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Research Article

Genetic Diversity and Population Structure Among an Assorted Group of Genotypes Pertinent to Reproductive Stage Drought Stress in Rice (*Oryza sativa* L.)

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Abstract

Water availability is one of the significant challenges in the present climate change scenario in rice cultivation. Consequently, the identification of suitable rice genotypes for drought tolerance through phenotypic and genotypic approaches has become indispensable in rice research. In the present experiment, 76 rice accessions were evaluated for morphological and yield attributed traits under drought stress at the reproductive stage for two years. The same set of genotypes was genotyped by using 36 SSR markers. The biplot graph has grouped the genotypes into two groups based on grain yield and spikelet sterility. Molecular genetic diversity (MGD) estimated the average number of the allele was 2.66 per locus and Polymorphism information content (PIC) of 0.67. Among the total 96 alleles, seven unique alleles and 18 rare alleles were found across 76 rice genotypes, and an expected heterozygosity or gene diversity ranged from 0.576 (RM237) to 0.01 (RM25368) with an average of 0.267. Further, STRUCTURE analysis of these genotypes were grouped into two sub-populations (at K=2) with a membership percentage of 56.7% in Pop1, and 43.3% in Pop2 and their fixation index (F_{ST}) values of subpopulations were 0.496 and 0.021 for Pop1 and Pop2, respectively. AMOVA results revealed that 3.6% of the total variation was identified among the populations and 96.4% was within the population. The genetic similarity estimation among 76 accessions varied from 0.0282 to 0.9679. The phenotypic and genotypic evaluation results revealed significant differences between the landraces and improved varieties for reproductive stage drought tolerance. Drought stress at the reproductive stage caused the significant reduction in plant height, grain yield, ear-bearing tillers per plant, total dry matter production, grain weight and increase in the percentage of spikelet sterility. Three drought-tolerant genotypes were identified viz., Jonha 349, Dhalasaita and N TeHao. Among the ten markers that showed a high level of allelic diversity, three viz., RM215, RM271, RM306, and RM237 were promising. Therefore, the identified donors and markers would be promising information for marker-assisted breeding

Keywords: Drought; Reproductive Stage; Molecular Markers; Genetic Diversity; Population Structure; Polymorphic Information Content

Introduction

Rice, as a staple food, covers half of the world's population. It is grown in more than a hundred countries, with a total harvested area of approximately 158 million hectares. Annually, about 500 MT of milled rice is produced [1], and 90% of this production is contributed by Asian countries. The escalating population would expect to increase by 16.44% (8.5 billion) by 2030, 27.40% (9.7 billion) by 2050 from the current population level of 7.3 billion [2,3]

. Therefore, food production has to be increased to over 40% by 2030 and 70% by 2050 to meet the requirement. Several yield-limiting rice constraints have been identified [4-6]. Among them, water availability and edaphic factors are highly responsible for yield loss [7]. Nowadays, rice is being cultivated under different water regimes, ranging from waterlogged areas to limited irrigated conditions [8]. Thus, drought performs a major yield-limiting factor for rice worldwide, particularly in Asian countries [9]. By nature, rice is a water-loving plant and very sensitive to water deficit conditions or drought. In Asia, about 34 Mha of rice is under rainfed lowland conditions, and about eight Mha is under upland conditions, which are regularly affected by the variable intensity of drought stress [10]. Further, drought-prone areas are gradually increasing in traditionally irrigated rice ecosystems [11], due to climate change. In India, out of 20.7Mha of rainfed rice (about 16.2 Mha in eastern India, of which 6.3 Mha of upland and 7.3 Mha of lowland) areas are highly drought-prone [12].

The impact of drought on rice depends on the severity, duration, and plant growth stage [7]. Drought alters the metabolic, physiologic, and molecular pathways in plants, which disturbs the equilibrium of the physiological process with water [13]. The appearance of stress and potential losses due to drought was measured by earlier researchers and estimated that yield loss would be 53-92% [14]. Among the various stages of rice, flowering and grain filling stages were the most sensitive stages [15], wherein, moderate stress at this stage would cause maximum yield to lose. However, the yield losses also depend upon the genetic constitution of plants and buffering capacity of an individual towards environmental variability [16]. Thus, the precise phenotyping of plants under drought and genotyping using molecular marker will assist the selection efficacy and deduct the environmental error. In certain situations of drought stress, morphologically diverse genotypes perform very much similar due to the lack of genetic variability at the DNA level [17]. Therefore, the of rice lines provides information on target traits that help select and develop superior cultivars for the drought-prone environment.

Simple sequence repeats (SSRs) are the most widely used DNA markers than other types of marker for the assessment of genetic variation [18]. Under drought at the reproductive stage, grain yield attributed traits would reveal differences among the genotypes [13]. Thus, it can provide a more direct and reliable selection of stress-tolerant donors in rice at the reproductive stage. This is very much necessary and a pre-requisite for the success of any drought-tolerant breeding program in rice. Based on the above facts, the present study was undertaken (i) to identify the best promising rice genotypes possessing reproductive stage drought tolerance and their utilization in the breeding program for the drought-prone locality, and (ii) estimation of the phenotypic and genotypic diversity of rice landrace and improved varieties.

Materials and Methods

Plant materials

Seventy-six rice genotypes consisting of 44 improved rice varieties, 29 traditional landraces, and three checks, i.e., Vandana and N22 as a tolerant check and IR 20 as a susceptible check, were used in the present study. The genetic materials were collected from the International Rice Research Institute (IRRI), Philippines, and Genebank of ICAR-National Rice Research Institute (NRRI), Cuttack (Table 1).

Field experiment and Stress environment

The field experiments were conducted at the experimental farm of the ICAR-National Rice Research Institute (NRRI), Cuttack, India (20°N, 86°E) during dry seasons of 2012 and 2013. The experiment was laid out in an alpha lattice design with three replications. The field was thoroughly prepared and leveled properly to avoid water stagnation during rainfall. This helped in imposing uniform drought stress in the field at the reproductive stage. Seedlings of 21 days old were transplanted in $6m^2$ (3m x 2m) plot size for each genotype and uniform plant populations were maintained in all the genotypes. The fields were properly managed to make it weed-free, and fertilizer was applied as N @ $80kg/ha^{-1}$, P_2O_5 @ 40 kg/ha^{-1} and K_2O @ 40 kg/ha^{-1}. The plants were irrigated uniformly up to four weeks after transplanting, later drought was imposed by holding irrigation for the next 30 days and beyond until the susceptible checks showed permanent wilting. The observations were recorded on yield and yield attributing traits using five randomly selected plants per genotype per replication at maturity. The quantitative traits observed were days to 50% days to flowering (DFF), plant height (cm)(PH), ear bearing tillers per plant (EBT), panicle length (cm)(PL), total dry matter production (gm)(TDM), harvest index (HI), spikelet sterility % (STL), kernel length (cm)(KL), kernel breadth (cm)(KB), 1000 grain weight(g) and plot grain yield (g).

DNA extraction and PCR analysis

The genomic DNA was isolated from the leaf tissues individu-

S. No	Genotypes	Type of variety	Structure group	S. No	Genotypes	Type of variety	Structure group
1	Basmati 370	TDL	ADM	39	Taichung Native 1	IMV	SG1
2	Dular	TDL	ADM	40	RD 25	IMV	SG1
3	N 22	TDL	ADM	41	IR 64	IMV	SG1
4	T 1	TDL	SG2	42	Ai Jiao Nan Te	TDL	SG1
5	Jhona 349	TDL	SG2	43	PSBRC 80	IMV	ADM
6	Kalakeri	TDL	SG1	44	Samba Mahsuri	IMV	SG1
7	Brown Gora	TDL	SG1	45	Vandana	IMV	ADM
8	Kasalath	TDL	SG2	46	Asse Y Pung	TDL	SG2
9	DhagadDeshi	TDL	ADM	47	Purbachi (Chinese 1)	TDL	SG2
10	Binnatoha	TDL	ADM	48	Koshihikari	IMV	ADM
11	Zhensan 2	TDL	SG2	49	Dee- Geo-Woo-Gen	TDL	SG2
12	BR 2	IMV	SG1	50	Niaw	TDL	ADM
13	T 136	IMV	ADM	51	Mahulata	TDL	ADM
14	BR 1	IMV	SG1	52	IR 55419-04	IMV	ADM
15	Davao	TDL	ADM	53	Satyabhama	IMV	SG2
16	Nan TeHao	IMV	ADM	54	Pyari	IMV	ADM
17	Dhalasaita	TDL	ADM	55	CR2702(IET 21627)	IMV	ADM
18	MueyNong (Wang Din)	IMV	SG1	56	SahabhagiDhan	IMV	SG2
19	DholiBoro	TDL	SG1	57	Naveen	IMV	SG2
20	Saita	TDL	SG1	58	Makmur	TDL	ADM
21	Jaya	IMV	SG1	59	IET 21625(CR 2698)	IMV	SG1
22	Sathi 34-36	IMV	SG1	60	CR2706	IMV	ADM
23	BR 21	IMV	SG2	61	Lalat	IMV	SG2
24	Black Gora (NCS 12)	TDL	SG2	62	IET 21692	IMV	ADM
25	Kalamkati	TDL	SG1	63	CR2699	IMV	SG1
26	Ratna	IMV	ADM	64	CR2707	IMV	SG1
27	CO 25	IMV	SG2	65	CR 143-2-2	IMV	ADM
28	Dubraj	TDL	SG2	66	Basmati 334	IMV	ADM
29	Sarjoo 50	IMV	ADM	67	RTS 4	IMV	SG1
30	Magawk Dong 269-7-7 (9)	IMV	SG1	68	KU 113-1	TDL	SG2
31	Annada	IMV	SG1	69	Dinorado	TDL	ADM
32	TAM CAU 9 A	TDL	SG1	70	UPLRI 4	IMV	SG1
33	Chau	TDL	SG1	71	BPI RI 10	IMV	SG1
34	MTU1010	IMV	SG1	72	NSIC Rc9	IMV	SG1
35	IRRI 123	IMV	SG1	73	Kinastano	IMV	ADM
36	NSIC Rc 192	IMV	SG1	74	IR 81896-B-B- 195	IMV	SG1
37	IR841-85-1-1-2	IMV	SG1	75	IR 20	IMV	ADM
38	IR74371-46-1-1	IMV	SG1	76	Swarna	IMV	SG2

Table 1: List of rice varieties/accessions used for the screening to drought tolerance at the reproductive stage and genetic diversity studies.

TDL- Traditional Landraces; IMV- Improved Rice Variety (SG-Structure group population; ADM-Admixture).

ally according to the modified CTAB method [19]. Thirty-six simple sequence repeat (SSR) markers distributed on all the 12 chromosomes of rice (3 per chromosome) used for genetic diversity analysis. These SSR markers were chosen based on their physical position on the 12 chromosomes of the rice genome according to the 'Gramene' database (http//:www.gramene.org). PCR was performed in a 96-well plate containing a total 10µL volume; 2 µL of DNA template, 1.5µL 10X PCR buffer (containing 200mMTris-HCl pH 8.3, 500 mMKCl, 15 mM $MgCl_2$), 1µL of 2.5mMdNTPs, 0.5µL of 5pmol forward and reverse primers (Sigma Genosys, USA), 0.3µL of commercial Taq polymerase and 4.2 µL of Nano-pure water. PCR amplification was performed with an initial denaturation of 5 min at 94°C, 35 cycles of 30 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C and extension of 7 min at 72°C (PTC-200 Thermocycler; Bio-Rad, Germany). Amplified products separated by 2.5% agarose gel electrophoresis for 2 to 2.5 h in 0.5X TBE buffer. Gels were stained in ethidium bromide solution and visualized under UV light using the gel documentation system (Alpha Imager, USA).

Morphological and molecular data analysis

The data were subjected to statistical analysis as per the method suggested by Gomez., *et al*. [20]. The computer-based software like CropStat 7.2 (http://bbi.irri.org/products), and POPGENE Version 1.32were used for the analysis. The molecular data entered in the form of single-individual genotypes with binary digits. By using POPGENE Version 1.32, the number of alleles per locus, average heterozygosity, genetic diversity (Nei genetic diversity), and Shannon's Information index (I) for each marker was calculated [21]. The informativeness of markers was assessed by calculating polymorphic information content according to Botstein., *et al*. [22].

Further, when an allele was found to be less than 5% of the study's germplasm, it was designated as a rare allele [23]. The dendrogram was constructed based on similarity coefficient by adopting the un-weighted pair group method of arithmetic averages (UPGMA) using MEGA6 software with 1000 replicates to establish the relationship among various genotypes [24]. Bayesian-based clustering method of analysis using the software package STRUCTURE [25] was performed to infer the number of populations (K) required for accurate data interpretation without prior information on the number of groups of accessions at which the individuals were studied. To derive the optimal number of groups (K), STRUCTURE was run with K varying from one to 10, with five runs for each K value. To determine the true value of K, ad hoc statistic ΔK was followed [26]. Parameters were set to 1,00,000 burn-in periods and 10,000 Markov Chain Monte Carlo (MCMC) replications after burn-in with an admixture and allele frequencies correlated model. The membership probabilities (Q) calculated from STRUCTURE ≥ 0.80 were used to assign rice accessions to clusters. Arlequin [27] assessed the presence of molecular variance within and between hierarchical population structure estimated by Structure via Analysis of molecular variance (AMOVA) with 1000 permutations.

Results

Variation in yield attributed traits and plant growth under drought at reproductive stage

The morphological and yield attributed traits were varied significantly due to drought imposed at the reproductive stage. The plot grain yield ranged between 8.80 g in Swarna and 205.39g in Dhalasaita during 2012. However, in the year 2013, Swarna exhibited 9.00 g,andDhalasaita recorded 183.41 g. Among the 76 genotypes studied, Dhalasaita (205.39 g), Black Gora (NCS 12) (158.52 g), and Annada (162.58 g) produced significantly more grain yield per plot than Vandana (check, 153.92 g) in 2012. The grain yield of tolerant check variety (N22) was 54.46 g in 2012; 51.35g in 2013 and susceptible check variety (IR 20) it was10.90g during 2012; 14.65g in 2013 (Table S1). Reproductive structures such as anther, stigma, pollen, and spikelet are very sensitive to water deficit stress. The seed set depends upon pollen availability at stigma receptivity, which is highly disturbed when water deficit occurs. Thus, spikelet sterility percent was recorded for all the genotypes. Five genotypes viz., T1(18.95%), Jhona 349(15.14%), Binnatoha(15.63%), Dhalasaita(15.28%) and PSBRC 80(15.59 %) in 2012 and 3 genotypes *viz*., Jhona 349(15.28%), Dhalasaita(16.60%) and PSBRC 80(17.30%) in 2013 had<20% of spikelet sterility.

Results of plant height indicated that the effect of drought stress on plant growth ranged from 53.50cm to 112.70 cm, with an average of 80.60 cm in 2012. Similarly, in 2013 plant height ranged between 56 cm and 111.05 cm with an average of 82.97cm. Significant differences were observed in plant height in both the years under drought stress (Table S1). Based on the grain yield per plot and spikelet sterility percent, 76 genotypes were classified arbitrarily into four groups (Table 2). It was found that four genotypes during 2012 and two genotypes during 2013 were found to yield more than 151gm $plot^{-1}$. On the other hand, six genotypes in the year 2012 and one genotype during 2013 exhibited >71% spikelet fertility. However, three genotypes such as Dhalasaita, Saita, and Vandana, were commonly identified as drought tolerant in both years,

while Dhalasaita exhibited the highest plot grain yield. Among the morphological traits studied, differences in plant height are visible under drought. Seven genotypes *viz*., Jhona 349, T 136, Dhalasaita, Saita, Black Gora (NCS 12), Annada and Vandana in 2012 and four genotypes *viz*., Kalakeri, Dhalasaita, Saita, and Vandana in 2013 exhibited semi-dwarf plant height (84-96 cm). Among the semi-dwarf genotypes Dhalasaita, Black Gora, Annada exhibited a higher grain yield than tolerant check variety Vandana in 2012. Whereas, in 2013 only Dhalasaita shown better performance than Vandana.

S. No	Traits	Categories	No. of genotypes based on plant height	
			2012	2013
1	Plot Yield (gm)	5-50 gm	26(64.13-95.5cm)	23(68.75-111.05)
		51-100 gm	28(65.8-99.8cm)	40(56-102.60cm)
		101-150 gm	18(53.5-112.7cm)	11(63-98.65cm)
		>151 gm	4(82-97.6cm)	2(69.05-82.15cm)
2	Spikelet sterility (%)	10-30%	23 (65.8-112.7cm)	23 (60.75-95cm)
		31-50%	36 (60.30-97cm)	39 (56-102.60cm)
		51-70%	11 (53.5-98cm)	13 (73.70-109.95cm)
		>71%	6 (65.7-90.60cm)	1 (111.05cm)

Table 2: Arbitrary classification of genotypes based on yield attributed traits in rice.

Traits and grouping pattern of genotypes

The 11 morphological traits of 76 genotypes were used for the genotype-by-trait biplot graph to identify drought-tolerant rice genotypes (Figure 1a and 1b). In the present experiment, PC1 and PC2 of the biplot graph accounted 74.77% and 9.68% of the variance respectively in 2012 and 67.71% and 16.22% of the variance in 2013, respectively. Principal component analysis (PCA) converted the variables into vectors were used to plot a distribution of the genotypes in the scatter diagram. The genotype-by-trait biplot indicated that the traits were grouped into two categories. The yield component traits were plotted on the right side, and days to 50% days to flowering and sterility percent were grouped on the left side. A similar trend was also observed in 2013. The biplot of the year 2012, plant height, panicle length, harvest index, ear bearing tiller, grain yield, and total dry matter indicated that the pairwise trait of drought-tolerant genotypes exhibited an acute angle among them located on the right side (second and third quarter) and they contain 43rice genotypes. The left out genotypes (33 number) were placed on the left side (first and fourth quarter) of the plot and grouped with days to 50% flowering and sterility percent. Out of 43 genotypes in the second and third quarters, six genotypes *viz*., Jhona 349 (134.78), Dhalasaita (205.39), Saita (136.06), Black Gora (158.52), Annada (162.58), and Vandana (tolerant check) (153.92) had produced more than 130g of grain yield. Similarly, 11 genotypes *viz*., T1 (18.95%), Jhona 349 (15.14%), Binnatoha (15.63%), Nan TeHao (21.04%), Dhalasaita (15.28%), Saita (24.12%), BR 21(22.99%), Black Gora(NCS 12) (24.64%), Annada (23.28%), PSBRC80 (15.59%), and Purbachi(Chinese 1) (22.87%) had less than 25% of spikelet sterility (Figure 1a). In 2013, 37 rice genotypes were plotted in the second and third quarters. Whereas 39 genotypes were plotted in the first and fourth quarters. Among the 37 genotypes, seven genotypes *viz*., Jhona 349 (118.30), Kalakeri (130.55), Nan TeHao (113.30), Dhalasaita (183.41), Black Gora (117.90), Vandana (161.59), and Mahulata (116.15) had more than 100g grain yield, and eight genotypes T1(21.60%), Jhona 349 (15.28%), Nan TeHao (22.40%), Dhalasaita (16.60%), BR 21 (25.65%), IR74371-46-1-1(24.05%), PSBRC 80 (17.30%) and Purbachi (Chinese 1) (24.25%) had less than 25% of spikelet sterility under drought condition (Figure 1b).

Association among the phenotypic traits

The angles between the vectors of the 2012 biplot graph variables showed both positive and negative relationships between the traits. Spikelet sterility percent with days to 50% flowering (r=0.318**) and total dry matter with grain yield (r=0.378**) had the acute angle; thus, exhibited a significant positive correlation between them. On the other hand, ear bearing tiller had a positive association with harvest index (r =0.400**), panicle length (r=0.380**), grain yield (r=0.442**) and total dry matter (r=0.569**). Spikelet sterility percent registered right/obtuse angle with grain yield (r=-0.535**), ear bearing tiller (r=-0.360**), panicle length (r=-0.3481**) and harvest index (r=-0.5789**), which represents a negative association between them. Similar trends of the relationship between the vectors/traits were also observed during 2013 (Figure 1).

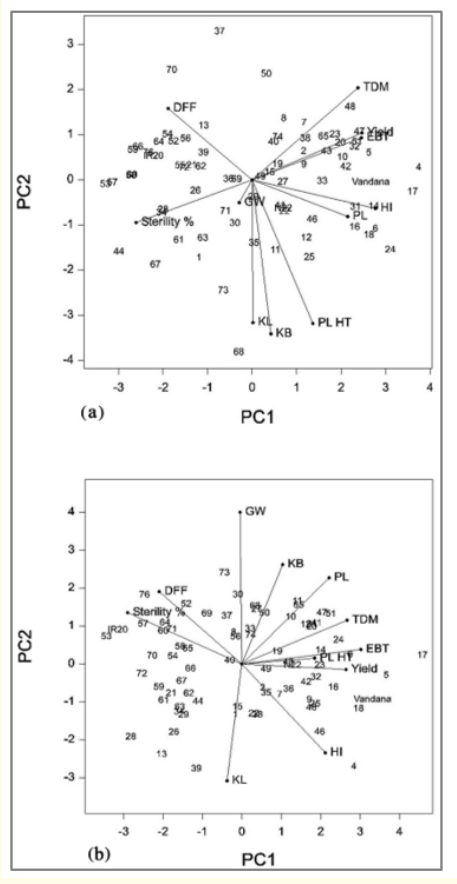

Figure 1: Spatial distribution of 76 rice genotypes under drought stress in 2012(a) and 2013 b) based on the genotype-by-trait analysis for the first two principal components.

The molecular genetic diversity of landraces and improved rice varieties

The 44 improved varieties, 29 landraces, and three checks of rice were genotyped using 36 SSR markers. Of the total 36 SSRs, 33 showed polymorphism and utilized to perform analyses. The rest three SSRs were found to be monomorphic, and they were not included in the analysis. The 33 markers generated 96 distinct alleles across 76 rice genotypes (Table 3).

The amplifies diverse allelic pattern of genotypes and PIC Value of SSR markers

The number of alleles per loci varied from two to four, with an average of 2.6 alleles per locus. Five markers, *viz.,* RM306, RM13097, RM22571, RM215, and RM271, showed the maximum of four alleles each. Whereas, RM341, RM13679, RM14981, RM17600, RM163, RM20150, RM20096, RM24542, RM25368, RM26423 and RM277 had two alleles per marker. The PIC value of the polymorphic markers ranged between 0.50 (RM25368) and 0.98 (RM215), with an average PIC value of 0.67 (Table 3). The markers RM22571, RM215, RM271, RM27861, RM28034, RM271, RM21352, RM19422, RM13097, RM237, RM11694 and RM306 had high allelic diversity. The expected heterozygosity or gene diversity (He) varied from 0.01 (RM25368) to 0.576 (RM237) with an average of 0.267 and Shannon's Information index ranged from 0.0396 (RM25368) to 0.9611 (RM237) with an average of 0.476. Among the total number of alleles, 7unique alleles, 18 rare alleles, 33 low-frequency alleles, and 35 high-frequency alleles across 76 rice genotypes with an average of 0.19, 0.51, 0.92 and 0.97, respectively were found. Two unique alleles in marker RM13097 and two rare alleles in the marker RM306 and RM21478 were observed. Shannon's diversity index values were in correspondence with the Nei's genetic diversity for all the loci. The gene flow (Nm) of traditional landraces was 0.016, while in improved varieties is 0.012. The average value of an effective number of alleles (Ne) in landraces was 1.50, and improved varieties were1.413. The Nei's Genetic diversity (He) in landraces was 0.274 and improved varieties were0.249. Whereas Shannon's diversity index (SII) in landraces was 0.482, and improved varieties were 0.444. This exhibited that, landraces exhibited higher values comparative to improved varieties.

Population structure and analysis of molecular variation

The population structure of the total 76 rice genotypes was analyzed by the Bayesian-based approach using Structure 2.0 software for 33 SSR markers. The number of subpopulations (K) was identified based on maximum likelihood and delta K (ΔK) value, and the total set of genotypes have been divided into two sub-populations (K=2), *i.e.*, Pop1 and Pop2 with a membership percentage of 57.90 and 42.10, respectively (Figure 2a, 2b). The expected heterozygosity was found to be high in Pop2 (0.3487) than Pop1 (0.17). Out of the 44 improved varieties and three checks, 68.3% of them were grouped in Pop1, while 31.7% were clustered in Pop2. Similarly, 45.1% of the landraces were grouped in Pop1, and 54.9% were in Pop2. The (F_{ST}) value of subpopulations were 0.496 and 0.021 for Pop1 and Pop2 respectively, while allele frequency divergence between the two sub-populations were 0.067 and an average expected heterozygosity was 0.170 for Pop1 and 0.348 for Pop2. By structure analysis, pure or admixture genotypes were categorized based on the score, >0.80 was considered as pure and <0.80 as an

S. No	Markers	Chr	Mol.wt	RPM	ONA	RA	ENA	EHMG	EHTG	SII	PIC
1	RM237	1	130	(CT)18	3	1	2.3629	0.4193	0.5768	0.9611	0.83
2	RM11694	1	193	(CT)34	3	0	1.6298	0.611	0.3864	0.6982	0.81
3	RM306	1	155	(GT)18(AT)8CT(GT)6	4	2	1.358	0.7346	0.2636	0.5416	0.83
4	RM341	2	172	(CTT)20	2	0	1.959	0.5072	0.4895	0.6826	0.74
5	RM13679	2	277	(TC)34	2	0	1.329	0.7508	0.2476	0.4135	0.64
6	RM13097	2	121	(GTT)14	4	0	1.258	0.7934	0.2051	0.435	0.81
7	RM14981	3	282	(AG)48	2	1	1.0821	0.9237	0.0758	0.1663	0.54
8	RM15806	3	393	(AT)11	3	1	1.5715	0.6339	0.3637	0.6453	0.77
9	RM15630	3	285	(GA)55	3	1	1.6657	0.5977	0.3996	0.6963	0.79
11	RM17600	4	478	(AAT)114	2	0	1.1108	0.8996	0.0997	0.2062	0.55
12	RM18270	5	621	(ATT)41	3	1	1.3086	0.7626	0.2358	0.4698	0.75
13	RM163	5	124	(GGAGA)4(GA)11C(GA)20	2	0	1.4638	0.6811	0.3168	0.4967	0.78
14	RM20150	6	277	(TC)16	2	0	1.1702	0.8536	0.1454	0.2762	0.61
15	RM19422	6	169	(CT)44	3	1	1.6337	0.6095	0.3879	0.6929	0.80
16	RM20096	6	396	(AT)52	2	0	1.1308	0.8835	0.1157	0.2315	0.58
17	RM20913	7	699	(TAA)42	3	0	1.439	0.6929	0.3051	0.5251	0.63
18	RM21352	7	240	(TC)25	3	0	1.75	0.5686	0.4286	0.7634	0.83
19	RM21478	7	128	(GAA)28	3	2	1.1263	0.8872	0.1121	0.2454	0.71
20	RM22825	8	160	(CT)28	3	1	1.2536	0.7964	0.2023	0.4025	0.74
21	RM22914	8	236	(GA)33	3	1	1.7326	0.5743	0.4228	0.6879	0.77
22	RM22571	8	120	(TCT)14	4	0	2.7832	0.3549	0.6407	1.157	0.96
23	RM316	9	192	(GT)8-(TG)9(TTTG)4(TG)4	3	1	1.1926	0.8374	0.1615	0.3483	0.74
24	RM24717	9	194	(AAT)28	3	0	1.3352	0.7473	0.251	0.4576	0.74
25	RM24542	9	266	(TA)51	2	0	1.4551	0.6849	0.3128	0.492	0.71
26	RM215	9	148	(CT)16	4	1	1.9787	0.502	0.4946	0.8137	0.98
27	RM25368	10	951	(TA)255	2	0	1.0132	0.9868	0.0131	0.0396	0.50
28	RM25817	10	168	(AG)30	3	0	1.142	0.8749	0.1243	0.2755	0.70
29	RM271	10	101	(GA)15	4	1	1.4966	0.666	0.3318	0.6877	0.85
30	RM26269	11	92	(TG)11	3	1	1.5809	0.6301	0.3675	0.634	0.78
31	RM26423	11	608	(AC)52	2	1	1.0278	0.9728	0.027	0.0724	0.54
32	RM277	12	124	(GA)11	2	1	1.1108	0.8996	0.0997	0.2062	0.56
33	RM27861	12	595	(AC)79	3	0	2.3374	0.424	0.5722	0.95	0.80
34	RM28034	12	290	(GGT)12	3	0	1.8025	0.5518	0.4452	0.7898	0.81
	Mean				2.6667		1.4609	0.7309	0.2673	0.4767	0.67

Table 3: Summary of different genetic variation parameters of 36 SSR marker loci for genotyping in the in 75 rice varieties. Chr-Chromosome; RPM-Repeat motifs; Mol.wt- Molecular weight of the band; ONA-Observed number of alleles; RA-Rare allele (alleles with a frequency lower than 5%); ENA-Effective number of alleles; EHMG-Expected homozygosity; EHTG-Expected heterozygosity; SII-Shannon's Information index; PIC-Polymorphic information content.

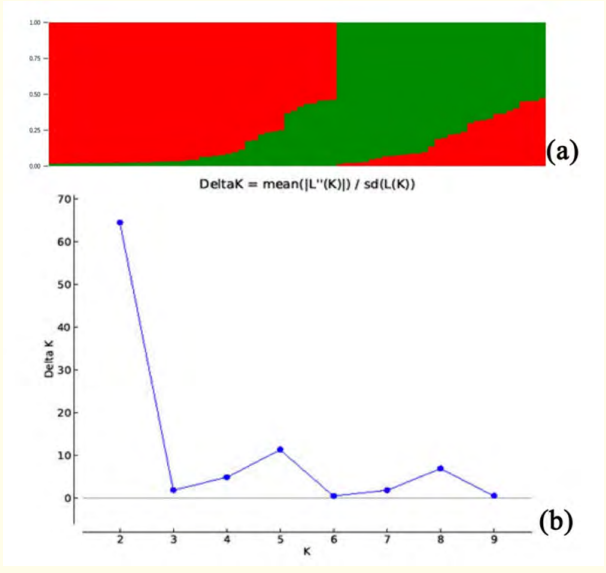

Figure 2: Pattern of variation of 76 accessions based on 33 SSR markers. (A) The K values are based on the run with the highest likelihood (Bar length represented accessions and improved rice varieties (B) Estimation of the population using LnP(D) derived delta K for determining an optimum number of subpopulations.

admixture. Further, the output of the program detected a lower value of alpha (α=0.276) and variance of likelihood (112.4) in the population set of traditional and improved rice varieties. The threshold has categories the 76 accessions into two populations of 44 and 32 each. Among the total genotypes, 49 were found to be pure with the proportion of 64.47% and 27 were admixtures with the proportion of 35.52% (Figure 2). The proportion of pure lines and landraces varies among the population. The pure lines in Pop1 consisted of 48.97% improved rice varieties and 16.32% traditional landraces. While in Pop2, 14.28% of improved lines and 20.40% of traditional landraces were found to be pure. In the case of admixtures, 17 genotypes were found to be improved, and ten belonged to traditional landraces. In total, genotypes of 76 were distributed into 42.10% in Pop1, 22.36% in Pop2 and rest of them (35.52%) as an admixture.

The molecular variance by AMOVA revealed that maximum variation was within the population of 96.4%, while subpopulation exhibited the variance of 3.6% and the F_{st} (0.036) was non-significant. Pairwise FST values showed significant differentiation among the pairs of sub-populations ranging from 0.0216 to 0.4965 suggesting that the two groups were significantly different from each other. The F_{ST} values and their distribution pattern show a clear differentiation of subpopulations from each other.

Genetic relatedness among the improved and traditional landraces of rice accessions

The UPGMA dendrogram constructed based on pair-wise genetic distance grouped the 76 genotypes into two major clusters I and II at 0.30 level (Figure 3). Cluster I contained the majority of the genotypes and was further divided into two sub-clusters Cluster Ia and Cluster Ib at 0.24 level, whereas the 70 genotypes were grouped in Cluster Ia and only one genotype (Niaw) was present in ClusterIb. The Cluster Ia having the highest number of genotypes and were further divided into 13 subclusters (A-M). Similarly, cluster II was divided into two sub-clusters as Cluster IIa and Cluster IIb at 0.25 levels, and one sub-cluster has two rice accessions as Nan TeHao, and Davao and another sub-cluster as CIIb contains three genotypes as Koshihikari, Sarjoo50, and Pyari, respectively.

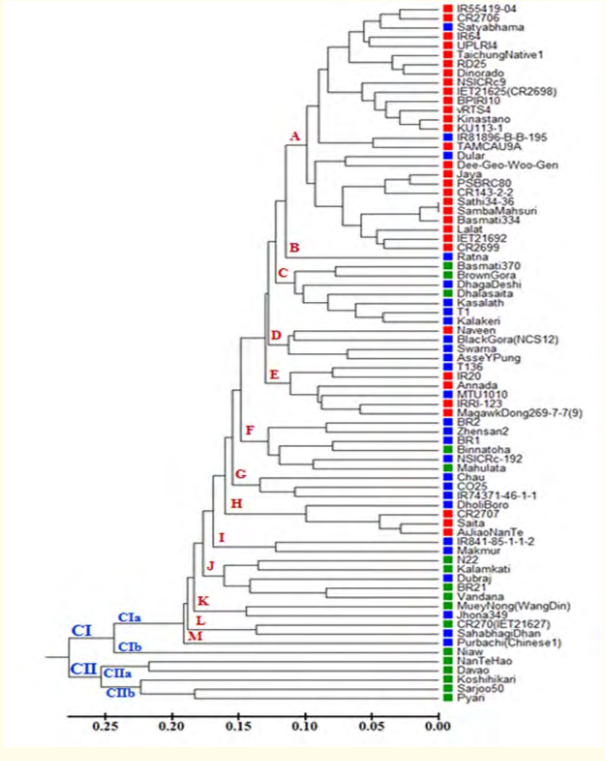

Figure 3: UPGMA of 76 rice genotypes based on Jaccard's genetic similarity coefficients (■pop2,■admix, ■pop1).

The genetic similarity (GS) between 45 improved rice varieties and 36 landraces of rice accessions varied from 0.0282 to 0.9679. The highest genetic similarity was observed between the traditional landraces of DholiBoro and improved varieties of Pyari (0.9679), while in lowest genetic similarity was observed among Basmati 334, Sathi 34-36 and Samba Mahsuri (0.0282), and also between KU 113-1 and Kinastano (0.0282). Notably, in case of traditional landraces of having a higher genetic similarity between Chau and Davao (0.8668) and lower genetic similarity between Ai Jiao Nan Te and Saita (0.0572), while in improved rice varieties higher genetic similarity was observed between CR2707 and Pyari (0.9216) and lowest genetic similarity between PSBRC 80 and Jaya (0.0431). The average genetic distances between the traditional landraces of 0.340 and 0.307 in improved rice varieties and the overall genetic distance are 0.326 in traditional landraces and improved varieties.

Discussion

Rice has evolved as a water-loving plant and very sensitive to water deficit stress. Water deficit stress is one among the major abiotic stresses, reduces the grain yield worldwide, particularly in arid and semiarid zones [28]. The varietal development for such stress is only possible by using tolerant genotypes as a parent while making the crossing program. The phenotyping on yield-attributing traits under drought and genotyping information is now very useful in reducing the false positive selection from a population. Thus, identification of stress-tolerant source line and utilization for crossing in a diverse group of lines would provide a rewarding result.

Genotypic responses and relationship between the traits

The PCA analysis revealed that plant height, EBT, panicle length, TDM, and HI were falling in a single group (highly correlated). Similarly, spikelet sterility percent and days to 50% flowering were in another group. However, kernel length, kernel breadth, and grain weight were in a separate group. This suggests that grain yield negatively correlated with spikelet sterility and positively correlated with EBT and TDM. Based on the PCA graph the genotypes Jonha 349, Dhalasaita and Na TeHao were fallen in the same group with Vandana over the year. The morphological characteristic of genotypes in quadrant II and III confers tolerance by an increase in panicle length, TDM, EBT, and harvest index compared to the genotypes in quadrant I and IV. Therefore, plants with these traits are expected to possess drought tolerance and grain yield under drought conditions during the reproductive stage [29,30]. The correlation between the spikelet fertility, harvest index, grain weight and spikelet per panicle significantly contributed to yield stability under drought stress.

On the contrary, five genotypes (T 136, IR 55419-04, Pyari, Sahabhagidhan, and UPLRI 4) registered more than 100g grain yield per plot in quadrant 1. This might be due to the moderate level of spikelet sterility and grain yield. Therefore, it suggests that reproductive stage tolerant is susceptible to drought and separate gene/mechanism might be involved in terminal drought tolerance. Spikelet sterility under drought stress is not only a highly informative indicator for the severity of water stress but also the most imperative determinant of grain yield under drought condition [31].

Identification of promising drought-tolerant genotypes

The reproductive stage drought tolerance is one of the important areas of rice breeding concerning anticipated climate change. In the present experiment, 76 rice genotypes were screened for reproductive stage drought tolerance at field conditions for two years (2012 and 2013). We found that Swarna is more susceptible to drought in comparison to IR-20 concerning grain yield under the reproductive stage. Over the years, Dhalasaita (landrace) produced the highest grain yield per plot (183.41-205.39 g). In both the years (2012 and 2013), drought hampered crop growth and development. A reduction in grain yield was observed almost in all the rice genotypes grown under stress condition. Further, Sabetfar., *et al*. [32], also reported the negative influence of drought over the yield. The stress reduces the plant height, grain yield, number of tillers, total dry matter and spikelet fertility percent due to water deficit stress. Based on the plot grain yield, the traditional landraces Dhalasaita was tolerant to reproductive stage drought in comparison to tolerant check variety Vandana over the years (Supplemental Table1).

Further, the identified tolerant genotype had a semi-dwarf plant height, an ideal selection trait. The can also evident form a negative correlation between plant height and spikelet sterility in both the years ($r = -0.128$ and $r = -0.296^*$). Drought stress during anthesis induces a higher percentage of spikelet sterility and ultimately reduces grain yield [33]. Therefore, spikelet sterility percent is an important criterion for selecting drought-tolerant genotypes at the reproductive stage. The best four genotypes exhibited <15% of spikelet sterility percent under stress conditions are Jhona 349, Binnatoha, Dhalasaita, and PSBRC 80. Under stress condition, leaf water potential reduces and increases the production of phytohor-

mone and ethylene [34,35] which, interfere with normal metabolic, physiological, and molecular pathways in plant leads to an increase in spikelet sterility [36,37]. Therefore, plant fails to emerge the panicle, poor anther dehiscence, and less viable pollen. Moreover, grain yield and spikelet fertility may not be a good choice for selection many times. To assure good selection, the expression of the traits and overall plant performance needs to be observed together with a yield to transfer the yield attributing traits into progenies easily. Thus, by results of grain yield performance and PCA analysis (which was based on the overall 11 morphological traits) three genotypes *viz*., Jhonha 349, Dhalasaita and Na TeHao were drought tolerant and most adapted to drought condition at reproductive stage.

Assessment of molecular genetic diversity at the genome level

In the current study, 96 alleles were resolved by 33 SSR polymorphic markers with an average of 2.6 alleles per locus. Among them, 5% were considered a rare allele and showed an allele frequency of < 5 % [23]. The PIC value of the polymorphic primers RM22571 and RM215located on chromosome 8 and 9 respectively showed the maximum value of 0.96 and 0.98, while in RM25368 on chromosome 10, RM14981 on chromosome 3 and RM26423 on chromosome 11 exhibited the minimum PIC value (< 0.54). Among the 33 markers, 11 SSRs *viz.,* RM27861, RM19422, RM28034, RM13097, RM11694, RM237, RM306, RM21352, RM271, RM22571, and RM215 had a high level of allelic diversity and found to be highly informative in the present experiment, having > 0.80 PIC value. In the present study, an average PIC value was found to be 0.67which is higher than reported in earlier studies 0.17 [38], 0.24 [4], 0.38 [39], 0.42 [40]. Among the 11 highly informative markers, RM237, RM306, RM271, and RM215were found to be linked with drought-tolerant QTLs. Marker RM306 was found to be associated with qDTY1.2, located on chromosome 1 at marker interval of RM259-RM306 [41], and RM271 found to be on chromosome 10 associated with qDTY10.1. reproductive-stage drought [42]. On the other hand, RM237 located on chromosome 1 associated with the relative number of spikelet's per panicle and leaf-drying score under drought [43]. The marker RM215 found to be multifaceted, which closely associated with panicle water potential [44], days to 50% flowering (DTF), relative water content [45], biological yield [46], root traits, plant water status and grain yield [47] under drought stress. Therefore, in the present study, selected SSR markers were found to be highly informative and suggest the presence of high genetic variability in the genomic regions of landraces and improved rice accessions, which can be useful for diversity analysis. Further, for a future crossbreeding program, these markers and donors can be utilized in marker-assisted selection to overcome the issue of drought in rice.

Genetic structure and relatedness of genotypes

The higher percentage of improved rice varieties were found to be grouped in pop 1, and landraces we found to be a major portion in pop 2. As the pop2 posses number of landraces in the higher side, it exhibits a higher proportion of heterozygosity. In the case of grain yield comparison between populations, pop2 having an average yield of 68.56g, which is slightly higher than with pop1 (65.22 g) with 40% spikelet sterility. Thus, this suggests that landraces were acting as the source for improving the stress tolerance in rice. However, admixtures were observed to have superior than pure lines in the aspect of average grain yield (76.02 g). This might be due to the alleles controlling the admixture's drought in the heterozygous state [4]. The lower value of alpha ($\alpha = 0.276$) signifying that the genotypes utilized for the present study might be from one population or another with few admixture individuals. The inferred ancestry specified that the genotypes studied under this experiment might have retained a partial allele for drought tolerance or susceptibility from the common ancestry by complex intercrossing among the germplasm of diverse genetic background. This line is in with earlier studies of various researchers [48]. Similarly, the admix genotypes Dhalasaita and Vandana were found to be drought tolerant and high yielding with low spikelet sterility, while among the pure lines, Saita was from pop1 was found to be tolerant. Therefore, the pure line Saita could be inducted into drought breeding program. The model-based structure analysis estimates and categories the genotypes as admixture/pure, largely based on pedigree information of the cultivars. In the present study, the most popular variety in Japan, Koshihikari has been defined as an admix. This evinces from the report of Yamasaki and Ideta [49] that the progenitor alleles inherited to cultivar of Koshihikari were unclear. The result of the STRUCTURE provided the organizing data for hierarchical AMOVA. AMOVA study indicated higher partitioning of variation within individuals and lower portion existed among populations. Estimates of the fixation indices revealed that Fst indicates little divergence existing between subgroups of the population. The population grouping through structure analysis was found to be similar to distance-based clustering of UPGMA.

The UPGMA dendrogram constructed based on pair-wise genetic distance grouped the 76 genotypes into two major clusters I

and II. The analysis was revealed that wide genetic variability observed among the genotypes of landraces and improved genotypes. Similarly, biplot has classified the genotypes into two groups as tolerant on the right side and susceptible to the plot's left side. Similarly, tolerant genotypes were clustered under Cluster Ia. Among the thirteen subclusters (A-M) within the Cluster of Ia, Cluster la-B and I grouped the genotypes having >50% spikelet sterility percent and <50% grain yield. Both these clusters grouped the improved genotypes susceptible to drought. Cluster Ia-C, K, and M have categorized high yielding genotypes with grain yield of >100 g. The genotypes clustered under these groups belonged to landrace of indigenous and exotic.

Conclusion

It is a pre-requisite to have a drought-tolerant source population in the drought-tolerant breeding program to improve the cultivar. Understanding the genetic diversity and population structure is also important to identify the relation between the genotypes and responsible traits. The information will access the precondition for choosing accessions appropriately and help design efficient breeding strategies. In the present study, genetic variation between genotypes for grain yield and yield attributing traits was observed. Drought stress at the reproductive stage caused a significant reduction in plant height, grain yield, ear-bearing tillers per plant, total dry matter production, test weight, and increased percentage of spikelet sterility. Three drought-tolerant genotypes were identified viz., Jonha 349, Dhalasaita, and N TeHao. The phenotypic variations had positively correlated with the genetic distance based on SSR markers. Ten markers showed a high level of allelic diversity and found to be highly informative. Among them, RM215, RM271, RM306, and RM237 were promising since they were linked to drought-related QTLs. Therefore, the identified donor and markers can be used in marker-assisted breeding for reproductive stage drought tolerant in rice.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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