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Physio-Biochemical and Microsatellite Based Profiling of Lowland Rice (*Oryza sativa* L.) Landraces for Osmotic Stress Tolerance

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ABSTRACT

Global climate changes accelerate a wide range of abiotic stresses leading to a series of physiological, biochemical and molecular changes that adversely affect the growth and productivity of rice (*Oryza sativa* L.). In this background, there is limited knowledge and profiling reports of lowland, less popular rice landraces in relation to their osmotic stress tolerance. Laboratory mediated physiological and biochemical screening for a selected set of 20 lowland rice landraces was done in induced drought and salinity stress conditions. Varietal genetic diversity and inter-relatedness were assessed by using osmotic stress tolerance linked forty-five rice microsatellite markers. For representing the allelic diversity of the studied microsatellite loci across the selected genotypes, a microsatellite panel was constructed and PIC values of all used microsatellite markers were calculated. The obtained database can be used for varietal identification, characterization and genetic information in relation to osmotic stress tolerance.

Keywords: Drought; Genetic Diversity; Salinity; Simple Sequence Repeat; Traditional Rice

1. Introduction

Rice (*Oryza sativa* L.) is the principal staple food as over 50% of the world's population depends on it for about 80% of their food requirements [1]. Rice is grown under diverse ecological conditions (irrigated, lowland, upland, coastal, drought and flood prone areas) in tropical Asian countries such as India. Global climate change accelerates a series of abiotic stresses, especially drought and salinity (osmotic stress), which impose serious threats for rice production worldwide [2]. By the end of the 1980 (post Green-revolution time), conventional breeding mediated rice yield's potential was increased in different experimental trials [3]. During this agricultural modernization era, a large number of rice landraces have been lost due to the invasion of high-yielding rice varieties [3]. Although less productive, these landraces have been reported to be highly tolerant to a number of biotic and abiotic stresses [4,5] and harbour a good number of other quality traits [6,7]. Unlike high-yielding varieties (whose variability is limited due to homozygosis), the rice landraces are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period of time [8,9]. For proper utilization and incorporation of these indigenous rice lines from the natural crop resources, identification of the allelic variant that results in plant phenotype is the utmost important. Conservation of these genetic resources is, therefore, much more useful since such lines have diverse gene pool providing the potentiality to withstand in varied environmental fluctuations. Their wide phenotypic and genotypic responses can also be considered for their varietal characterization and proper identification [10]. In this context, molecular evaluation in form of genetic diversity for available landraces has become important in modern-day crop improvement [11,12]. Such rice genetic resources were being used for incorporating genetic variability into rice breeding programs to generate new cultivars with broadened genetic bases [3] and allow new useful allelic combinations [13,14]. Genetic profiling among the diverse rice lines presently grown in West Bengal (a state of In-

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dia) has been assessed by a number of workers [15-19] using various molecular marker systems. Microsatellites, also known as simple sequence repeats (SSRs), a recent and popular marker system, are abundant and well distributed throughout the rice genome [20-22], being used in rice for genomic diversity analysis [23-25]. Several traits linked rice SSR markers have been established for drought [26,27], salinity [28,29] and submergence [30,31] tolerance. Besides the molecular characterization at DNA level, physiological performances under induced osmotic stresses and quantity of bioactive compounds (proline, chlorophyll, total protein, salicylic acid, catalase, peroxidase etc.), having a significant role in adaptation to altered environmental stress conditions, are also necessary for the proper characterization of rice lines in relation to stress tolerance.

In spite of erosion of a number of rice landraces, West Bengal is still today a rich source of indigenous rice lines having the hidden genetic potential that needs to be explored [16]. There were no previous reports on the stud-

ied rice landraces for their osmotic stress tolerance properties. Keeping this as an objective of the present research, a total number of 20 lowland indigenous less popular lines' suffixes with "Sail" were genotyped for the first time with 45 osmotic stress tolerance linked reported SSRs in addition to physiological and biochemical screening to make a systematic and multi-informative profile of selected varieties in relation to osmotic stress tolerance.

2. Materials and Methods

2.1. Plant Materials

A total of 20 indigenous, lowland, less popular West Bengal rice landraces suffix with "Sail" were selected for this investigation. The detailed description of these landraces is given in **Table 1**.

2.2. Physiological Screening

For physiological screening, drought stress was induced by varying concentrations (−0.5, −1.0 and −1.5 MPa) of

Table 1. Details of the studied rice landraces.

Sl. no.	Local name	Our accession no.	Special character (s)
1	Lati Sail	VB 24	Lowland, medium grained rice
2	Mallik Sail	VB 25	Lowland, long-slender grained rice
3	Chapri Sail	VB 39	Lowland, long-medium grained rice
4	Balaram Sail	VB 40	Brown spot resistant, long-medium grain
5	Kabirraj Sail	VB 44	Long straw, labile starch and has medicinal values, long-slender grained
6	Lat Sail	VB 47	Lowland, medium grained rice
7	Laxmi Sail	VB 48	Lowland, long-medium grained rice
8	Malik Sail	VB 50	Lowland, long-slender grained rice
9	Mihinagra Sail	VB 52	Lowland, long-slender grained rice
10	Nagra Sail	VB 53	Brown spot resistant, long-medium grain
11	Nekrai Sail	VB 54	Lowland rice with medium grain
12	Neizer Sail	VB 55	Lowland rice with extra long-slender grain
13	Punjab Sail	VB 59	Lowland rice with long-medium grain
14	Rani Sail	VB 60	Lowland, long-slender grained rice
15	Ratan Sail	VB 61	Lowland rice with long-slender grain
16	Raghu Sail	VB 62	Bacterial leaf blight resistant, long-slender grain
17	Rup Sail	VB 63	Typical drought tolerant, medium-slender grained traditional lines of Bankura and Purulia districts of West Bengal
18	Sindur Sail	VB 64	Lowland, long-slender grained rice
19	Seeta Sail	VB 65	Medium slender white grained rice
20	Sankar Sail	VB 67	Medium drought tolerant traditional line with long-slender grain

Polyethylene Glycol 6000 (PEG) [32] and salinity was induced by three different concentrations (100, 200 and 300 mM) of sodium chloride (NaCl) solution [33]. Stresses were imposed during seed germination and seedling growth for the studied landraces after the mercuric chloride mediated surface sterilization. Germination percentage (G%) and seedling growth (in form of shoot and root length) were monitored and recorded on 15th day after seed sowing. Grains were considered germinated when both the plumule and radical had extended to more than 2 mm [34].

2.3. Biochemical Profiling

Estimation of some selected bioactive compounds commonly varying with different degree of osmotic stresses was quantified. Surface sterilized rice grains were allowed to germination and growth in a plastic pot contained artificial soil and set for 20 days at 28°C ± 2°C with 9 h photoperiod. Then the plantlets were transferred to culture tubes for treatment with 20 ml solutions of different PEG and NaCl concentrations (as used in physiological screening) and set for 24 h. Then, proline, chlorophyll and total protein contents were quantified from the fresh leaf tissues using standard protocols [35-37].

2.4. Statistical Analysis

For different physio-biochemical parameters the estimated values were analyzed statistically. Mean values of studied traits (germination percentage, root and shoot length); proline, total chlorophyll and total protein contents were subjected to analysis of variance (ANOVA) using Microsoft Excel version 10.0 as per the method of Panse and Sukhatme [38] to test the significance at 5% level of probability ($P < 0.05$) for each character which employed to obtain, assemble, classify and to interpret voluminous quantitative data.

2.5. Genomic DNA Isolation, PCR Amplification and SSR Genotyping

Leaf and other aerial plant tissues from five days old germinating seedlings were used for isolation of rice genomic DNA following a prestandarized protocol [39] of our laboratory. The isolated genomic DNA samples were adjusted to 25 ng/μl concentration. Based on available published reports [18,40-42], forty five osmotic stress tolerance linked SSR markers were selected. The detailed information of the selected SSR markers was obtained from the Gramene database (www.gramene.org). PCR amplification, polymorphism screening and analysis of the amplified products were done [18]. Individual alleles (in the form of different mol. wt. of the amplified product

from a single loci) of all SSR markers were scored to prepare a binary matrix based on the presence (1) or absence (0) of a particular allele. A pair-wise genetic similarity coefficient matrix between all possible pairs of the studied genotypes were calculated from such 1/0 matrix using SPSS (version 10.0) and a dendrogram was constructed based on Ward's method using complete linkage between groups and Euclidean distance using STATISTICA software. Polymorphism information content (PIC value) of each SSR was also determined following a simplified formula:

$$PIC_i = 1 - \sum P_{ij}^2 ,$$

where $i = 1$ to n, P_{ij} represents the frequency of jth allele for the ith band scored for a particular marker [43].

3. Results

3.1. Physiological Screening

Germination percentage: Seed G% of the studied rice landraces on 15th day was significantly low at the highest concentrations of both PEG (−1.5 MPa) and NaCl (300 mM). With increasing doses of stressors (PEG and NaCl), G% gradually decreased when compared with the control set (**Figures 1(a)** and **(b)**). The significant exception found for three genotypes (Lati Sail, Punjab Sail and Rup Sail) showing higher G% than the control for lower doses. Although, both PEG and NaCl inhibit seed germination, PEG showed more inhibitory effects than that of NaCl. Malik Sail, Neizer Sail and Rani Sail showed highest germination (100%) in all osmotic stress conditions. Chapri Sail, Punjab Sail, Rup Sail and Sankar Sail showed 100% germination in all NaCl doses, but not in PEG. Days to seed germination was also delayed with the increasing concentration of stressors.

Growth pattern: The phenotypic responses in form of shoot and root length in induced osmotic stresses (by PEG and NaCl treatment) were varied greatly. Shoot length in control was maximum (5.7 cm) in Malik Sail, whereas the minimum (3.2 cm) was obtained for Mallik Sail. Overall shoot growth in three different doses of PEG were more than the values found in three different NaCl concentrations as presented in **Figures 2(a)** and **3(a)**, respectively. Shoot length in all concentrations of PEG and NaCl were lower than the control ones. In **Figures 2(b)** and **3(b)**, it has been shown that root length was lower in both Mallik Sail and Laxmi Sail (4.5 cm) and highest in Chapri Sail (7.1 cm). Compared to the control, lower concentration of PEG (−0.5 MPa) and NaCl (100 mM) increased the root length. The subsequent higher doses of stressors inhibit the root growth. This reduction in root length was higher in PEG rather than in NaCl doses.

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(a)

(b)
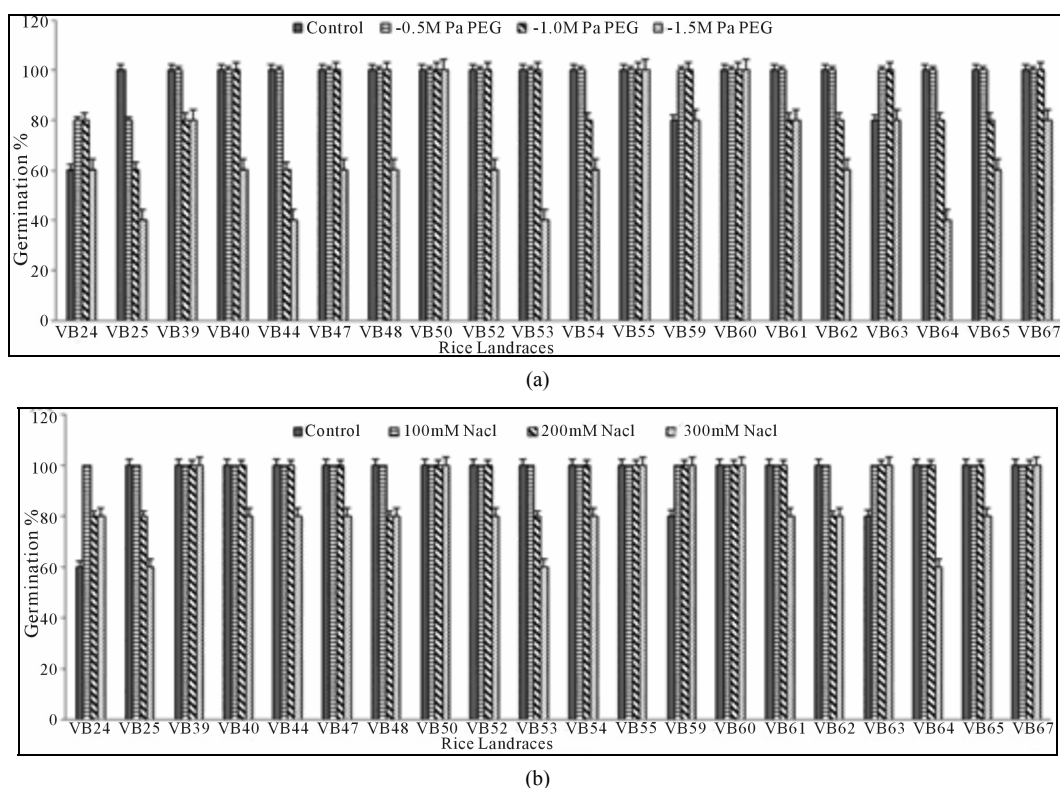
Figure 1. Graphical representation of rice seed germination percentage of 20 studied landraces in drought (a) and salinity (b) stress conditions induced by polyethylene glycol (PEG) and sodium chloride (NaCl), respectively.
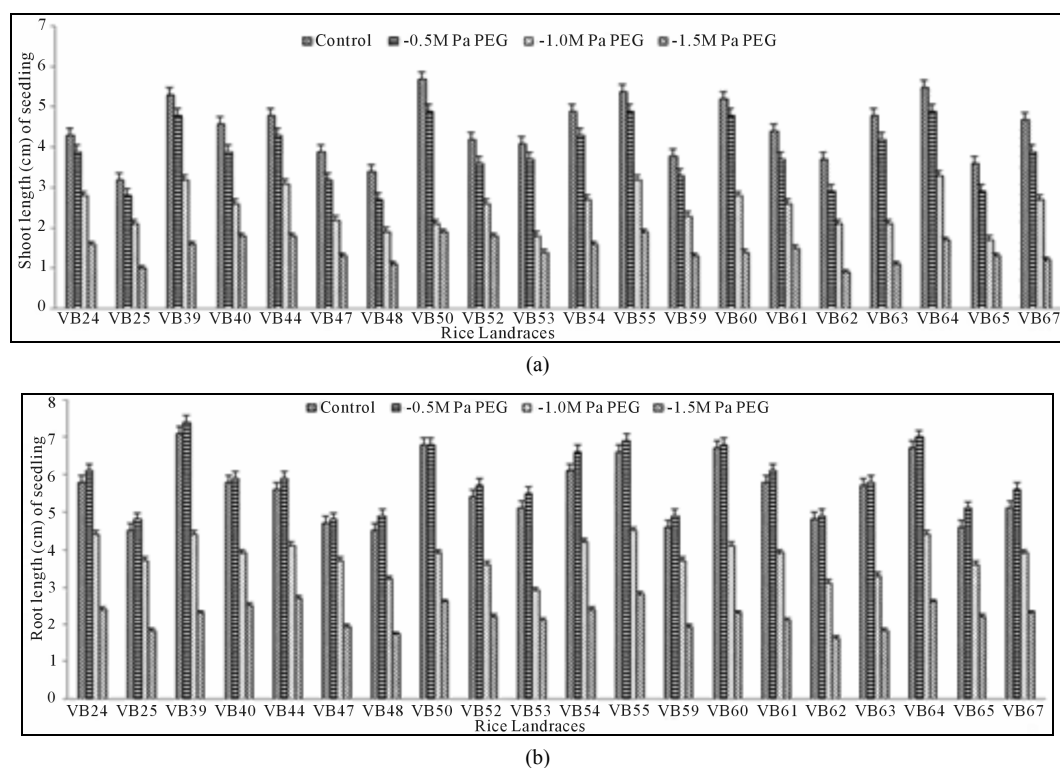

(a)

(b)

Figure 2. Graphical representation showing shoot (a) and root (b) length of 15 days old germinating rice seedlings grown in different concentrations (−0.5, −1.0 and −1.5 MPa) of polyethylene glycol (PEG).
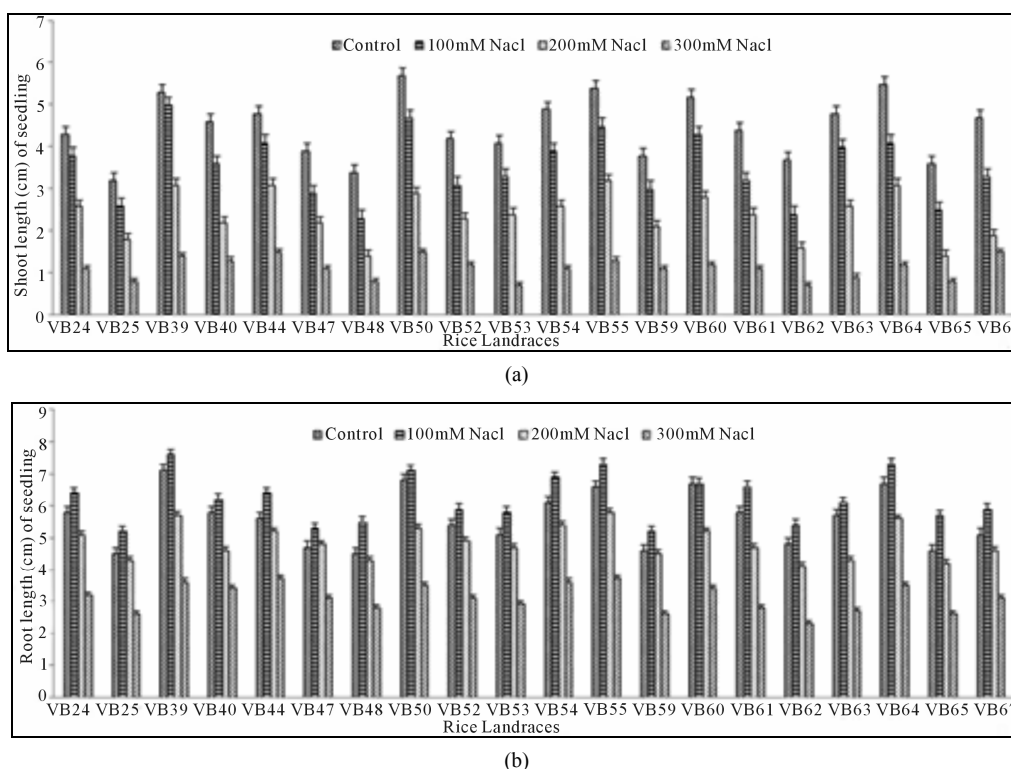
(a)

(b)

Figure 3. Effect of different NaCl concentrations (100, 200 and 300 mM) on shoot (a) and root (b) length of 15 days old germinating rice seedlings.

3.2. Biochemical Profiling for Proline, Chlorophyll and Protein Content

Chlorophyll (mg), proline (µg) and total protein (mg) content per g of mature normal leaf tissue, in control and all stress conditions, showed a considerable variation for studied twenty rice landraces (**Table 2**). Among the all rice cultivars, Sankar Sail showed the highest and Nekrai Sail showed the lowest quantity of chlorophyll in all the concentrations of PEG and NaCl. Protein content in the lower osmotic stresses, *i.e.* −0.5 MPa PEG and 100 mM NaCl, reveal that Laxmi Sail has gave the highest values (95.2 and 109, respectively) and Chapri Sail has gave the lowest values (56.7 and 58.2, respectively).

3.3. Analysis of Variance (ANOVA)

The analysis of variance revealed highly significant ($p < 0.05$) differences among the physio-biochemical responses (**Table 3**) in different osmotic stress conditions (in both PEG and NaCl).

3.4. SSR Based Molecular Characterization

Fingerprinting results reveal that 38 SSR primers, out of total 45, are polymorphic and the rest seven (RM 8094, RM 10745, RM 315, RM 162, RM 10927, RM 3231 and RM 296) are monomorphic. Polymorphism has been identified from 38 primer pairs and that is shown in **Table 4**. A total of 122 alleles were detected, of which 12 were found to be rare. The number of alleles per locus ranged from 2 to 5, with an average of 3.21 alleles across the 45 loci. Based on the revealed allelic size differences, microsatellite panel (**Figure 4**) represents the SSR based whole amplicon variability amongst the studied landraces. A total of 12 rare alleles (present only in a single genotype) were identified. The lowest amplicon size belonged to OSR 2 (57 - 66 bp) and the highest amplicon size belonged to RM 10772 (427 - 512 bp). The large size of the monomorphic amplicon size was highest (173 bp) for RM 10745 and lowest (105 bp) for RM 162. Highest allelic frequency (90%) were revealed by both RM 212 (111 bp) and RM 219 (165 bp) and lowest allelic frequency (35%) were observed for RM 3412 (200 bp), RM 206 (139 bp and 167 bp), RM 328 (270 bp), RM 215 (148 bp) and RM 257 (137 bp). It was noted that out of 5 alleles of RM 206, two alleles (139, 167 bp) having the same (35%) frequency. Similar results was found for RM 8115, RM 10890 and RM 107; where the former two loci showed 2 alleles with same frequency (50%) and the later (RM 107) showed alleles with same frequency (40%).

Polymorphism information content or PIC values for 38 polymorphic primers varied from 0.180 (for RM 212

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Table 2. Quantified proline, chlorophyll and total protein content from the fresh leaf tissues maintained at different stressing conditions.

Varieties	Control set			PEG									NaCl								
				−0.5 MPa			−1.0 MPa			−1.5 MPa			100 mM			200 mM			300 mM		
	PRO	CL	PT	PRO	CL	PT	PRO	CL	PT	PRO	CL	PT	PRO	CL	PT	PRO	CL	PT	PRO	CL	PT
VB24	50	0.94	67.3	57.3	0.91	66.1	64.7	0.87	62.6	73.8	0.73	58.3	54.6	0.91	63.6	59.2	0.81	56.8	64.1	0.78	51.2
VB25	52.8	0.81	79.4	67.8	0.75	74.4	77.1	0.70	67.2	87.3	0.65	62.7	57.3	0.79	77.6	62.3	0.69	70.8	72.6	0.61	66.8
VB39	52.8	2.43	60.1	61.3	2.19	56.7	69.5	2.16	52.8	76.0	1.92	47.3	56.7	2.28	58.2	61.2	2.21	54.4	72.6	2.03	53.1
VB40	47.2	0.67	82.4	56.7	0.64	70.2	59.2	0.55	61.4	75.6	0.40	60	53.1	0.66	78.6	55.8	0.61	67	65.8	0.59	64.5
VB44	70.1	0.77	84.6	79.2	0.71	72.4	91.2	0.64	66.1	98.4	0.57	57.8	74.6	0.75	77.6	76.4	0.69	67.2	82.4	0.65	63.3
VB47	40.2	1.44	99	53.2	1.26	82.8	59.7	1.03	77.6	74.0	0.91	69.6	49.1	1.33	89.6	51.0	1.19	80.4	62.5	0.98	72.7
VB48	54.4	1.46	122	59.7	1.13	95.2	63.2	0.99	74.3	79.3	0.96	67.8	55.2	1.26	109	63.1	1.14	81.2	67.3	1.05	79.6
VB50	51.6	0.98	99.8	62.3	0.88	88.9	69.5	0.81	80.5	83.8	0.73	73.4	60.7	0.91	92.3	66.3	0.85	83.7	74.4	0.77	77.9
VB52	60.4	1.40	77.2	65.9	1.05	71.8	68.8	0.89	66.3	84.7	0.77	61.1	62.4	1.25	74.1	65.6	0.92	72.6	72.1	0.86	67.9
VB53	43.6	1.18	85.8	50.4	1.04	78.6	55.3	0.85	71.3	78.9	0.59	63.4	48.2	1.11	81.6	62.8	0.98	76.4	69.6	0.65	69.3
VB54	57.2	0.45	87.6	62.3	0.41	81.6	75.4	0.37	73.9	81.0	0.31	64.2	65.7	0.44	85.1	69.1	0.41	78.7	79.3	0.39	73.4
VB55	54.4	1.25	93.8	59.2	0.90	90.8	72.8	0.84	84.4	78.9	0.77	78	61.3	1.06	92	68.4	0.93	88	77.2	0.86	83
VB59	55.6	1.17	96.6	60.3	1.09	79	73.6	0.89	71.2	79.9	0.82	64.8	62.4	1.14	86.4	69.6	0.99	81.1	78.6	0.92	70.5
VB60	56.4	0.89	96	59.8	0.85	86.2	66.2	0.69	77.6	72.1	0.79	72.8	61.7	0.83	92	69.2	0.81	87.4	79.3	0.77	73.2
VB61	47.2	1.32	79.3	53.1	1.15	71.2	65.5	0.94	68.4	71.7	0.81	62.3	59.3	1.20	75.7	67.8	1.08	72.1	78.0	0.92	69.6
VB62	57.6	1.49	87.6	59.3	1.06	79	63.4	0.91	71.4	69.7	0.77	63.2	63.7	1.26	82.2	69.2	1.02	76.5	76.4	0.85	71.1
VB63	65.2	1.24	88.8	73.2	1.02	87.2	79.6	0.99	86	86.9	0.81	80.4	69.4	1.05	86.8	72.8	1.00	83.6	78.2	0.93	79.6
VB64	53.2	1.26	74.2	73.5	0.98	72	80.3	0.78	65	85.6	0.64	59.3	69.2	1.02	72.4	71.7	0.95	68.3	79.8	0.84	63.2
VB65	41.2	0.93	71.8	79.4	0.73	65.3	82.7	0.67	59.5	86.3	0.59	55	70.2	0.77	68.9	73.9	0.71	62.7	79.5	0.67	58.9
VB67	51.6	2.98	81.4	77.7	2.86	72.6	82.3	2.71	62.6	88.2	2.58	58.4	71.5	2.92	77.1	74.2	2.67	72.3	80.8	2.31	68.2
Mean	53.14	1.25	85.74	63.58	1.08	77.1	71	0.96	70.01	80.61	0.86	63.99	61.32	1.15	81.04	66.48	1.03	74.06	74.53	0.92	68.85
SD	7.36	0.58	13.58	8.80	0.54	9.64	9.27	0.54	8.53	7.10	0.51	7.92	7.35	0.56	11.41	6.47	0.52	9.44	5.95	0.46	8.34
SE	1.65	0.13	3.04	1.97	0.12	2.15	2.07	0.12	1.91	1.59	0.11	1.77	1.64	0.12	2.55	1.45	0.12	2.11	1.33	0.10	1.87

PRO = Proline (in µg/g); CL = Chlorophyll (in mg/g); PT = Protein = Protein (in mg/g).

SSRs	VB24	VB25	VB39	VB40	VB44	VB47	VB48	VB50	VB52	VB53	VB54	VB55	VB59	VB60	VB61	VB62	VB63	VB64	VB65	VB67
RM1287	121	137	137	148	121	148	148	148	137	148	137	148	148	148	121	148	137	121	121	121
RM8094	147	147	147	147	147	147	147	147	147	147	147	147	147	147	147	147	147	147	147	147
RM3412	183	238	200	224	213	224	183	200	200	213	200	224	213	224	200	183	238	200	200	213
RM10745	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173
RM10764	136	80	136	80	80	136	136	136	156	136	136	136	136	136	136	136	136	136	136	136
RM10772	447	512	447	427	447	447	447	447	427	447	512	447	447	447	447	447	447	447	447	447
RM140	250	210	250	250	250	309	272	272	272	272	272	309	309	272	250	272	272	272	272	250
RM493	261	130	261	228	261	228	200	178	178	228	178	200	178	178	178	178	178	178	178	178
RM315	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139
RM223	136	158	158	158	158	158	158	170	158	158	136	153	153	158	158	158	153	153	158	158
RM212	111	111	121	111	111	111	111	111	111	111	121	111	111	111	111	111	111	111	111	111
RM162	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
RM206	167	167	131	167	167	152	139	131	167	167	139	143	139	167	139	139	139	143	139	131
OSR2	66	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	66	66	57	57
RM209	137	137	144	124	144	124	144	124	124	124	137	137	137	124	124	137	137	137	137	137
RM287	97	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	97	97	105	105
RM10720	207	221	207	214	214	214	214	214	221	221	192	214	214	214	214	214	214	214	192	192
RM10748	91	91	91	91	91	91	91	91	91	97	97	86	86	97	91	91	97	91	91	97
RM 8115	115	115	115	115	115	115	115	115	115	115	107	107	107	107	107	107	107	107	107	107
RM10843	161	161	161	150	150	161	161	161	161	150	161	150	161	161	161	161	150	150	150	150
RM562	284	225	240	260	225	240	240	225	225	240	240	240	240	260	260	240	240	260	260	260
RM10890	262	262	249	249	262	249	249	262	262	249	249	249	249	249	249	262	262	262	262	262
RM7075	109	117	117	109	117	117	117	117	109	117	117	117	130	130	130	130	130	130	130	130
RM10927	151	151	151	151	151	151	151	151	151	151	151	151	151	151	151	151	151	151	151	151
OSR28	144	144	144	144	144	144	144	134	130	134	134	134	134	134	134	134	134	134	134	134
RM136	113	94	94	94	86	86	86	86	86	86	86	86	86	86	86	86	86	86	94	94
RM107	292	270	253	253	253	253	239	239	239	253	239	239	239	253	239	239	253	253	292	292
RM553	177	167	167	167	167	158	158	158	158	158	154	154	154	154	154	154	154	154	177	167
RM3825	154	154	154	148	154	154	161	161	154	154	154	154	148	154	154	148	154	148	148	154
RM201	170	145	136	145	136	136	136	136	136	126	136	136	136	136	136	145	145	145	145	136
RM271	117	94	94	94	94	94	94	94	94	83	83	83	94	94	83	94	94	94	83	83
RM321	176	163	176	176	176	176	176	176	176	176	176	176	176	176	176	176	176	176	202	202
RM537	245	203	245	218	218	203	164	164	203	218	218	245	218	218	218	245	245	218	218	245
RM3231	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146
RM302	146	146	146	146	146	146	153	146	153	146	146	146	153	146	153	160	160	160	146	146
RM212	95	98	98	106	106	106	106	106	106	98	98	98	106	106	106	106	106	106	106	106
RM328	234	218	242	234	270	242	255	270	270	242	270	255	234	255	270	270	234	270	242	218
RM316	186	186	186	186	193	193	193	193	193	193	193	186	186	193	193	193	193	193	186	186
RM5443	145	154	145	154	154	154	154	154	145	154	154	154	134	134	145	124	124	124	124	145
RM215	122	122	122	148	148	131	131	131	131	131	148	148	148	148	148	164	164	164	131	164
RM242	241	208	241	241	241	208	241	241	208	165	208	165	208	208	226	226	241	241	241	241
RM257	154	145	126	154	126	126	137	145	145	145	145	154	137	137	154	137	137	137	137	137
RM219	173	165	165	173	165	165	165	165	165	165	165	165	165	165	165	165	165	165	165	165
RM288	111	111	111	111	111	111	111	111	111	111	121	121	121	121	111	111	111	111	111	111
RM296	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139

ALLELE A   ALLELE B   ALLELE C   ALLELE D   ALLELE E

Figure 4. Microsatellite panel of studied rice landraces against used 45 SSRs.

and RM 219) to 0.765 (for RM 3412 and RM 328) with an average PIC value of 0.524. As overall observation, the SSR loci RM 3412 followed by RM 328, RM 206 and RM 493, were found to be superior for analysis of allelic diversity in this study. Similarity matrix revealed that the similarity coefficient value ranged from 0.0 (between Balaram Sail-Nekrai Sail and Rup Sail-Sindur Sail) to 0.98 (between Lati Sail and Nagra Sail). The dendrogram (**Figure 5**) showed four major clusters: Cluster I, II, III and IV. Within the first cluster, Chapri Sail and Kabirraj Sail are very much close to each other; whereas in second cluster, Rup Sail and Sindur Sail are closely related. In Cluster-III and IV, the pairs of Rani Sail-Ratan Sail and Neizer Sail-Punjab Sail are closely linked, respectively.

4. Discussion

Analysis of variance (ANOVA) indicates the presence of considerable amount of variability amongst the different studied cultivars for all studied physio-biochemical parameters. Reduction in seed G% was higher in PEG compared to NaCl indicates that at equivalent osmotic potential, seed germination was better in NaCl than the PEG as reported by Gulzar and Khan [44]. The explanation of the higher inhibitory effects of PEG than NaCl lies in ion or solute entry into the seed [45].

It was clear from the above results that increased concentration of both PEG and NaCl reduce seedling growth. Such type of stress tolerance is characterized by an extensive root growth and small reduction of shoot growth in stressed conditions [46]. Pradhan *et al*. [47] reported that root length and rootlet number were increased due to severe moisture stress.

Castillo *et al*. [48] and Cha-um *et al*. [49] have been reported that the quantity of different biochemical components like protein, chlorophyll, proline, etc. in mature rice tissues were changed under drought and/or salinity stress conditions. Chlorophyll pigment also tends to decline rapidly under osmotic stresses [50]. Presence of high proline content is a good index for osmotic stress tolerance and under stressed condition, the cellular protein degrades and consequently, the proline content increases [51].

Range of the SSR genotyped amplified alleles (2 - 5 per locus) was much similar to the earlier studies on another set of rice genotypes [17,18]. The overall poly-

Table 3. Analysis of variance of the physio-biochemical parameters in PEG and NaCl treatments for twenty rice genotypes.

Parameters	SOV	df	MSS	F-value	p-value
Germination %	Genotypes	19	369.77	3.526291[*]	1.4
	Treatments	6	2636.19	25.13958[*]	7.75
	Error	114	104.86		
Shoot length	Genotypes	19	1.82	17.40367[*]	2.37
	Treatments	6	31.32	298.9713[*]	2.71
	Error	114	0.105		
Root length	Genotypes	19	2.17	21.91527[*]	1.61
	Treatments	6	46.12	465.9547[*]	1.01
	Error	114	0.099		
Proline	Genotypes	19	290.87	16.1[*]	5.25
	Treatments	6	1646.21	91.12[*]	3.75
	Error	114	18.07		
Chlorophyll	Genotypes	19	1.93	298.24[*]	7.58
	Treatments	6	0.37	57.61[*]	2.99
	Error	114	0.0064		
Protein	Genotypes	19	603.56	36.98[*]	7.11
	Treatments	6	1128.21	69.13[*]	1.1
	Error	114	16.32		

SOV = source of variation, df = degree of freedom, MSS = mean sum of square, [*]indicates significant at 5% level of probability ($p < 0.05$).
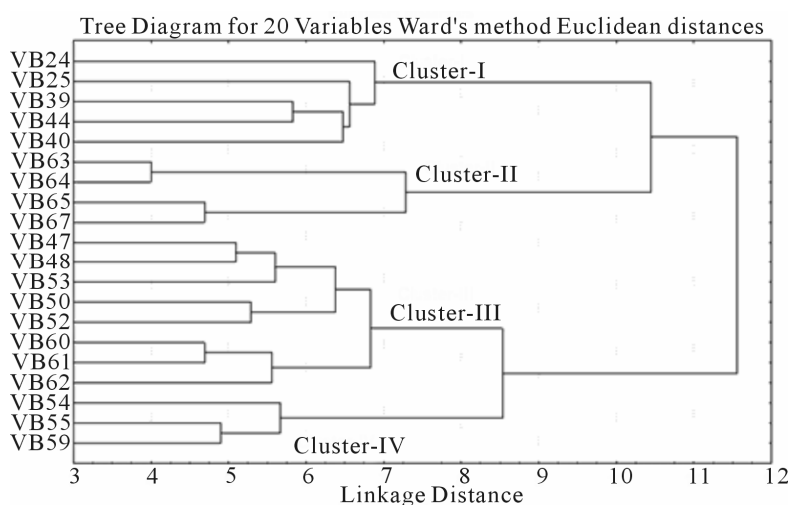
Figure 5. Clustering of twenty rice landraces based on 45 SSR fingerprint.

morphic frequency of biallelic markers was higher than the tri-, tetra- and pentaallelic SSRs [11]. The number of SSR amplified alleles and their PIC values also depend upon the repeat motif and the repeat sequence of the microsatellites [52]. Temnykh *et al*. [53] showed that (CTT) and (AT) rich repeat motifs amplified with higher efficiency and revealed greater overall polymorphism. In this context, it was observed that SSRs (RM 493, RM 206 and RM 3412) with (CT)-rich di or tri-nucleotide repeats were effective for producing the highest number

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Table 4. Amplicon size range, total scorable bands, total alleles, number of unique alleles, polymorphic alleles and percentage of polymorphism for each SSR marker.

SSRs	No. of alleles amplified	No. of rare allele	Allelic size (bp)		Major allele		PIC
			Range	Difference	Size (bp)	Frequency (%)	
RM 1287	3	0	121 - 148	27	148	40	0.655
RM 3412	5	0	183 - 238	55	200	35	0.765
RM 10764	3	1	80 - 156	76	136	80	0.335
RM 10772	3	0	427 - 512	85	447	80	0.340
RM 493	5	1	130 - 261	131	178	55	0.640
RM 140	4	1	210 - 309	99	272	55	0.610
RM 223	4	1	136 - 170	34	158	55	0.595
RM 212	2	0	111 - 121	10	111	90	0.180
RM 206	5	1	131 - 167	36	139, 167	35	0.720
OSR 2	2	0	57 - 66	09	57	85	0.255
RM 209	3	0	124 - 144	20	137	45	0.615
RM 287	2	0	97 - 105	18	105	85	0.255
RM 10720	4	0	192 - 221	29	214	55	0.625
RM 10748	3	0	86 - 97	11	91	65	0.505
RM 8115	2	0	107 - 115	08	107, 115	50	0.500
RM 10843	2	0	151 - 161	10	161	60	0.480
RM 562	4	1	225 - 284	59	240	45	0.665
RM 10890	2	0	249 - 262	13	249, 262	50	0.500
RM 7075	3	0	109 - 130	21	117	45	0.615
OSR 28	3	1	129 - 144	15	134	65	0.485
RM 136	3	1	86 - 113	27	86	65	0.515
RM 107	4	1	238 - 292	54	239, 253	40	0.655
RM 553	3	0	155 - 177	22	155	60	0.540
RM 3825	3	0	147 - 162	15	153	65	0.505
RM 201	4	1	125 - 170	45	135	60	0.545
RM 271	3	1	83 - 118	35	94	65	0.485
RM 321	3	1	162 - 202	40	175	85	0.265
RM 537	4	0	164 - 245	81	218	45	0.725
RM 302	2	0	146 - 156	10	146	65	0.455
RM 212	2	0	96 - 106	10	106	70	0.420
RM 328	5	0	217 - 270	53	270	35	0.765
RM 316	2	0	185 - 193	08	193	60	0.480
RM 5443	4	0	123 - 154	31	154	40	0.685
RM 215	4	0	121 - 164	43	148	35	0.725
RM 242	4	0	165 - 242	77	242	50	0.640
RM 257	4	0	126 - 154	28	137	35	0.665
RM 219	2	0	165 - 173	08	165	90	0.180
RM 288	2	0	110 - 121	11	110	80	0.320
TOTAL	122	12	-	-	-	-	19.91
AVERAGE	3.21	0.32	-	-	-	-	0.524

of alleles as well as highest values of PIC than other SSR markers. While variation in the size of SSR alleles results from a change in the number of repeats is the consequence of sequence changes at the primer binding site(s) [54]. The genetic relationship among the studied rice genotypes after SSR based molecular profiling has also partially confirmed by the physio-biochemical screening in relation to osmotic stress tolerance. Malik Sail and Rani Sail showed 100% germination in all stressor (PEG and NaCl) concentrations and were placed in a single cluster and thus initially proved as stress tolerant genotypes. Rup Sail and Sankar Sail showed 100% germination in only NaCl conc., but not in PEG and were placed in an another cluster. For selection of the parental rice genotypes for the development of a mapping and/or breeding population, Laxmi Sail, Mihinagra Sail, Ratan Sail and Raghu Sail were proved as stress susceptible cultivars and were placed in one cluster. On the other hand, Rup Sail-Sindur Sail and Punjab Sail-Neizer Sail pairs were found to be closely linked together in other two clusters, respectively. For future rice breeding program, the probable parental combinations derived from the partially completed analyses will be further studied for selection of the proper parental genotypes.

5. Conclusion

The physiological screening and fingerprint profile, with the aid of quantified biochemical compounds in different stress tissue of the selected traditional rice lines are the first attempt to make their overall multi-informative profiling in relation to osmotic stress tolerance. Osmotic stresses, mainly drought and salinity, adversely affected the germination percentage as well as shoot and root length of germinated seedlings which are necessary to have optimum plant growth for crop production. Chlorophyll and protein contents were declined in increasing stress doses compared to the control; whereas proline was accumulated at the higher concentration in the mature leaves with an elevated stress dose. The 45 sets of SSR markers provide a positive assessment by their ability to produce unique DNA fingerprinting profile of traditional rice genotypes leading to their genetic relationship and diversity. Microsatellite profiling combined with physiological and biochemical screening revealed that Malik Sail and Rani Sail showed full germination at both osmotic stress (due to PEG and NaCl) conditions and were recommended as tolerant genotypes; whereas Laxmi Sail, Mihinagra Sail, Ratan Sail and Raghu Sail were proved to be susceptible to drought and salinity stress for all aspects. The obtained data can be used for varietal identification and the construction of a database of such vulnerable rice landraces of West Bengal. This investigation would be more significant and useful if it can be used for more numbers of trait linked SSR markers spanning all twelve rice chromosomes.

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