\*, -0.675\*\*\*, -0.720\*\*\* and -0.811\*\*\*, respectively. These parameters also showed highly significant negative association with the score taken after 15 days of treatment. The association between scoring after 10 days of treatment and after 15 days of treatment was highly positively significant ( $r = 0.662$ \*\*\*).

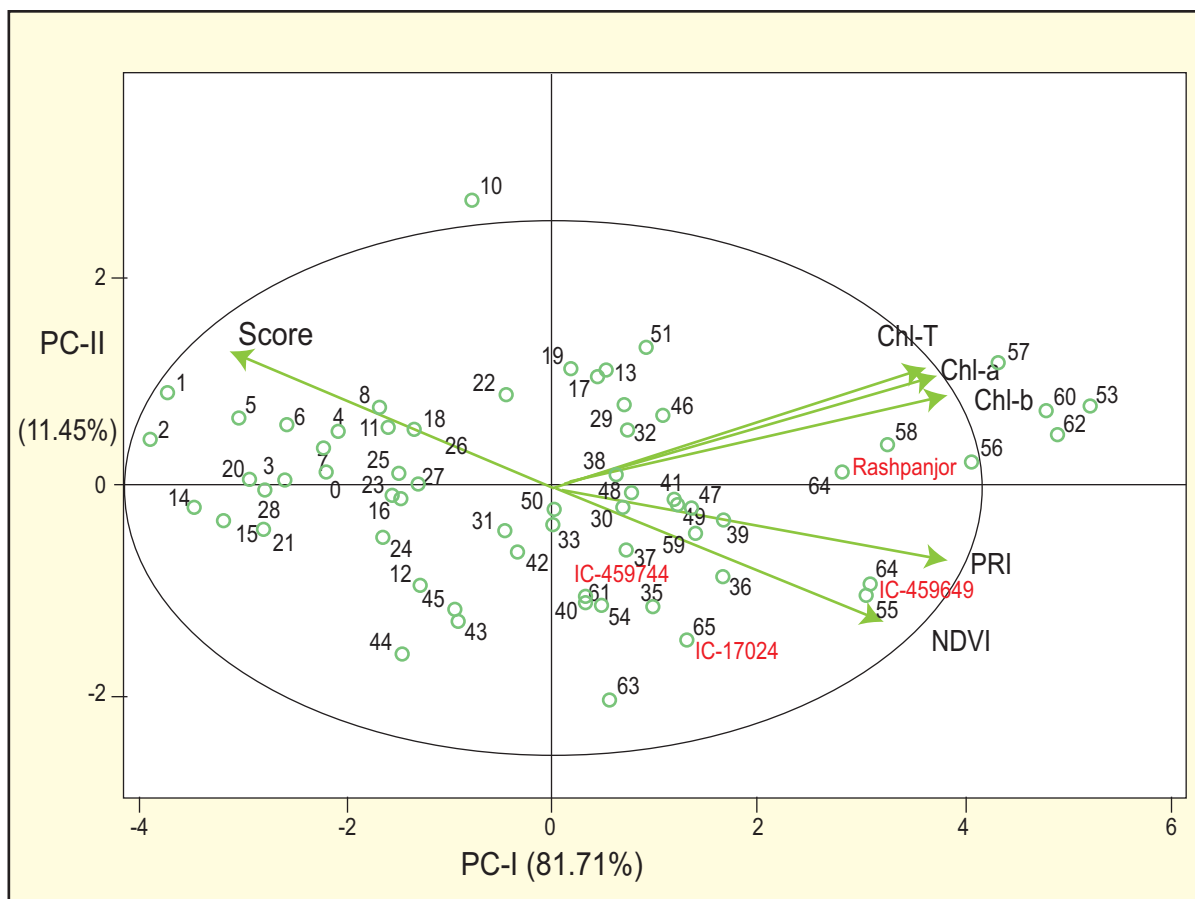
### Principal Component Analysis and Biplot Construction

PCA was done taking data of NDVI, PRI, Chl *a*, Chl *b*, Chl T and scoring at 10 days after partial submergence with saline water of 65 rice accessions. The first two principal components explained around 93% of the total variability among the germplasm accessions. Eigen vectors data (data not given) revealed that the positive effect of Chl *a* (0.42), Chl *b* (0.43), Chl T (0.43), NDVI (0.37), and PRI (0.41) and negative effect of score (-0.38) at 10 days after treatment contributed significantly to the first principal component (PC-I), and the negative effect of NDVI and PRI and the positive effect of Chl *a*, Chl *b*, Chl T and score contributed to PC-II. The Eigen vector size also depicted that Chl *a*, *b*, total chlorophyll and PRI contributed equally followed by NDVI and susceptibility score to the positional distribution of genotypes in the biplot based on PCA (Fig. 4). Rashpanjor, a popular rice genotype in coastal saline area tolerant to partial submergence and salinity belonged to a quarter of biplot with both positive effect of PC-I and PC-II. But due to

**Table 4. Correlation among different pigment associated parameters with scoring under combined effect of stagnant flooding and salt stress**

Parameters	Chl <i>a</i>	Chl <i>b</i>	Chl T	NDVI	PRI	Score 10d
Chl <i>b</i>	0.982***	---	---	---	---	---
Chl T	0.999***	0.990***	---	---	---	---
NDVI	0.619***	0.634***	0.624***	---	---	---
PRI	0.731***	0.768***	0.745***	0.822***	---	---
Score 10d	-0.666***	-0.695***	-0.675***	-0.720***	-0.811***	---
Score 15d	-0.423***	-0.484***	-0.439***	-0.501***	-0.643***	0.662***

\*\*\*, significance at 0.001 level; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl T, total chlorophyll; NDVI, Normalized Difference Vegetative Index; PRI, Photochemical Reflectance Index; Score 10d, 10 days after combined effect of stagnant flooding and salt stress.



**Fig. 4.** Diversity analysis among sixty-four rice genotypes based on various phenotypic traits such as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl T), normalized difference vegetative index (NDVI), photochemical reflectance index (PRI) and susceptibility score by principal component analysis under partial submergence with salt stress.

its position near to equatorial line in biplot, Chl *a*, Chl *b*, total chlorophyll, PRI and NDVI all are supposed to be with nearly equal contribution to its low scoring. In contrary tolerant rice genotypes for both complete and partial submergence with salty water, IC-459744, IC-459649 and IC-17024 belonged to a quarter of the biplot with positive effect of PC-I and negative effect of PC-II.

### Discussion

Identification of specific genotype(s) within the cultivated gene pool and their utilization in crop improvement is still the most trusted and successful approach (Reddy *et al.*, 2009; Sarkar *et al.*, 2009; Chattopadhyay *et al.*, 2014; Singh *et al.*, 2016). Coastal areas are more vulnerable than inlands. Multiple abiotic stresses are common in the same growing season depending on the rainfall pattern, high wind and higher tidal wave. Rice is the only crop that could be grown in this hostile

environment when all other crops get lost (Mahata *et al.*, 2010). Genotypes tolerant to complete submergence under saline environment are identified (Figs. 1-2, Table 1). FR13A, a genotype tolerant to submergence but susceptible to salinity (Sarkar *et al.*, 2013) showed 90% survival under submergence with salt stress where as Rashpanjor, a genotype tolerant to salinity but susceptible to submergence totally succumbed under such conditions. Highly submergence tolerant genotypes showed less elongation compared to susceptible genotypes (Fig. 3). This is in conformity with earlier findings (Sarkar and Bhattacharjee, 2011; Ray *et al.*, 2017). Floodwater quality largely determines the survival under submergence (Das *et al.*, 2009). In this situation though floodwater was saline, the effect was as usual like normal water (Xu *et al.*, 2006; Sarkar and Panda, 2009). Due to the lack of active transpiration under submergence, Na<sup>+</sup> built up inside the plant body was inhibited and thus effect of salinity was not noticed under complete submergence



with salt stress. In the same hypothesis, FR13A though susceptible to salinity could survive submergence stress under saline environment (Sarkar and Ray, 2016).

Genotypes showed tolerant reaction under submergence with salinity were not necessarily tolerant to partial submergence with salinity (Table 2). FR13A succumbed after 7 days of the imposition of the partial submergence with salt stress. Like FR13A, two highly submergence tolerant genotypes such as IC-574839 and IC-451031 showed susceptible reaction and after 10 days of the treatment were almost dead (Table 2). Rashpanjor which showed susceptible reaction under submergence with salinity exhibited tolerant reaction under partial submergence with salinity. This shows that salinity tolerant genotypes have an edge to withstand the partial submergence with salt stress over the salt-susceptible genotypes. Damage to plant under partial submergence with salinity increased with time. Thirteen genotypes showed tolerant reaction (score 3) after 10 days of partial submergence with salt stress. However, none of the genotypes showed sign of tolerant reaction after 15 days of the said stress (Table 2). Greater damage due to salinity with run of time was reported earlier (Singh and Sarkar, 2014).

The different physiological parameters such as chlorophyll levels, values of NDVI and PRI differed significantly under the combined stress of partial submergence and salt stress (Table 2). Few genotypes maintained greater values of these parameters compared to other genotypes. Restricting degradation of vital organ under stress is important for survival and growth as observed in any stress tolerance studies including this investigation. The genotypes were grouped into four categories with the score of 9, 7, 5 and 3 after 10 days of partial submergence with salty water. Average values of different pigment content, light radiance (NDVI) and light reflectance (PRI) under different scoring groups differed greatly (Table 3). The values of these parameters were maximum in the group with score 3, followed by 5, 7 and 9. Degradation of pigment level due to salinity as well submergence with salinity stresses were reported earlier (Yan *et al.*, 2013; Singh and Sarkar, 2014; Sarkar and Ray, 2016). Different parameters showed distinct relationship among themselves (Table 4). Positive significant association of chlorophyll content and NDVI values with PRI was reported earlier (Hmimina *et al.*, 2014; Soudani *et al.*, 2014). Highly significant negative association of these parameters with scoring after 10

and 15 days of treatment revealed that maintenance of greater values of these parameters were the sign of plant fitness under stress.

Two dimensional biplot derived through PCA allowed to differentiate between genotypes with different tolerance levels (Fig.4). Some moderately tolerant genotypes co-localized with the vector for chlorophyll content traits in a cluster of the biplot (Fig. 4). But comparatively more tolerant genotypes with low susceptibility score localized in the just opposite cluster. The same cluster was dominated by PRI with long vector size. This indicated that PRI was the main determinant of tolerance for these genotypes.

### Conclusion

Rice genetic resources tolerant to partial and complete submergence with salt stress are available among the tested genotypes which were mainly collected from coastal areas of India. The genotypes tolerant to submergence with salt stress were not always tolerant to partial submergence with salt stress. Genotypes tolerant to both partial and complete submergence with salt stress identifies in this investigation are of great value in developing the high yielding varieties tolerant to multiple abiotic stresses. The results show that the Photochemical Reflectance Index (PRI) provides a non-invasive and rapid method for investigating stress effects on photosynthetic machinery better compared to chlorophyll content and Normalized Difference Vegetative Index (NDVI).

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