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RESEARCH ARTICLE

Genetic Diversity of Lowland Rice (*Oryza sativa* L.) Genotypes in Relation to Germination Stage Oxygen Deficiency Tolerance

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Abstract

Rice genotypes tolerant to germination stage oxygen deficiency (GSOD) can reduce weed infestation and can help in maintaining optimum plant stand in direct-seeded rice. Out of 43 rainfed lowland rice genotypes, AC41620A with greater establishment ability and vigor index was found to be the best under submergence at the germination stage. Further, genetic diversity was studied with 47 polymorphic simple sequence repeats (SSR) markers. Allele numbers varied between 2 and 4 and all together 11 unique alleles were identified in 10 genotypes with six SSR markers such as RM339, RM1187, RM10695, RM6840, RM1183 and RM7180. Polymorphic information content value was more than 0.7 in RM235, RM219 and RM11 indicating the usefulness of these markers for genetic studies associated with GSOD sensitivity. The results from the study are useful for marker-assisted selection as well as QTL discovery for GSOD tolerance.

Keywords: Allelic diversity, Anaerobic germination potential, Germplasm and SSR markers**.**

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Introduction

Any event which diminishes rice production is a serious threat to food security. Rice is one of the important food crops, yet rice cultivating farmers are in distress condition due to the increasingly high cost of cultivation and climatic aberrations. Rice crops in the same season are experiencing drought, submergence, and stagnant floods, apart from salinity stress in coastal areas (Kumar and Ladha *et al.,* 2011; Sarkar and Ray *et al.,* 2016). The adaptation to extremely variable conditions in rice offers a scope to combat the current challenges imposed by variable abiotic stresses, as well as a means to cope with the adverse effects of climate change, to secure food and livelihood. Rice genetic resources are vast, yet a fraction of it has been utilized for crop improvement programs which may be attributed to a lack of proper phenotyping and knowledge on stress-sensitivity and other important agronomic traits (Xie *et al*., 2015; Reynolds et *al*., 2021). Germplasm description has now gained analytical power for resolving the genetic basis of trait variation, diversity patterns, and their adaptations in definite or variable environments. Tapping the genetic diversity, and development of climate-proof rice cultivars is possible, as genes associated with tolerance of various abiotic stresses are available within the cultivated gene pool, offering considerable opportunities for genetic improvement. The approach involving the identification of tolerant germplasm and associated QTLs/genes has paid rich dividends in developing stress-tolerant high-yielding cultivars for commercial utilization (Neeraja *et al*., 2007; Thomson *et al*., 2010; Ismail *et al*., 2012; Chattopadhyay *et al*., 2014).

Rice is cultivated either through direct seeding or transplanting mode. The transplanting mode of rice cultivation is mainly practiced under irrigated and favorable rainfed lowland conditions whereas direct seeding is practiced in both upland and rainfed lowlands where assured water supply is lacking. The transplanting mode of rice cultivation is highly water intensive, cumbersome and laborious as compared to direct-seeded rice (Kaur and Singh, 2017; Adigbo *et al*., 2018). Moreover, direct-seeded rice emits less greenhouse gas as compared to transplanted rice (Aulakh *et al.,* 2001). Though direct seeding is highly beneficial, practicing direct seeding is not increasing as expected due to fear of high weed infestation and optimum plant stand (Sarkar *et al.,* 2012; Kaur and Singh *et al.,* 2017). The problem is aggravated if seeds are sown beneath the soil surface and stagnation of water occurs due to untimely rain or natural flooding. Standing water of 5-10 cm depth creates a hypoxic/anoxic zone beneath the soil surface, which jeopardizes germination and sometimes total land becomes barren. All the above factors imply that cultivation through direct seeding is risky. To overcome such problems seed priming/seed coating with calcium peroxide was proposed (Ota *et al.,* 1982; Sarkar *et al.,* 2012). The calcium peroxide-coated seeds create an oxidized zone in the vicinity of the seeds through the release of oxygen which helps in proper germination and establishment of rice under flooded soil conditions. The treatments need extra investment and are still not so popular among the farmers except in Japan. Due to soil flooding, oxygen limitation during germination occurs (Ray *et al*., 2016). The tolerant rice genotype has the capacity to germinate and extend its coleoptiles even in the complete absence of oxygen (Vijayan *et al.,* 2018) – a phenomenon termed anaerobic germination (AG). The present study showed that anaerobic germination potential (AGP) or tolerance to germination stage oxygen deficiency (GSOD) and seedling vigor varied among rice genotypes. Genetic diversity among the rice genotypes tolerant to GSOD was studied employing SSR markers so that plant breeders could choose the genotypes of interest in developing GSOD-tolerant high-yielding cultivars suitable for direct seeding under soil flooding.

Materials and Methods

Plant materials

In the present investigation, we took 43 rice genotypes generally grown in flood-prone rainfed lowland ecosystems of West Bengal, Odisha and Kerala in India.

Phenotyping for germination stage oxygen deficiency tolerance

The seeds harvested during the preceding year were used for the experiment. Approximately 10 g seeds were put in brown paper packets with several small holes and these packets were further kept inside an oven at 48 ± 2°C. After five days the packets were taken out from the oven and kept in ambient conditions. Later on, these seeds were used for experimental purposes.

Seeds were sown 1.0 cm below the soil surface in a polypropylene tray (37.5 × 35 × 13.0 cm) containing a 2 cm depth of dried and pulverized soil. Submergence was provided with 10 cm water immediately after sowing. This water depth was maintained for 3 weeks. The seeds that germinated underwater and successfully pushed their leaf/coleoptile tips above the water surface after submergence treatment were considered as successfully established plants and accordingly establishment % was determined (Sarkar *et al*., 1999; Ismail *et al*., 2009). Plant height and shoot dry weight were measured to assess the variation in seedling vigor. Vigor index was calculated following Zhu *et al*., (2010). Vigour Index = Establishment (%)* Seedling length.

Extraction of genomic DNA and SSR assay

DNA was extracted from the leaves of 20-day-old seedlings using a plant genomic DNA isolation mini kit (Xcelris, Ahmedabad, India) following the standard procedure as described by the DNA isolation kit manufacturer. DNA concentration and quality were determined with agarose gel electrophoresis. Premix Taq polymerase of Xcelris (version 2.0) was used which contained Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer at optimal concentration for efficient amplification of DNA templates by PCR. Premix Taq version 2.0 contained blue dyes that allowed monitoring of progress during electrophoresis. The PCR reaction mixture of 25 µL quantity contained 2X Premix Tag (version 2.0) 10 µL, 10 µM forward primer 1-µL, 10 µM reverse primer 1-µL, DNA solution 2 µL (12.5 ng μL^{-1}) and doubled distilled water 11 µL. Over 47 primers were selected based on their ability to give positive, clear and polymorphic banding patterns in selected two genotypes such as AC41620A (tolerant) and FR13A (susceptible).

Statistical Analysis

The banding patterns obtained from molecular analysis were scored as presence (1) or absence (0). All the bands (polymorphic and monomorphic) were taken into account for similarity calculation to get proper genetic similarity value (Gherardi *et al*., 1998). Jaccard's coefficient of similarity was measured and a dendrogram based on the similarity coefficient generated by the un-weighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal *et al.,* 1973) and the SAHN clustering analysis. The analyses were done using the computer package NTSYS-PC (Rohlf *et al.,* 2000). The differences among the genotypes for various parameters were tested by ANOVA. Comparison of means was done by the least significant difference test when the *F* value showed at least $p < 0.05$ level of significance in *CropStat* (International Rice Research Institute, Manila, Philippines).

Table 1: Plant establishment, seedling length and dry weight after 21 days of submergence at germination stage

S. No.	Name	Establishment (%)			Seedling length (cm)			Shoot dry weight (mg Plant^{-1})		
		2014	2015	Average	2014	2015	Average	2014	2015	Average
1	JRS8	40	35	37	30	32	31	23	24	23
2	JRS20	35	37	36	30	32	31	27	28	27
3	JRS21	63	45	54	33	32	32	24	24	24
4	JRS155	48	28	38	33	31	32	26	26	26
5	JRS182	63	58	60	30	31	30	29	29	29
6	JRS196	38	28	33	29	29	29	26	26	26
7	AC393	83	63	73	31	33	32	21	26	23
8	AC813	58	42	50	30	30	30	12	21	16
9	AC917	73	60	66	32	27	29	20	25	22
10	AC1151	70	52	61	31	33	32	23	24	23
11	AC34352	58	35	46	34	36	35	30	24	27
12	AC34245	70	50	60	31	35	33	26	34	30
13	AC34280	65	39	52	32	35	33	21	25	23
14	AC40331	30	23	26	30	33	31	27	31	29
15	AC40331A	35	37	36	33	36	34	28	21	24
16	AC40638	48	58	53	31	30	30	38	25	31
17	AC40346	68	68	68	27	35	31	24	20	22
18	AC41622A	48	67	57	26	29	27	21	24	22
19	AC41647	73	47	60	37	30	33	31	27	29
20	AC41644A	53	62	57	25	34	29	18	29	23
21	AC41620A	90	85	87	30	31	30	23	37	30
22	AC41620	65	75	70	28	34	31	26	29	27
23	AC41644B	68	60	64	29	26	27	24	22	23
24	AC34289	46	77	61	27	39	33	19	31	25
25	AC41644	60	52	56	35	24	29	28	18	23
26	AC39393	20	40	30	37	28	32	24	24	24
27	AC39384	28	38	33	35	28	31	28	17	22
28	AC39390	48	63	55	28	28	28	25	24	24
29	AC39397	40	42	41	41	28	34	28	20	24
30	AC39418	43	60	51	34	36	35	31	29	30
31	AC39406	35	30	32	41	27	34	24	24	24
32	AC39460	53	38	45	36	35	35	23	27	25
33	Pantara	48	42	45	29	30	29	23	25	24
34	Panikekua	70	45	57	34	41	37	24	28	26
35	AC39416A	70	58	64	34	37	35	28	30	29
36	Kamini	58	45	51	28	37	32	16	25	20
37	Ravana	55	37	46	40	36	38	23	27	25
38	Paloi	50	30	40	31	35	33	35	28	31
39	Pokkali	48	40	44	28	30	29	13	17	15
40	Talmugra	55	47	51	35	33	34	31	26	28
41	Morisal	39	43	41	21	20	20	18	14	16
42	Rashpanjor	70	66	68	39	29	34	31	26	28
43	FR13A	18	13	15	25	30	27	24	22	23
LSD $p < 0.05$		13	11	---	4	3	---	5	6	---

Results

Variability in anaerobic germination traits

Soil flooding during germination significantly decreased the establishment of the seedlings (Table 1). The average of two-year data showed that plant establishment (%) varied significantly under submergence. It ranged from 15 to 87% among different genotypes. FR13A was highly susceptible whereas AC41620A is highly tolerant at germination stage submergence. Establishment was 60 to 87% in thirteen genotypes namely AC41620A (87%), AC393 (73%), AC41620 (70%), Rashpanjor and AC40346 (68%), AC917 (66%), AC41644B and AC39416A (64%), AC1151 and AC34289 (61%), JRS182, AC34245 and AC41647 (60%). Medium establishment (40–59%) was observed in 18 genotypes. Nine genotypes exhibited 20 to 39% establishment. Average seedling length varied from 20 cm (Morisal) to 38 cm (Ravana) whereas average dry weight per plant varied between 15 (Pokkali) and 31 mg (AC40638). Wide variations in vigor index were observed among the 43 rice genotypes (Fig. 1). Vigour index was maximum in AC41620A (2610), followed by AC393 (2336), Rashpanjor (2312), AC39416A (2240), AC41620 (2170), Panikekua (2109) and AC40346 (2108). The vigor index was merely 405 in susceptible genotype FR13A. The vigor index was below average (1588) in nineteen genotypes whereas the values of vigor index were above average in 24 genotypes.

SSR marker characteristics, Allelic diversity, PIC values and unique Alleles

The molecular diversity analysis data showed that 47 simple sequence repeats (SSR) markers amplified 123 loci covering all the chromosomes of rice except chromosome number 10 (Table 2). Most of the polymorphic primers were linked to chromosome 1 (17 primers) chromosome 9 (7 primers), and 2 and 12 (5 primers). The amplicon size varied from 90 bp (RM3753) to 400 bp (RM10864) and the average alleles per locus were 2.61. The PIC values ranged from 0.088 (RM339) to 0.757 (RM235) with a mean of 0.488. PIC values were more than 0.7 in the case of three primers viz., RM235, RM219 and RM11 (Table 2). Allelic numbers per locus showed a highly significant negative association with major allelic frequency (%) and a positive association with PIC values (Fig. 2). Major allelic frequency (%) showed a highly significant negative association with PIC values (Table 3). Six primers such as RM339, RM1187, RM10695, RM6840, RM1183 and RM7180 indicated the presence of unique alleles in ten rice genotypes. The allelic size of these primers ranged from 130 bp to 170 bp. RM1187 identified two unique alleles in AC41644 and JRS8, whereas RM10695 identified one unique allele in AC41644, which was a GSOD-tolerant genotype. Marker RM339 identified two unique alleles in GSOD tolerant AC41620 and GSOD susceptible Pantara.

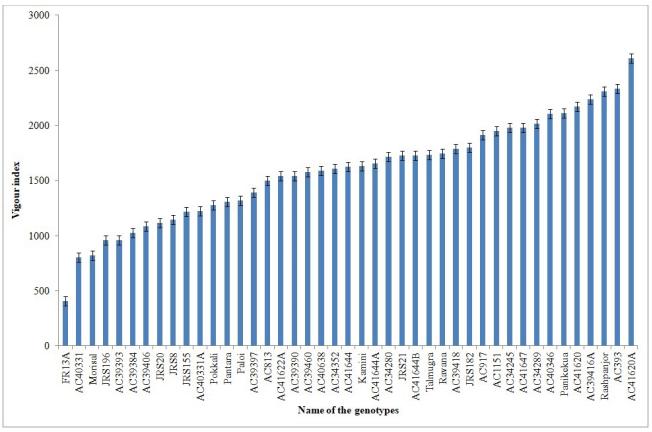

Fig. 1: Vigour index of 43 rice genotypes. Bar represents critical difference at $p<0.05$

Clustering of genotypes

The average similarity among the studied genotypes was 0.509. The maximum similarity was observed between AC40331 and AC40331(A) with a similarity value of 0.775 (Supplementary Table S1). The genotypes were divided into two clusters (Fig. 3). Cluster A comprised three genotypes namely JRS20, JRS196 and FR13A and cluster B contained the rest 40 genotypes. The Cluster B was further divided into two sub-clusters such as B1 and B2. Cluster B1 consisted of only 2 genotypes namely Panikekua and Ravana and cluster B2 again sub-clustered into two groups. The sub-cluster B2A monotypic with only one genotype i.e. Pantara. B2B was further sub-divided into two groups with 27 genotypes in group B2B1 and 10 genotypes in group B2B2. All 27 genotypes under group B2B1 were again grouped into two groups with 19 (B2B1I) and 8 (B2B1II) genotypes.

Discussion

Greater seedling vigor under normal as well as under submergence conditions is a useful trait in direct-seeded rice (Vu *et al*., 2015). After germination, fast seedling growth suppresses weed infestation and pushes the coleoptiles above the water surface very quickly which improves the crop stand. An optimum crop stand is the guarantee of good yield (Sarkar, 2012). The present investigation revealed that 13 genotypes were highly capable of germinating under submergence with establishment % under submergence varying from 60 to 87%. Likely, there was great variation in seedling length and dry weight. Identification of new genetic resources opens up new avenues for crop improvement programs. Prospects of identification of new genes/ quantitative trait loci (QTLs) have been increased ultimately helping to develop stress-tolerant crops (Haussmann *et al*., 2004; Burke *et al*., 2009). New QTLs have been identified from rice genetic resources, which imparted tolerance to submergence (Neeraja *et al*., 2007), salinity (Thompson *et al*., 2010; Chattopadhyay *et al*., 2014), drought (Sandhu *et*

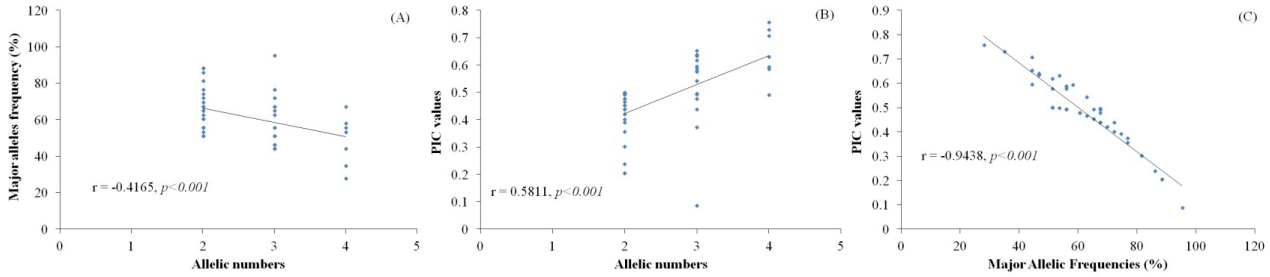

Fig. 2: Association among allelic numbers with that of major allelic frequency (Fig. 2A), PIC value (Fig. 2B) and between PIC value and Major allelic frequency (Fig. 2C). 'r' is the correlation coefficient

al., 2019) and other abiotic stresses. Genotypes tolerant to germination stage oxygen deficiency (GSOD) or with greater anaerobic germination potential (AGP) were identified earlier (Yamauchi *et al*., 1993; Sarkar *et al*., 1999; Angaji *et al*., 2010; Barik *et al*., 2019), however, identification of robust QTLs were not yet achieved (Angaji *et al*., 2010). Kim and Reinke *et al*., 2018 reported that the presence of three QTLs such as *qAG1a*, *qAG1b* and *qAG8* associated with AGP showed 50% establishment whereas establishment % was reduced to 32 to 36% when only two QTLs were present. Establishment % did not show any significant association with seedling length and dry matter accumulation per plant. Seedling length, however, showed a significant positive association with dry matter accumulation (r = 0.472, *p< 0.01***). This showed that establishment %underwater was the main hurdle rather than seedling length and dry weight (Miro *et al*., 2017). The vigor index ranged from 405 in susceptible genotype FR13A to 2610 in highly tolerant genotype AC41620A. The vigor index was more than 2000 points in seven genotypes such as AC393, Rashpanjor, AC39416A, AC41620, Panikekua, AC40346 and AC34289. Yamauchi and Winn *et al.,* (1996) found a highly linear correlation between establishment % in a field or a laboratory with a vigor index. In the present investigation, we also noticed a highly significant association between establishment % and vigor index (r = 0.946, *p <0.001****). The association between seedling length and vigor index (r = 0.368, *p <0.05**) and dry weight and vigor index (r = 0.304, *p <0.05**) was also significant.

Allelic variations of 47 polymorphic markers across 43 genotypes revealed two to four alleles with an average of 2.61 alleles per marker. Allelic variation describes the changes that happened in due course of time due to the influence of both micro- and macro-environment on a particular genotype and its adoption to the said environment (Vemireddy *et al*., 2019). It also describes the genetic changes that occur at a specific locus on a chromosome. Allelic variation is greatly exploited in crop improvement (Shao *et al*., 2019). Major allelic frequency ranged from 27.9 (RM235) to 95.3% (RM339). The association between allelic numbers and major allelic frequency (%) was negative (Fig. 2A), suggesting that the genotypes collected from eastern Indian coastal rainfed lowland areas were highly diverse.

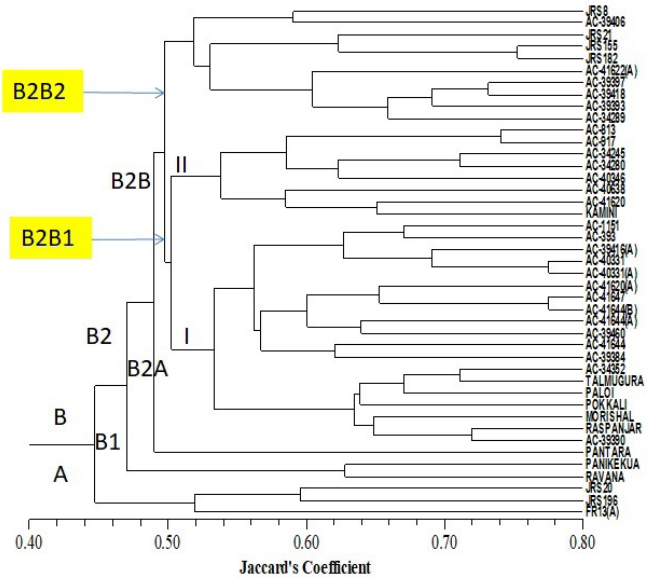

Fig. 3: Clustering of 43 rice genotypes based on 47 SSR markers

The association between major allelic frequency (%) and PIC value was negative (Fig. 2C). Lower % of major allelic/gene frequency determines the divergent nature of the plant or the presence of variant genes of the same locus (Morton *et al*., 2001). The results were comparable with Vu *et al*., (2015) who studied the diversity of Vietnamese rainfed lowland rice genotypes.

Markers with more than 0.5 PIC values were considered highly informative and could be employed to detect more alleles within germplasm accessions (Vu *et al*., 2015). Out of 47 SSR markers, only 15 markers scored more than 0.5 PIC values. PIC value showed a highly significant positive association with allelic numbers (Fig. 2B). Unique alleles are important to make distinctions among different genetic groups of the same species (Szczecińska *et al*., 2016). Unique alleles have appeared in both GSOD tolerant and susceptible genotypes with PIC values ranging from 0.088 to 0.631. Among the ten genotypes, that showed the presence of unique alleles, a few such as AC41644, Panikekua and Kamini were medium-tolerant and AC41620 was tolerant to GSOD (Table 1). The remaining genotypes were either tolerant to submergence e.g. FR13A (Jambhulkar *et al*., 2018) or tolerant

Table 2: Forty-seven SSR markers characteristics across 43 rice genotypes

Primers	*5'<-----Sequence----->3'*	*Chr. no.*	*Position (Mb)*	*Size (bp)*	*Allelic no.*	*Major allelic / gene frequency (%)*	*PIC*
RM 235	F: AAGCTAGGGCTAACGAACGAACG R: TCTCCATCTCCATCTCCATCTCC	12	26.17	130-160	4	27.9	0.757
RM 219	F: CGTCGGATGATGTAAAGCCT R: CATATCGGCATTCGCCTG	9	11.17	200-240	4	34.9	0.730
RM 11	F: ATCGGTGCTTGGCTGGATAGC R: CCACCTTCTTCTCCTCCTCTTCC	7	19.20	120-150	4	44.2	0.708
RM8094	F: AAGTTTGTACACATCGTATACA R: CGCGACCAGTACTACTACTA	1	11.24	180-200	3	44.2	0.653
RM 23877	F: TGCCACATGTTGAGAGTGATGC R: TACGCAAGCCATGACAATTCG	9	6.35	300-400	3	46.5	0.640
RM 3769	F: CTGAAATCTGTGAAAGCCTGAACG R: GCTGGTGACAAACTGCATCTTCC	9	11.69	200-220	3	46.5	0.634
RM 6840	F: CGACTGGAAGAAGGGATCATGG R: CACACTACCAAGACTCCGCTATGG	1	43.16	130-160	4	53.5	0.631
RM 208	F: AGTACCACCACCATTCTCTGCAAGC R: TCGATTGGCCATGAGTTCTCG	2	35.16	160-200	3	51.2	0.619
RM 3753	F: GCACAGTGAATGAGCTAAGAACACG R: TCCAATACGATAAGTGGCTGATGG	7	23.61	90-120	3	44.2	0.596
RM 6386	F: ATCATCGTCGTCATCCTCTCTCC R: CGTCCAGTTCGTAGGCGTATAAGG	12	25.03	130-160	4	58.1	0.594
RM 444	F: TGCATCTTTCACCGTAGTCCTAGC R: CTTGCTGGAGCTCGTAGATGC	9	5.87	400-450	3	55.8	0.588
RM 1183	F: AGCCATTTCCAGCAGTTCATGC R: CCAGTTTCAAGGCTCGGAAGC	1	30.97	140-170	4	55.8	0.586
RM 518	F: AAGACACAAGCAAACAGCTCAACC R: AAGCTTGCTTGGTTCAAGAGAGG	4	2.02	160-180	3	55.8	0.578
RM 259	F: GAAGTGCTCCCTAAACTTGTTGC R: TTATGGAGGATGGATTCGAAGG	1	7.44	150-170	3	51.2	0.577
RM 12292	F: ATGAGACGATGAAAGCCTCAAGC R: GTGGGACAAGCAAATTGAAACG	1	43.22	140-160	3	62.8	0.543
RM 263	F: AATCTATGGACCTGGGAGGAACC R: TGACGAGAGTGCTACGTTTGAGC	2	25.89	180-200	2	51.2	0.500
RM 582	F: TCTGTTGCCGATTTGTTCG R: AAATGGCTTACCTGCTGTCTC	1	9.19	210-220	2	51.2	0.500
RM 7338	F: CGCATGGATCAATCAATAGTGG R: CAAGTGCTGCTACTCTGTCTCTTGG	7	15.33	320-330	2	51.2	0.500
RM 5793	F: TGGACACAACACATTCCATCTCC R: TCAGCTTTCTTCTTTCTCCCAAGC	7	17.44	140-150	2	53.5	0.498
RM 253	F: CCATCTCTGCCTCTGACTCACC R: TCCTTCAATGGTCGTATCTTCTCC	6	5.44	130-150	3	67.4	0.497
RM 520	F: ACGATAACGCCGACATCACTGG R: GCTAAGCATCCACGGTTTCTCTCC	3	30.72	140-150	2	55.8	0.493
RM 28766	F: ACCAACCAGTCACTGATCTTAGC R: ACCTAACGAAAGAGATCGAACC	12	26.65	100-130	3	65.1	0.493
RM 28748	F: TGAGAACACGCTCTTGAGTATTGC R: TGTTGTGTGCCTTACAGCAGAGG	12	26.44	440-450	2	55.8	0.493
RM 23911	F: TGCCTGCACTTATCTCTTGATGC R: GATGAACCTAAAGGGCAGTTTCC	9	7.15	260-270	2	55.8	0.493
RM 3808	F: CAGTGGCGTGGAGAGAAATTTGG R: CTCACCTGCGACAGCAAGATCG	9	20.25	280-300	2	55.8	0.493

RM 10695	F: CCTTCGACTCCATGAAACAAACG R: TCTCTTTGCCCTAACCCTATGTCC	1	10.98	140-170	4	67.4	0.492
RM 11701	F: CTGGTGGAGTTGCAGTGCCTCTAGC R: CCTTGCTGCTTTCTCATTGAAACTGG	1	32.02	200-220	2	60.5	0.478
RM 11008	F: TTTGGATGGTCATTAGCCTCTGG R: ATCAACCTTGCATGCTGTCTTCC	1	17.97	110-120	2	60.5	0.478
RM 10864	F: GAGGTGAGTGAGACTTGACAGTGC R: GCTCATCATCCAACCACAGTCC	1	14.24	400-600	2	60.5	0.478
RM 1187	F: CTACTGAGCCATCGCATGAGTGC R: TAGTTGTCTCCTGCCGTTGTTGG	5	23.13	140-160	3	67.4	0.478
RM 5378	F: GCTGCGTTCTACTACTAGCCTACCC R: GCGCTCAATTAGAGTTGAGTTTGG	2	29.89	150-160	2	62.8	0.467
RM 149	F: GGAAGCCTTTCCTCGTAACACG R: GAACCTAGGCCGTGTTCTTTGC	8	24.72	240-250	2	65.1	0.454
RM 468	F: AAAGATCCGTGTCCTCAATCAGC R: CCTAAAGCCCTTCCTTGTTGTGG	3	32.47	510-520	2	65.1	0.454
RM 232	F: CCGGTATCCTTCGATATTGC R: CCGACTTTTCCTCCTGACG	3	15.92	140-160	3	72.1	0.440
RM 341	F: CAAGAAACCTCAATCCGAGC R: CTCCTCCCGATCCCAATC	2	75.00	180-190	2	67.4	0.439
RM 28759	F: CTCTCTGTTCATCACTGGCTTCG R: GAGAATCGTGTTGCAGAAGTTGC	12	26.51	180-200	2	67.4	0.439
RM 104	F: GGAAGAGGAGAGAAAGATGTGTGTCG R: TCAACAGACACACCGCCACCGC	1	27.54	110-120	2	67.4	0.439
RM 206	F: ATCGATCCGTATGGGTTCTAGC R: GTCCATGTAGCCAATCTTATGTGG	11	21.63	140-150	2	69.8	0.422
RM 12288	F: AGCTCGGCCCTTGTGCTTCC R: GCTGGCCCATCAGAGTCAGAGC	1	43.21	150-160	2	72.1	0.402
RM 10685	F: TATCGGACTCTACTGAAACACC R: GTGTACTCCCTGCATTTCTAGG	1	10.90	150-160	2	74.4	0.392
RM 7180	F: GTGTTTATAGGGGTGCCACG R: TGTTGGTGGTGCAGGTAAAG	1	34.10	160-180	3	76.7	0.374
RM 10694	F: TTTCCCTGGTTTCAAGCTTACG R: AGTACGGTACCTTGATGGTAGAAAGG	1	10.97	250-260	2	76.7	0.357
RM 3475	F: ATGTTGTCGAGTCGTGGTAATGC R: TATTCCTCGGTGTATGGGTCTCC	1	26.04	170-180	2	81.4	0.303
RM 5526	F: CACATGATCCTCCACCCACTAGC R: GCCTGGCCTCTCTTATCTGTCTACC	9	7.26	150-180	2	86.0	0.240
RM 6318	F: AAGTGCCTCGAATTACACATCTCC R: GCTGCTTCTGTCCAGTGAGACC	2	24.44	170-180	2	88.4	0.206
RM 11125	F: CCAAGAACCCTAGCTCCCTCTCC R: TCGACGAGATCCTCCTCGTAAACC	1	20.54	200-220	2	88.4	0.206
RM 339	F: GTAATCGATGCTGTGGGAAG R: GAGTCATGTGATAGCCGATATG	8	72.2	140-160	3	95.3	0.088
Mean					2.62	61.4	0.488

to stagnant flooding e.g. JRS196 (Kuanar *et al*., 2017), or tolerant to salinity e.g. Pantara, Kamini and Paloi (Sarkar *et al*., 2013; Singh and Sarkar *et al*., 2014). This investigation revealed that genotypes with multiple allelic variations were a great source of new genes and could be utilized for plant breeding programs.

Genetic variation has two distinct advantages to a population. It allows some genotypes to fit into the

Table 3: Unique alleles as amplified by SSR markers in different rice genotypes

S. No.	*Markers*	*Unique Alleles No.*	*Allele size (bp)*	*Genotypes with unique Alleles*
1	RM339	1	160	Pantara
2	RM339	1	140	AC41620
3	RM1187	2	140	JRS8, AC41644
4	RM10695	1	140	AC41644
5	RM6840	2	130	Kamini, FR13(A)
6	RM1183	2	170	JRS20, JRS196
7	RM7180	2	180	Panikekua, Paloi
Total		11		Ten genotypes

environment while maintaining the endurance of the populace from an evolutionary point of view whereas it also enables to design of a crop suitable for a specific environment. A study of genetic diversity and relationship among the 43 rice genotypes using 47 SSR markers further revealed that three genotypes such as JRS8, JRS196 and FR13A constituted one group (Group A) and the rest 40 genotypes constituted another group (Group B). The genotypes in group A were susceptible to GSOD but possessed some unique features (Table 3). UPGMA method of analysis showed that the rice genotypes highly tolerant to germination stage oxygen deficiency (GSOD) were distantly placed compared to susceptible genotypes. Some genotypes that are tolerant to salinity but have good AGP potential such as Rashpanjor, Paloi, Talmugra, and Morishal are distantly placed as compared to GSOD tolerant genotypes such as AC41620 and AC41620A. Judging the similarity and dissimilarity, it can be said that the genotypes reported here possessed unique character and could be utilized in designing the plant suitable for direct seeding conditions even in areas where salinity is a threat to rice cultivation.

Conclusion

The study revealed that the genotypes collected from the eastern Indian states of Odisha, West Bengal and Pokkali region of Kerala showed a high degree of genetic diversity to GSOD tolerance. Genotypes such as Rashpanjor, Paloi, and Talmugra tolerant to both salinity and GSOD would be of great use in coastal rainfed lowland areas. SSR markers that gave high numbers of allelic diversity with a PIC value of more than 0.5 could be employed for marker-assisted selection as well as QTL discovery for GSOD tolerance.

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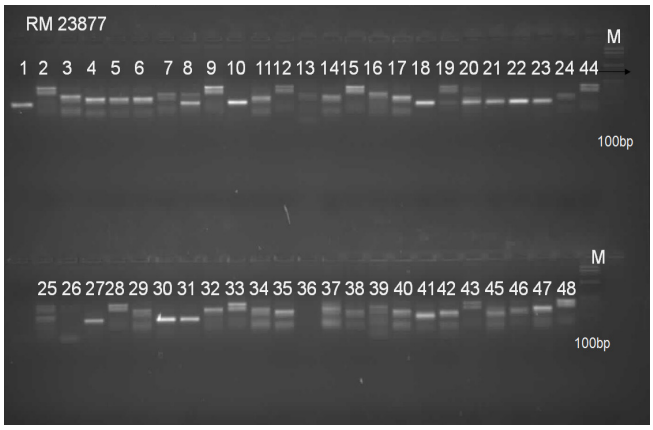


Fig. S1: SSR Marker RM 23877

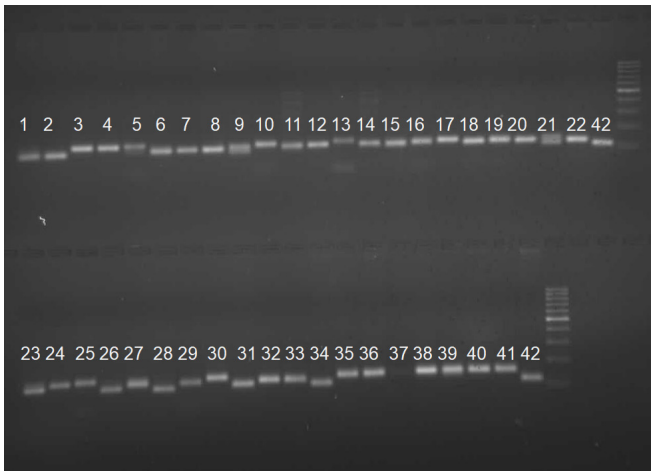


Fig. S2: SSR Marker RM 3753

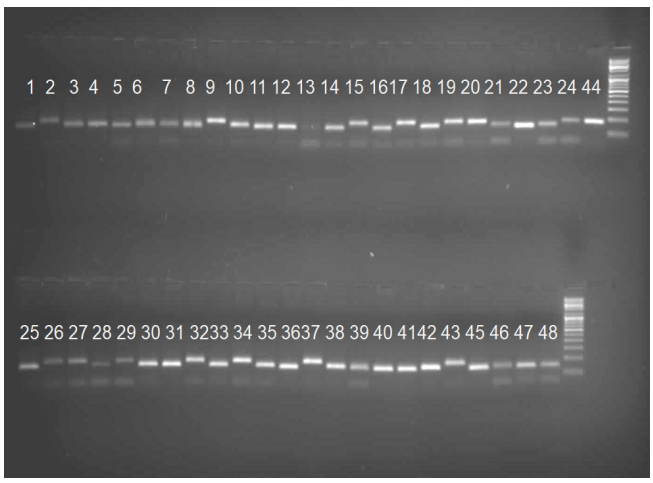
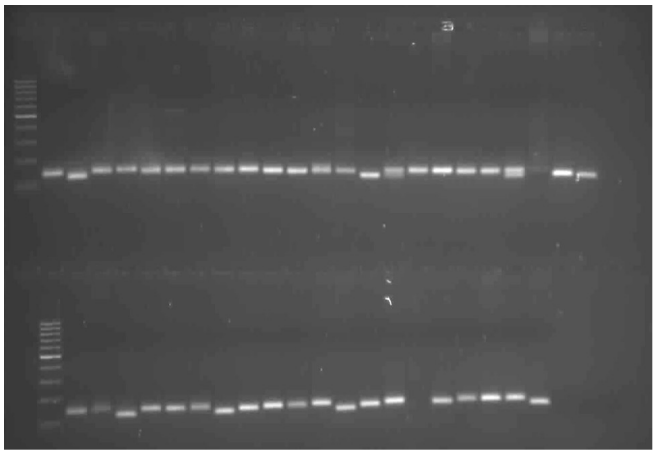
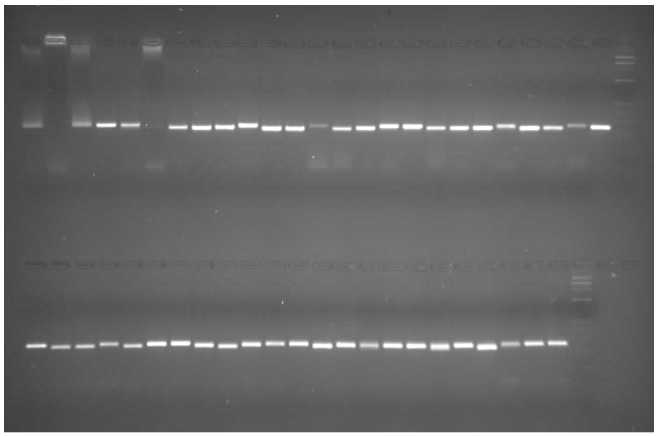


Fig. S3: SSR Marker RM 28759



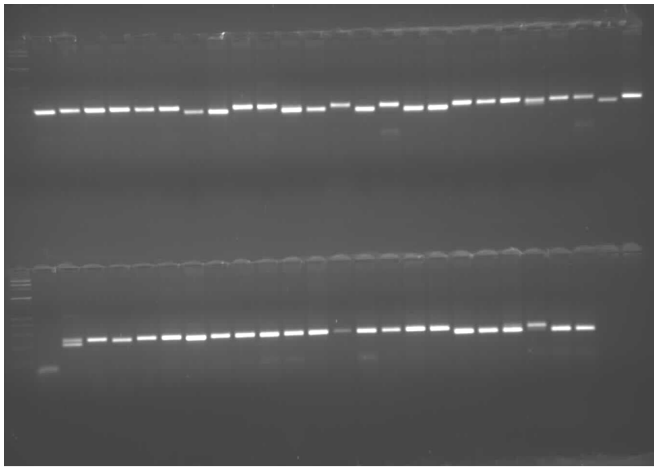
RM 253

Fig. S4: SSR Marker RM 253



RM 520

Fig. S5: SSR Marker RM 520



RM 10694

Fig. S6: SSR Marker RM 10694

Loading sequence of varieties are –LANE-1

1-JRS8,2-JRS20,3-JRS21,4-JRS155,5-JRS182,6-JRS196,7-AC 813,8-AC 917,9-AC 1151,10-AC 393,11-AC 34245,12-AC 34280,13-AC 40638,14-AC 40346,15-AC39416(A),16-AC 41620,17-AC 41620(A),18-AC 41647,19-AC 40331,20-AC40331(A),21-AC 41644(A),22-AC 41644(B),23-AC 41644®,24-AC 39384,
44- FR13(A)

Loading sequence of varieties are –LANE-2

25-AC 41622(A),26-AC 39397.27-AC 39418,28-AC 34289,29-AC 39393,30-AC 39406
31-AC 39460,32-AC 34352,33-KAMINI,34-PANTARA,35-PANIKEKUA,36-PALOI,37-POKKALI,38-RAVANA,39-MORISHAL,40-RASPANJAR,41-TALMUGURA,
42-AC 39390,43-AC 41620(A),45-FR13(A),46-NAVEEN,47-SWARNA,48-SWARNASUB1

Fig. S7: Loading Sequence of All Rice Genotypes As per Their Serial Number.