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Appraisal of genetic diversity and population structure in assorted rice genotypes for early seedling vigour trait linked markers

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ABSTRACT

Early Seedling Vigour (ESV) is an important trait for early establishment of rice crop in the direct seeded condition. In the present study, genetic diversity in a set of 91 rice accessions of improved varieties, landraces and wild rice accessions of Oryza nivara and Oryza rufipogon were assessed by 52 microsatellite markers associated with early seedling vigour QTLs. A total of 82 alleles were amplified with an average of 2.34 alleles per locus and their PIC values ranged from 0.374 to 0.071 with an average of 0.33. The model based population structure approach grouped the total rice accession into two distinct populations ranged between 0.104 (population 1) and 0.334 (population 2), while allele frequency divergence between two populations was 0.105. The phylogenetic analysis grouped the genotypes into five major clusters and 12 sub-clusters. Results indicated that, these rice genotypes exhibited a high genetic diversity and could be useful in rice improvement programme specific to ESV.

Key words: *rice, early seedling vigour, microsatellite markers, genetic diversity, population structure, cluster analysis*

Rice (*Oryza sativa* L.) is the foremost cereal crops of the world and primary food crop of half of the world's population. Globally, 158.8 million ha of rice is grown with production of 738 million tons (FAO 2013), among them 90% of rice grown and consumed in Asia. As on today, rice production represents 30% of the world cereal production. It has doubled in the last 30 years, due to the introduction of new varieties, but its present growth barely follows consumption. In the year 2025, 4.6 billion people would depend on rice for their daily nourishment, compared with three billion today. The ever increasing rice demand with shrinking natural resources is a great challenge to plant breeders and biotechnologist.

Weed is as old as agriculture, and from the very beginning farmers realized the interference of weed with crop productivity, which led to the co-evolution of agro-ecosystems and weed management. Weeds are the greatest yield-limiting constraint to rice. The risk of yield loss from weeds in direct-seeded rice is greater than transplanted rice (Rao *et al*. 2007). The modern method of irrigation, practicing herbicide application, deployment of new varieties with precocity and high yield, and exaltation of labor cost in direct seeding has become inevitable. High seedling vigour is an important trait for direct seeding, as it can enhance crop establishment and increases the plant's ability to compete against weeds (Dingkuhn *et al*. 1999; Rao *et al*. 2007).

Early seedling vigour (ESV) is an essential component of plant development under most environmental conditions (Hund *et al*. 2004). In arid environments, crop varieties with early seedling vigour and good stand establishment tend to maximize use of available soil water, resulting in increased dry matter accumulation and improved grain yield (Liu *et al*. 2014;

Mahender *et al*. 2015). However, genetic improvement of early seedling growth, information on genetic variation in traits related to early seedling vigour and also knowledge concerning the relationships among various seedling vigour traits is necessary to understand for improvement of grain yield under direct seeded rice (DSR).

The information on the magnitude of genetic variability and the extent to which the desirable characters are heritable is required for successful crop improvement programme (Ravi *et al*. 2003; Das *et al*. 2013; Choudhury *et al*. 2013; Mizan *et al*. 2015; Yan *et al*. 2015). SSR markers (or microsatellites) due to their co-dominant and highly polymorphic nature offer an easy, accurate, and quantifiable measure of the genetic variation within crop plants. Therefore, the SSR markers have been proved to be an ideal for making genetic maps, markers assisted selection and studying genetic diversity in diverse rice germplasms (Mahender *et al*. 2014; Anandan *et al*. 2016; Tarang *et al*. 2016; Pradhan *et al*. 2016). In this context, estimating genetic diversity by molecular maker in the absence of environmental influence is of great value in crop improvement to identify diverse genotype with possibility of getting transgressive segregants. As ESV is an important trait contributor in the case of direct seeded rice against weed competitiveness and nutrient absorption. Studying genetic diversity for ESV with ESV linked markers would be the most appropriate in the present agricultural scenario and this could be the prominent report from India. The present investigation has been carried out to estimate the extent of genotypic variability among representative accessions of improved varieties, landraces and wild rice accessions (*Oryza nivara* and *Oryza rufipogon*) using ESV trait linked SSR markers.

MATERIALS AND METHODS

Plant materials

Ninety-one rice accessions (including 14 improved varieties, 37 landraces and 40 wild rice) were obtained from Rice Genebank, National Rice Research Institute (NRRI), Cuttack (Table 1). The collected seeds were raised in trays under net house for sample collection.

SSR genotyping

Genomic DNA was isolated from fresh leaf of the rice accessions by following the method of CTAB (Murray and Thompson 1980). The final DNA concentration was adjusted to 30mg/ μ lfor polymerase chain reaction (PCR) analysis.

A total of 52 ESV trait QTLs linked SSR markers were sampled from the reports of earlier mapping population studies and the distribution of markers covered all the chromosomes (Mahender *et al*. 2015; Anandan *et al*. 2016) to assess the genetic diversity of rice accessions. The details of molecular markers information were collected in public domain databases of www.gramene.org.

The PCR reaction mixture contained 30ng templates DNA, 10pM concentration of each of the primers, 2.5mM dNTPs, 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mMKCl, 1.5 mM $MgCl_{2,}$ and 0.01 mg/ml gelatin) and 5U of *Taq* DNA polymerase in a volume of 10ml. The reaction mixture was initially denatured for 3 min at 94^0C, and, then, subjected to 30 cycles of 30 sec denaturation at 94^0C, 30 sec annealing at 50-60^0C (varies according to primers) and 60 sec extension at 72^0C; and a final extension for 5 min at 72^0C. PCR products were resolved on 3% agarose gel. After electrophoresis, the gel was visualized under UV and photographed using Alpha Innotech gel documentation system (Flour ChemTM 5500, Alpha Innotech, USA).

Molecular data analysis

The genetic diversity parameters such as number of alleles per locus, major allele frequency, observed heterozygosity and polymorphic information content (PIC) were estimated using the POWERMARKER Ver3.25 (Liu and Muse 2005). Allele frequency represents the frequency of particular allele for each marker. Heterozygosity is the proportion of heterozygous individuals in the population. Polymorphic information content that represent the amount of polymorphism within a population was estimated based on Botstein *et al*. (1980).

To assess the genetic structure, model based approach and distance based approach were used. The gene diversity analysis provides an unbiased estimation of genetic variation at any given locus. The allelic data were subjected to estimation of genetic distances among genotypes using simple matching coefficients by bootstrapping 10,000 times and they were clustered

Table 1. List of rice accessions/varieties used for the genetic diversity analysis

Sl.No.	Rice varieties	Type	S. No	Rice varieties	Type
1	AC 100062(A)	WD	47	Dular	LR
2	AC 100062(B)	WD	48	B1	LR
3	AC 100062(C)	WD	49	B6	LR
4	AC 100107	WD	50	B8	LR
5	AC 100117	WD	51	B10	LR
6	AC 100142	WD	52	B11	LR
7	AC 100169	WD	53	B12	LR
8	AC 100175	WD	54	B13	LR
9	AC 100193	WD	55	B15	LR
10	AC 100219(A)	WD	56	B16	LR
11	AC 100219(B)	WD	57	B17	LR
12	AC 100281	WD	58	B18	LR
13	IR36	IM	59	B19	LR
14	AC 100296	WD	60	B20	LR
15	Heera	IM	61	B21	LR
16	Khitish	IM	62	B22	LR
17	Phalguni(A)	IM	63	B23	LR
18	Phalguni(B)	IM	64	Boff16	LR
19	Kamesh	IM	65	AC 100006	WD
20	Neela	IM	66	AC 100010	WD
21	Abhisek	IM	67	AC 100015	WD
22	CR Dhan 103	IM	68	AC 100026	WD
23	Vandana	IM	69	AC 100032(A)	WD
24	Kalinga - 3	IM	70	AC 100032(A)	WD
25	Brown gora	LR	71	AC 100035	WD
26	SadaBahar	IM	72	AC 100087	WD
27	CR Dhan 40	IM	73	AC 100121	WD
28	Sekri	LR	74	AC 100123	WD
29	SukhaPanki	LR	75	AC 100124	WD
30	ChakhaoAubi	LR	76	AC 100133	WD
31	Kabuk Phou(A)	LR	77	AC 100135	WD
32	Kabuk Phou(B)	LR	78	AC 100203	WD
33	Leima Phou	LR	79	AC 100281	WD
34	Baman Phou	LR	80	AC 100209(B)	WD
35	Buluharana	LR	81	AC 100282(A)	WD
36	Kumbhi Phou	LR	82	AC 100283	WD
37	Long manabi(A)	LR	83	AC 100284	WD
38	Long manabi(B)	LR	84	AC 100285(B)	WD
39	Akhiyaturfa	LR	85	AC 100295	WD
40	Gini	LR	86	AC 100301	WD
41	Arupathamkuruvai(A)	LR	87	AC 100309	WD
42	Arupathamkuruvai(B)	LR	88	AC 100326	WD
43	Kasalath	LR	89	AC 100328	WD
44	Harishankar(A)	LR	90	AC 100329	WD
45	Harishankar(B)	LR	91	AC 100337(B)	WD
46	Sarathi	IM			

LR-Landraces; **IM**-Improved varieties; **WD**-Wild rice **L**-50bp ladder; **1-91** order of the rice genotypes

using neighbor joining method (Felsenstien, 1985). Further, Principal Coordinates Analysis (PCoA) was performed and the first two principal components were used to represent the genetic distance among the genotypes in graphical form. Both the clustering analysis and PCoA were done using DARwin software ver 5.0 (Perrier *et al*. 2003; Perrier and Jacquemoud-Collet 2006). A hierarchical analysis of molecular variance (AMOVA) of improved varieties, landraces and wild rice accessions were calculated to partition genetic

diversity within and among accessions by Arlequin software (Excoffier *et al*. 2005) with 1000 permutations. F statistics, which include F_{IT}, deviations from Hardy-Weinberg expectation across the whole population, F_{IS} deviation from Hardy-Weinberg expectation within rice accessions and F_{ST}, correlation of alleles between different rice accessions were calculated using Arlequin.

Model based approach was utilized with Structure ver 2.3.4 software (Pritchard *et al*. 2000). To derive the number of population clusters (K), STRUCTURE was run with K varying from 1 to 10, with five runs for each K value. Values of *L(K)* (log posterior probability of the data) returned by STRUCTURE were averaged across simulations for each *K* value by adapting the method given by Evanno et al. (2005). The 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. Further, to determine the true value of K, a plot of the second order rate of change in *L(K)* between values of *K* (ΔK) was formed. A threshold of 80% genetic membership coefficient was used to assign genotypes to the respective population. Genotypes lesser than the threshold were designated as 'admixtures'.

RESULTS AND DISCUSSION

Genetic variability of 91 accessions of rice was evaluated for the estimation of genetic diversity parameters (Table 2) (Fig.1). Of the total of 52 SSR markers used for genotyping the total rice accessions, 35 markers showed polymorphism and were used in all analyses performed subsequently. The remaining 17 markers were not polymorphic and hence were excluded. The 35 markers generated a total of 82 distinct alleles across 91 rice genotypes with an average of 2.34 alleles per locus. The number of alleles varied from 2-4 with an average number of alleles amplified per marker was 2.34. The maximum numbers of alleles (4) were amplified with RM336 and three alleles were amplified with ten markers as RM3839, RM161, RM9, RM148, RM340, RM16, RM252, RM8085, RM106 and RM341. The size of the amplified fragments ranged from 70bp to 750bp. The PIC value of the polymorphic primers RM9, RM264, RM252, RM106, RM7389 and RM253 showed more than 0.374, while in RM223 and RM3839 showed the lowest PIC value as 0.071 with an average PIC value of 0.33**.**

Out of 35 SSRs, RM230 didn't show any heterozygosity and in the remaining of SSR markers, it ranged from 0.011 (RM3839, RM334 and RM263) to 0.769 (RM161) with an average of 0.231. Further, the gene diversity ranged from 0.074 (RM223 and RM3839) to 0.500 (RM106, RM252, RM264, RM9) with an average of 0.431. The major allele frequency (MAF) at the SSR loci varied between 0.500 (RM252) and 0.962 (RM3839 and RM223), with an average value of 0.635 of the 91 rice genotypes shared common allele per locus.

AMOVA has clearly brought out significant differences among various genotypes evaluated (Table 3). The AMOVA analysis revealed 14% ($P< 0{\cdot}001$) of total genetic variation among the three different rice populations, 36% ($P< 0{\cdot}001$) among the accessions and 50% ($P< 0{\cdot}001$) within rice accessions. The inbreeding co-efficient F_{IS} (0.418) and F_{IT} (0.502) were found to be 1.00.

Cluster analysis

The Cluster analysis was carried out to assess genetic distance and the dissimilarity matrix-using neighbor joining method. In the Unrooted tree, genotypes were grouped into five major clusters (Fig. 2) (Table 4) and were again distributed into 12 sub-clusters. Among the five major clusters, the highest number of accessions contained in Cluster I with 35 rice genotypes, which includes 30 wild rice accessions with four landraces (Baman Phou, Leima Phou, Kabuk Phou (B), Harishankar (A) and one improved variety Kalinga-3. Further, the cluster 1 was sub grouped further into cluster 1a (21), cluster 1b (9), and cluster 1c (5). The second highest number of genotypes were recorded in Cluster III which contained 24 rice genotypes belonging to improved rice varieties and landraces (Fig. 2) (Table 4). The lowest number of rice accessions was observed in Cluster V. The cluster V consists of only two rice accessions B6 (landrace) and AC100175 (wild species).

Population structure and principal component analysis

According to STRUCTURE analysis results based on Bayesian clustering approach model, a significant population structure was detected among the 91 rice

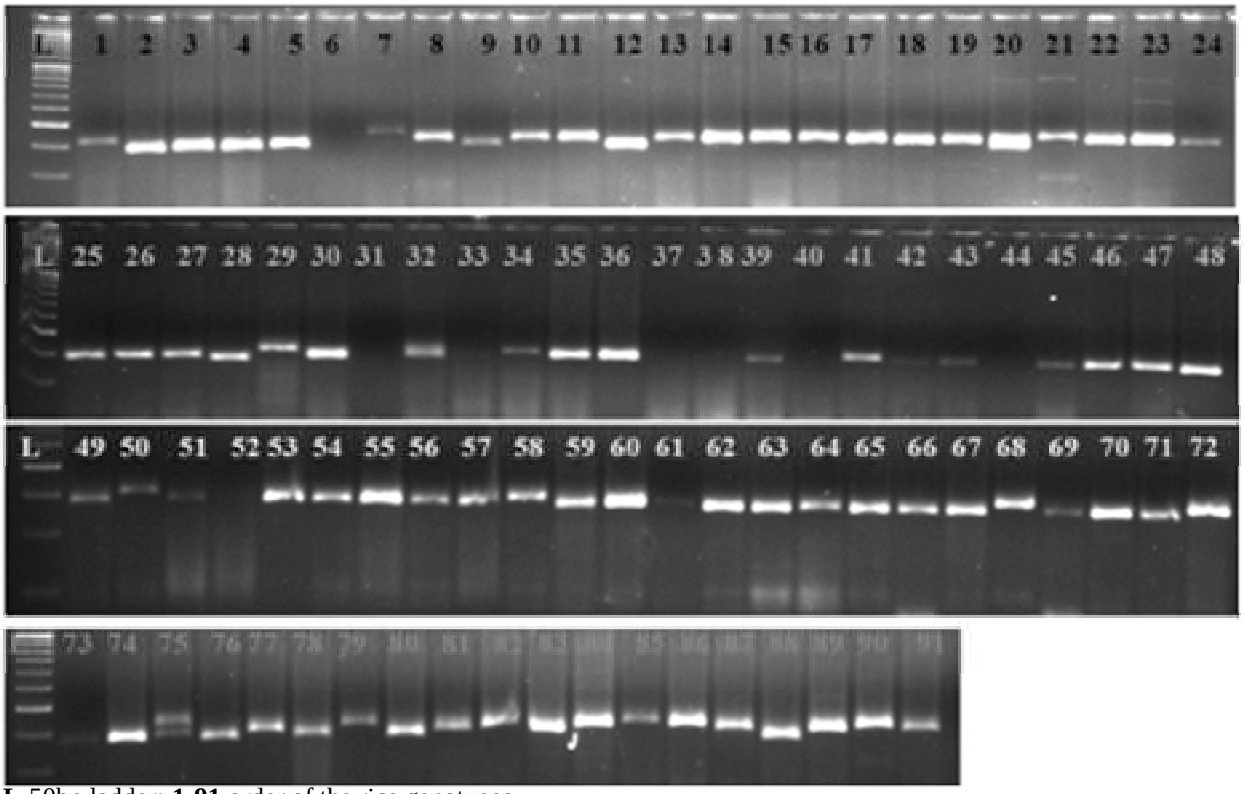
Table. 2. Details of SSR molecular marker loci used for genotyping of 91 rice accessions and their genetic diversity parameters

S. No	Markers	Chr	Motifs	MAF	TNA	GD	Ho	PIC
1	RM9	1	(GA)15GT(GA)2	0.511	3	0.5	0.473	0.375
2	RM7075	1	(ACAT)13	0.527	2	0.498	0.242	0.374
3	RM1339	1	(AG)22	0.813	2	0.304	0.022	0.258
4	RM8085	1	(AG)26	0.533	3	0.498	0.121	0.374
5	RM221	2	(TC)4T3C3(TC)(CT)2	0.703	2	0.417	0.11	0.33
6	RM263	2	(CT)34	0.555	2	0.494	0.011	0.372
7	RM106	2	(GAA)5	0.511	3	0.5	0.495	0.375
8	RM341	2	(CTT)20	0.681	3	0.434	0.264	0.34
9	RM218	3	(TC)24ACT5(GT)11	0.703	2	0.417	0.11	0.33
10	RM148	3	(TG)12	0.593	3	0.483	0.484	0.366
11	RM85	3	(TGG)5(TCT)12	0.736	2	0.388	0.022	0.313
12	RM16	3	(TCG)5(GA)16	0.527	3	0.498	0.505	0.374
13	RM168	3	T15(GT)14	0.599	2	0.48	0.407	0.365
14	RM7389	3	(GATA)7	0.516	2	0.499	0.286	0.375
15	RM3839	4	(GA)23	0.962	3	0.074	0.011	0.071
16	RM13	5	(GA)6-(GA)16	0.527	2	0.498	0.022	0.374
17	RM26	5	(GA)15	0.687	2	0.43	0.385	0.338
18	RM87	5	(CTT)3T(CTT)11	0.819	2	0.297	0.319	0.253
19	RM161	5	(AG)20	0.549	3	0.495	0.769	0.373
20	RM334	5	(CTT)20	0.599	2	0.48	0.011	0.365
21	RM249	5	(AG)5A2(AG)14	0.615	2	0.473	0.549	0.361
22	RM252	5	(CT)19	0.5	3	0.5	0.692	0.375
23	RM340	6	(CTT)8T3(CTT)14	0.665	3	0.446	0.187	0.346
24	RM225	6	(CT)18	0.637	2	0.462	0.088	0.355
25	RM253	6	(GA)25	0.516	2	0.499	0.022	0.375
26	RM125	7	(GCT)8	0.923	2	0.142	0.154	0.132
27	RM336	7	(CTT)18	0.593	4	0.483	0.725	0.366
28	RM264	8	GA)27	0.505	2	0.5	0.044	0.375
29	RM230	8	(AGG)4(GA)9A(AG)13	0.538	2	0.497	0	0.374
30	RM223	8	(CT)25	0.962	2	0.074	0.077	0.071
31	RM258	10	(GA)21(GGA)3	0.56	2	0.493	0.044	0.371
32	RM3428	11	(CT)18	0.566	2	0.491	0.055	0.371
33	RM224	11	(AAG)8(AG)13	0.703	2	0.417	0.176	0.33
34	RM21	11	(GA)18	0.615	2	0.473	0.176	0.361
35	RM19	12	(ATC)10	0.659	2	0.449	0.022	0.348
Mean	**0.635**	**2.34**	**0.431**	**0.231**	**0.332**			

RM-Rice Microsatellite;**Chr**-Chromosome**; TNA**-Total Number of Alleles**; MAF**-Major Allele Frequency; **GD**- Gene Diversity; **Ho**-Observed Heterozygosity; **PIC**-Polymorphic Information Content

accessions of improved varieties, landraces and wild rice. The optimal number of groups were determined by the maximum likelihood, and *k* was set at 2 implying two structural groups were identified in the panel (Fig. 2). In population1, 53 genotypes were grouped, population 2 contains 24 genotypes and rest (14 genotypes) was identified as admixture. The genotype with score >0.80 was considered as pure and <0.80 as admixture.The fixation index (Fst) values of two population ranged between 0.104 (population 1) and 0.334 (population 2), while allele frequency divergence between two population was 0.105. The mean value of admixture alpha (á) was 0.16,which suggests that most of selected landraces and improved cultivars of each subpopulation had a common ancestry with few admixture individuals. The genetic diversity among the 91 rice accessions were visualized by PCoA in the scatter plot (Fig. 2). The PC1 accounted for about 12.96% and PC2 accounted for 8.96% of the total genetic variance (totaling 21.92%). In PCoA, genotypes were presented in colors corresponding to the clusters observed in unrooted tree.

Genetic diversity plays a major role in survival and adaptability of a species, as changes happen in



L-50bp ladder; **1-91** order of the rice genotypes

Fig. 1. Genotyping of 91 rice accessions with polymorphic microsatellite marker RM3428.

environment, organism needs to make changes in the phenotype that enables it to adapt and survive in unfavorable conditions. Species with good amount of genetic diversity among populations have more variations to choose the fittest alleles. Species with little genetic diversity are at a greater risk of extinction. Broadening the genetic base of core breeding material requires the identification of diverse parents for hybridization with cultivated rice.

The SSR markers are a DNA-based co-dominant marker, which offers greater variability in crop germplasm that makes them more suitable in molecular breeding. This marker has been successfully applied in rice to decipher genetic diversity (Singh *et al*. 2010; Das *et al*. 2013; Singh *et al*. 2013; He *et al*. 2014; Surapaneni *et al*. 2016; Anandan *et al*. 2016; Edzesi *et al*. 2016; Pradhan *et al*.2016).The 35 polymorphic SSR markers used for this study revealed a clear and consistent amplification profile. The total numbers of 82 alleles were identified from the polymorphic ESV trait QTL linked SSR marker with 91 rice genotypes and an average allele number of 2.34. In an earlier report, Pervaiz *et al*. (2009) assessed genetic variability of 35 Asian rice cultivars using 32 SSR markers and they observed 144 alleles with an average of 4.5 alleles per locus. In similar way, Rahman *et al*. (2010) screened 28 local rice varieties with seven primers and found 82 alleles with an average of 11.7 alleles per locus. Jiang *et al*. (2004) also reported similar observation of number allele per locus ranged from three to as high as 22 with an average of 7.8 alleles per locus. Israt *et al*. (2014) detected 321 alleles from 30 landraces and high yielding varieties using 27 SSR markers and the number of alleles per locus generated by each marker varied from 6 to 21 alleles with an average number of polymorphic alleles per marker 11.89. Recently, Mizan *et al*. (2015) identified 76 alleles in 24 rice germplasms using nine simple sequence repeat (SSR) primers with an average of 8.44 alleles per locus. Tarang *et al*. (2016) studied genetic diversity in 64 rice (*Oryza sativa* L.) genotypes, including breeding lines and improved varieties using 17 SSR

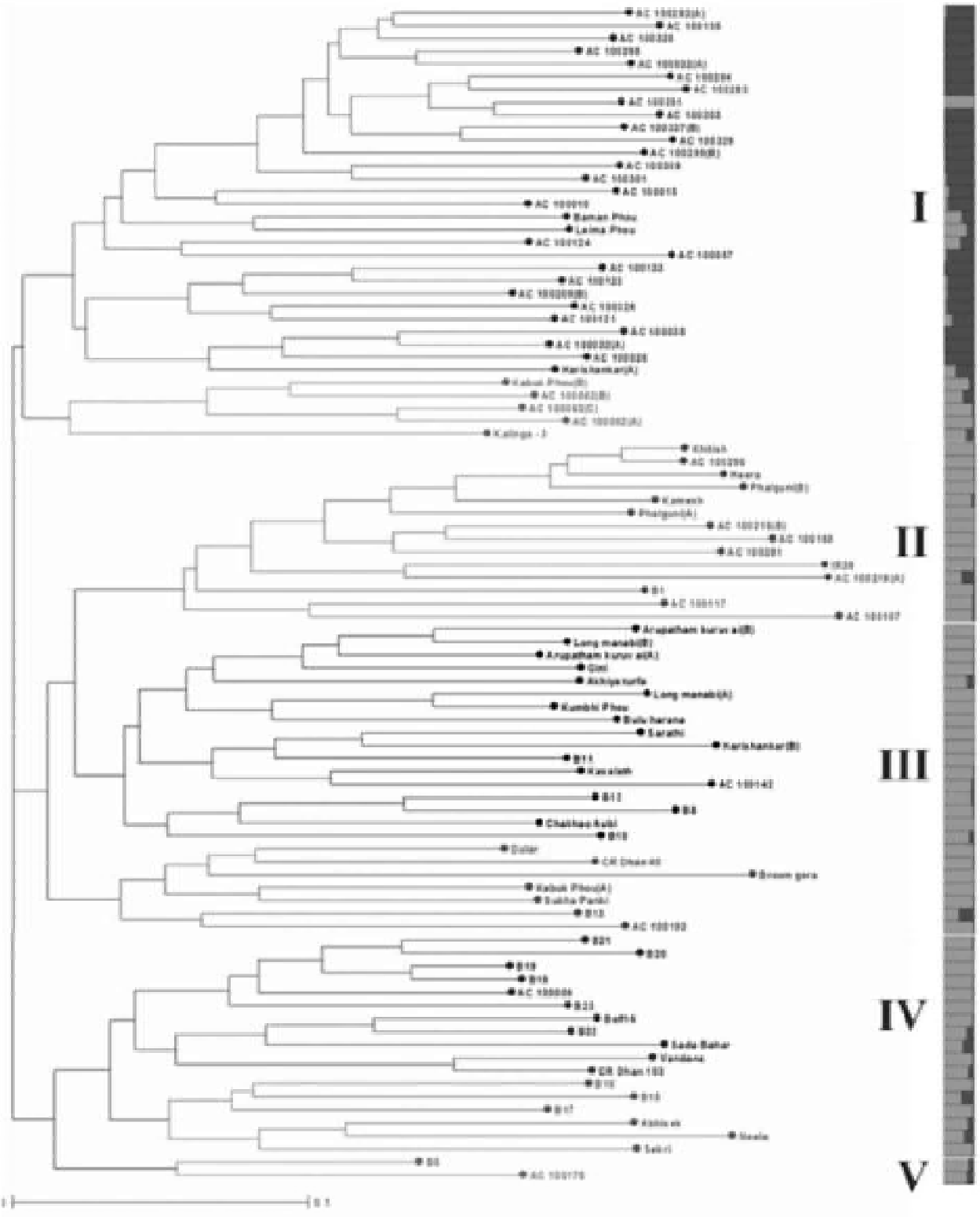


Fig 2. Genetic grouping of 91 rice genotypes. A. Neighbour-joining tree showing five distinct clusters; B. STRUCTURE grouping of rice genotype into two clusters

Table. 3. Analysis of molecular variance (AMOVA) based on the 35 SSR loci of 91 rice accessions

Source of variation	df	SS	MS	Est. Var.	%	P-value
Among Pops	2	138.778	69.389	1.158	14%	<0·001
Among Indiv	88	866.651	9.848	2.905	36%	<0·001
Within Indiv	91	367.500	4.038	4.038	50%	<0·001
Total	181	1372.929	83.276	8.102	100%	

d.f- degree of freedom; **SSD**- sum of squared deviations; **Est. Var**- variance component estimates; **% -**total percentage of total variation

Table. 4. Distribution of rice genotypes into different clusters based on ESV QTLs associated simple sequence repeat (SSR)

S.No	Clusters	Sub-clusters	No. of genotypes	Name of the varieties/accessions
1	I	3	21	AC100282(a), AC100135, AC100328, AC100295, AC100032(A), AC100284, AC100283, AC100281, AC100203, AC100337(B), AC100329, AC100285(B), AC100309, AC100301, AC100015, AC100010, Baman Phou, Leima Phou, and AC100124, AC100087
			9	AC100133, AC100123, AC100209(B), AC100133, AC100123, AC100123, AC100209(B), AC100326, AC100121, AC100035, AC100032(A), AC100026, and Harishankar (A)
			5	Kabuk Phou (B), AC100062 (B), AC100062(C), AC100062 (A) and Kalinga-3.
2	II	1	14	Khitish, AC100296, Heera, PhalgunI(B), Kamesh, Phalguni(A), AC100219(B), AC100169, AC100281, IR36, AC100219(A), B1, AC100117, and AC100107
3	III	4	8	ArupathamKuruvai(A), Long manabi(A), ArupathamKuruvai(B), Gini, Akhiyaturfa, Longmanabi(B), Kumbi Phou, Buluharana
			5	Sarathi, Harishanjer(B), B11, Kasalath, AC100142,
			4	B12, B8, ChakhaoAubi, B10
			7	Dular, CR Dhan 40, Brown gora, kabuki Phopu (A), sukhaPanki, B13, AC100193.
4	IV	3	6	B21, B20, B19, B18, AC100006, B23
			5	Boff6, B22, SadaBahar, Vandana, CR Dhan 103
			6	B16, B15, B17, Abhisek, Neela and Sekri
5	V	1	2	B6 and AC100175

markers. The amplified alleles were ranged from 2 to 4 alleles per locus.

In our study, maximum numbers of alleles, four were amplified with RM336 and three alleles were amplified by each one of the following markers *viz.*, RM3839, RM161, RM9, RM148, RM340, RM16, RM252, RM8085, RM106 and RM341(Table 2). The variability in the number of alleles identified per locus might be due to utilizing diverse rice genotypes as landraces, improved varieties and wild rice. These variations in multiple allele level indicate that, SSR marker is crucial for identification of rice accessions at molecular level. Similar results were observed in previous fingerprinting and diversity studies, having 1 to 8 alleles with an average of 4.58 alleles for various classes of microsatellite (Siwach *et al*. 2004) and 3 to 9 alleles, with an average of 4.53 alleles per locus of 30 microsatellite markers (Hossain *et al*. 2007).

In case of PIC value, the polymorphic primers RM9, RM264, RM252, RM106, RM7389 and RM253 showed more than 0.374 PIC value, while in RM223 and RM3839 showed the lowest PIC value as 0.071 with an average PIC value of 0.33 (Table 2). Similarly, diverse level of PIC value were observed earlier as 0.48 (Ashfaq *et al*. 2012), 0.25 (Singh *et al*. 2013), 0.48 (Zhang *et al*. 2011), 0.17 (Mahender *et al*. 2014) 0.68 (Mizan *et al*. 2015), 0.24 (Anandan *et al*. 2016), 0.84 (Israt *et al*. 2014), 0.38 (Tarang *et al*. 2016) in diverse set of rice germplasm. The reason for the varied level of factors that affects PIC value in a breeding and molecular research might be due to collection size, diversity of the collection, breeding pattern of the species and location of primers in the genome.Therefore, the SSR markers those generate higher number of alleles and having higher PIC values could be used for future rice diversity analysis.

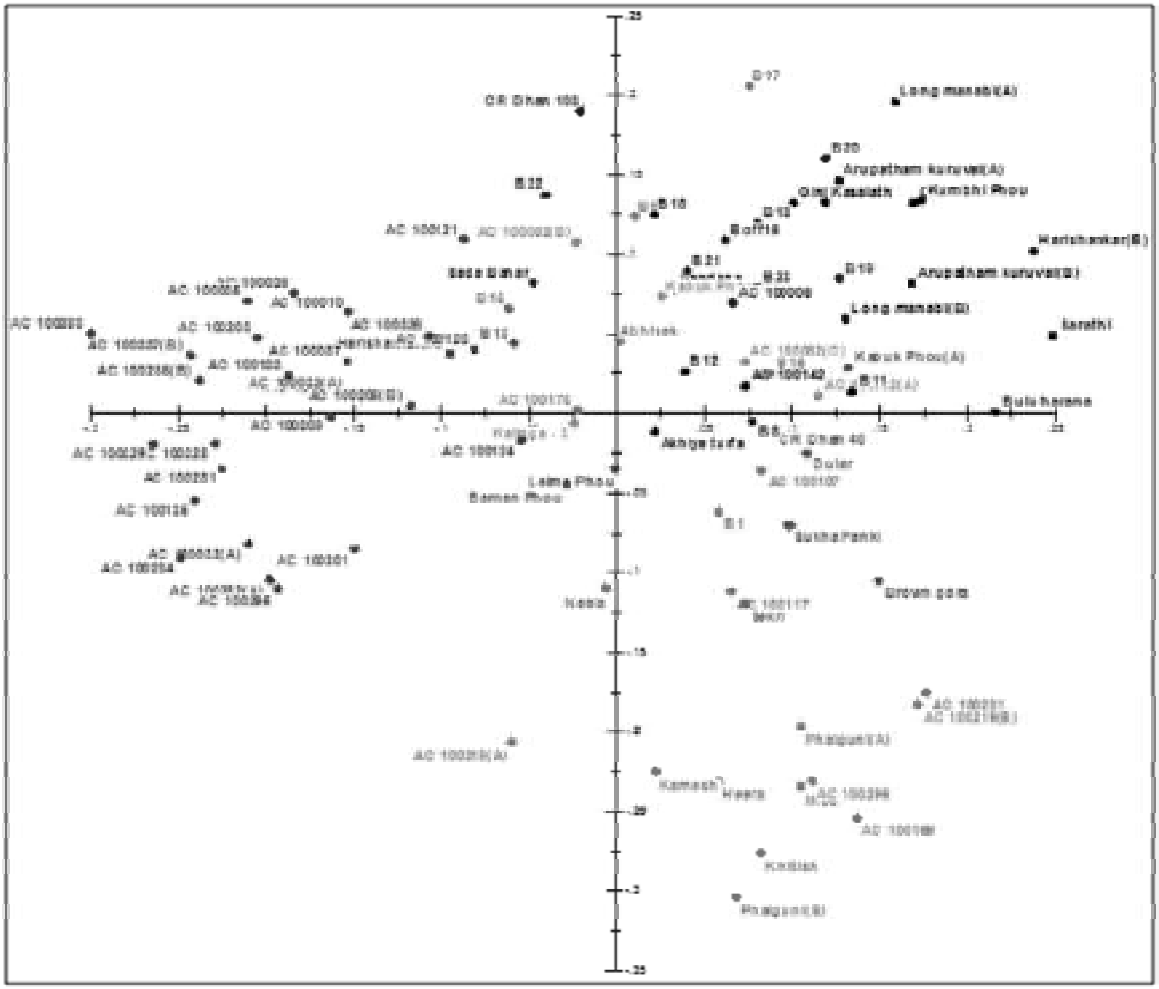


Fig. 3. Principal coordinate analysis of 91 rice genotypes based 35 SSRs (genotypes represented in colors corresponding to the cluster observed in unrooted tree).

The highly polymorphic markers such as RM336 (Germination rate (GR), Shoot dry weight (SDW), Shoot length (SL)-Huang *et al*. 2004), RM3839 (Shoot dry weight (SDW)- Cheng *et al*. 2013), RM161 (Root length (RL), Total dry weight (TDW), Early seedling vigour (ESV), Field vigour (FV), Germination rate (GR)-Zhou *et al*. 2006; Lu *et al*. 2007), RM9 (Germination rate (GR), Shoot dry weight (SDW), Shoot fresh weight (SFW)-Wang *et al*. 2010), RM148 (Germination rate (GR), Shoot length (SL), Early seedling vigour (ESV), Field vigour (FW)-Zhang *et al*. 2005; Zhou *et al*. 2006; Lu *et al*. 2007), RM340 (Shoot length (SL), Germination percentage (GP)-Zhang *et al*. 2005;Wang *et al*. 2010), RM16 (Shoot length (SL), Shoot dry weight (SDW), Root length (RL), Germination rate (GR)- Zhang *et al*. 2005), RM252 (Early seedling vigour (ESV), Germination percentage (GP), Germination rate (GR), Root activity (RA)-Cui *et al*. 2002; Lu *et al*. 2007; Wang *et al*. 2010), RM8085 (Shoot length (SL)-Abe *et al*. 2012), RM106 (Germination rate (GR)- Diwan *et al*.2013) and RM341(Germination rate(GR)-Diwan *et al*.2013; Diwan *et al*. 2013) are related to QTLs associated with early seedling vigour traits in rice. In the present study, which was carried out with landraces, improved cultivars and wild rice genotypes, the markers displayed polymorphism of the QTL linked markers suggesting the close relationship between the markers and the traits. This can help in use of these markers in the marker assisted breeding programme to identify superior genotypes for early seedling vigour traits under DSR.

Gel image of the present study showed production of multiple alleles in 91 rice accessions. The presence of multiple alleles suggests that, these markers could be used effectively in molecular characterization of different rice accessions. Despite the production of multiple alleles, they were robust enough to distinguish specifically diverse genotypes or different accessions of the studied genotypes. In the present study, out of 35 SSRs, RM230 didn't show any heterozygosity and in the remaining markers it ranged from 0.011 (RM3839, RM334 and RM263) to 0.769 (RM161) with an average of 0.725 and gene diversity ranged from 0.074 (RM223 and RM3839) to 0.500 (RM106, RM252, RM264, RM9) with an average of 0.431. Similarly, Mizan *et al*. (2015) found an average gene diversity of 0.714 of 24 genotypes, ranging from 0.6188 to 0.7908. Recent study of Anandan *et al*.(2016)found that,observed heterozygosity (H_o) ranged from 0.04 (RM3839) to 0.97 (RM148) with an average heterozygosity across all 39 loci was 0.42 from the 96 rice lines of landraces and improved varieties. Tarang *et al*. (2016) reported an average value of genetic diversity of 0.71 (0.21 to 1.37) as measured by Shannon's index indicating a considerable genetic diversity and heterogeneity within different selected varieties. Further, the genetic heterozygosity of SSRs was found to be 0.28.

In the present study, we attempted to classify the rice accessions by grouping them in relation to the genetic diversity between the genotypes. The STRUCTURE analysis revealed the optimal grouping of the genotypes into two clusters with78 genotypes as pure and 13 were identified as admixture. The fixation index (Fst) value of two population ranged between 0.104 (population 1) and 0.334 (population 2), while allele frequency divergence between two population was 0.105. Similarly, Courtois *et al*. (2012), Das *et al*. (2013), Anandan *et al*. (2016), Surapaneni *et al*. (2016) and Pradhan *et al*. (2016) were also reported varying number of (2 to 8) subpopulations from 425, 91, 629, 23, 240 number of accessions respectively.

In PCoA analysis, PC1 accounted for 12.96% and PC2 accounted for 8.96% of the genetic variance, totaling 21.92%.Two-dimensional scaling obtained using PCoA analysis also showed the same grouping pattern as UPGMA and sorted most of the cultivars into five major clusters distributed across the quadrants. This technique has been used to partition rice genotypes based on variation in molecular data generated by ESV QTLs trait associated molecular markers. In similar way, Seetharam *et al*. (2009); Maji and Shaibu (2012); Gana *et al*.(2013); Nachimuthu *et al*.(2015) also exploited the genetic variance of the first PC for classifying the rice genotypes.

Cluster analysis is a powerful method in the evaluation of genetic relationship studies (Randi and Lucchini 2002).Clustering based on polymorphic ESV QTL trait associated SSR markers classified the total rice accessions into improved varieties and wild rice accessions. The neighbour-joining tree cluster analysis of 91 rice genotypes, grouped them into five distinct clusters (Table 3). The major distinct clusters showed an additional sub-clusters identified within them. The UPGMA diagram generated through marker data information revealed that the genotypes derivatives of genetically similar type clustered together. In the earlier observations of cluster analysis, Yu *et al*. (2003) found that three major clusters and 9 sub-clusters using parental lines of 193 rice accessions and also Chakravarti *et al*. (2006) classified the rice genotypes into 11 distinct groups. Recently, Kumbhar *et al*. (2015) classified 50 rice genotypes comprising landraces, local selections, and improved varieties into 5 clusters and 11 sub-clusters using with SSR and ISSR markers.

The PIC values of the present study revealed that RM9, RM264, RM252, RM106, RM7389 and RM253 might be the best markers for identification of early seedling vigour traits and diversity estimation of rice genotypes. Physiological, morphological, biochemical and molecular genetic diversity analysis in a large germplasm collection will be relevant for the successful implementation of the various breeding approaches. In summary, it can be concluded that, a combination of integrated morpho-physiological and modern breeding approaches can help the researchers (plant breeders and biotechnologist) to select better genotypes for complex traits like early seedling vigour. In this context, SSR markers provided an adequate power of resolution to identify the superior genotypes from the germplasm pools, as they can serve as a potential tool in both identification and characterization of different genotypes. This allows breeders to track genetic loci controlling early seedling vigour traits in rice effectively.

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REFERENCES

Abe A, Takagi H, Fujibe T, Aya K, Kojima M, Sakakibara H, Uemura A, Matsuoka M and Terauchi R 2012. OsGA20ox1, a candidate gene for a major QTL controlling seedling vigor in rice. Theor Appl Genet 125(4):647-57

Anandan A, Mahender A, Pradhan SK and Singh ON 2016. Population structure, Genetic diversity and association mapping of traits associated QTLs in eco-geographical regions of rice (*Oryza sativa* L.) genotypes for early seedling vigor. PLoS ONE 11(3): e0152406

Ashfaq MS and Khan A 2012. Genetic diversity in basmati rice (*Oryza sativa* L.) Germplasm as revealed by microsatellite (SSR) markers. Russian J Genet 48(1): 53–62

Botstein D, White RL, Skolnick M and Davis RW 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Amer J Hum Genet 32: 314–331

Chakravarthi KB and Naravaneni R 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L); Afr J Biotechnol 5:684-688

Cheng XX, Cheng JP, Huang X, Lai YY, Wang L, Du WL, Wang ZF and Zhang HS 2013. Dynamic quantitative trait loci analysis of seed reserve utilization during three germination stages in rice. PLoS ONE 8(11):e80002

Choudhury B, Khan ML and Dayanandan S 2013. Genetic structure and diversity of indigenous rice (*Oryza sativa*) varieties in the Eastern Himalayan region of Northeast India. Springerplus 2(1): 228

Collard BCY, and Mackill DJ 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century; Philos Trans R Soc Lond B Biol Sci 363: 557-572

Courtois B, Frouin J, Greco R, Bruschi G, Droc G and Hamelin C 2012. Genetic diversity and population structure in a European collection of rice. Crop Sci 52(4): 1663–1675

Cui KH, Peng SB, Xing YZ, Xu CG, Yu SB and Zhang Q 2002. Molecular dissection of seedling-vigor and associated physiological traits in rice. Theor Appl Genet 105:745-753

Das B, Sengupta S, Parida SK, Roy B, Ghosh M, Prasad M and Ghose TK 2013. Genetic diversity and population structure of rice landraces from Eastern and North Eastern States of India. BMC Genet 14(1): 71

Dingkuhn M, Johnson DE, Sow A and Audebert AY 1999. Relationships between upland rice canopy characteristics and weed competitiveness. Field Crops Res 61:79–95

Diwan J, Channbyregowda M, Shenoy V, Salimath P and Bhat R 2013. Molecular mapping of early vigour related QTLs in rice. Res J Biol (1) 24-30

Edzesi WM, Dang X, Liang L, Liu E, Zaid IU and Hong D 2016. Genetic Diversity and Elite Allele Mining for Grain Traits in Rice (*Oryza sativa* L.) by Association Mapping. Front. Plant Sci 7:787.

Evanno G, Regnaut S and Goudet J 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14(8): 2611–2620.

Excoffier L, Laval G and Schneider S 2005. Arlequin version 3·0: an integrated software package for population genetics data analysis (Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, University of Berne, Switzerland)

FAO 2013. faostat.fao.org/site/339/default.aspx

Felsenstien J 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–91.

Gana AS, Shaba SZ and Tsado EK 2013. Principal component analysis of morphological traits in thirty-nine accessions of rice (*Oryza sativa* L.) grown in a rain fed lowland ecology of Nigeria. J Plant Breed Crop Sci 5:120–126

He J, Zhao X, Laroche A, Lu Z-X, Liu H and Li Z 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers Plant Sci. 5:484.

Hossain MZ, Rasul MG, Ali MS, Iftekharuddaula KM and Mian MAK 2007. Molecular characterization and genetic diversity in fine Grain and aromatic landraces of rice (*Oryza sativa* l.) Using Microsatellite markers. Bangladesh J. Genet Pl. Breed 20(2): 01-10, 2007

Huang Z, Yu T, Su L, Yu SB, Zhang ZH and Zhu YG 2004.

Identification of chromosome regions associated with seedling vigor in rice. Acta Genetica Sinica 31:596-603

Hund A, Fracheboud Y, Soldati A, Frascaroli E, Salvi S and Stamp P 2004. QTL controlling root and shoot traits of maize seedlings under cold stress. Theor Appl Genet 109(3):618–629

Israt N, Mohiuddin AKM, Sultana Shahanaz and Jannatul Ferdous 2014. Diversity analysis of indica rice accessions (*Oryza sativa* L.) using morphological and SSR markers. Annals Biol Res 5 (11):20-31

Jiang L, Hou MY, Ming WC and Min WJ 2004. Quantitative trait loci and epistatic analysis of seed anoxia germinability in rice (*Oryza sativa*). Rice Sci 11:238–244

Kumbhar S, Kulwal P, Patil JV, Sarawate C, Gaikwad A Jadhav A 2015. Genetic Diversity and Population Structure in Landraces and Improved Rice Varieties from India. Rice Science 22(3): 99-107

Liu H, Saddam H, Zheng M, Sun L, Shah F, Huang J, Cui and Nie L 2014. Progress and constraints of dry direct-seeded rice in China. J Food Agric Environ 12(2):465–472

Liu K and Muse SV 2005. Power Marker: Integrated analysis environment for genetic marker data; Bioinformatics. 21: 2128–2129

Lu XL, Niu AL, Cai HY, Zhao Y, Liu JW, Zhu YG and Zhang ZH 2007. Genetic dissection of seedling and early vigor in a recombinant inbred line population of rice. Plant Sci 172:212–220

Mahender A, Dash GK, Padmini S, Rao GJN 2014. Physiological and molecular profiling of rice genotypes under drought stress. Oryza 51(1): 12-24

Mahender A, Anandan A and Pradhan SK 2015. Early seedling vigour, an imperative trait for direct seeded rice: an overview on physio-morphological parameters and molecular markers. Planta 241:1027–1050

Maji AT and Shaibu AA 2012. Application of principal component analysis for rice germplasm characterization and evaluation. J Plant Breed Crop Sci. 4(6):87–93

Mizan FR, Rahman MM, Halder J, Rehana S, Begum S and Islam MM 2015. Genetic diversity and SSR marker assisted salt screening of rice (*Oryza sativa* L.) Biosci Bioeng Commun 1: 29-37

Murray MG and Thompson WF 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325

Nachimuthu VV, Muthurajan R, Duraialaguraja S, Sivakami R, Pandian BA and Ponniah G 2015. Analysis of population Structure and Genetic Diversity in Rice Germplasm Using SSR Markers: An Initiative Towards Association Mapping of Agronomic Traits in *Oryza Sativa*. Rice. 8: 30

Pervaiz ZH, Rabbani MA, Pearce SR and Malik SA 2009. Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. Afr J Biotechnol 8: 5641–5651

Perrier X, Flori A and Bonnot F 2003. Methods for data analysis. In: Hamon P, Seguin M, Perrier X, Glazmann JC, editors. Genetic diversity of cultivated tropical plants. Montpellier: Science Publishers Inc and Cirad, pp. 31–63

Perrier X and Jacquemoud-Collet JP 2006. Darwin software, http://darwin.cirad.fr/darwin (September 22, 2009).

Pradhan SK, Barik SR, Sahoo A, Mohapatra S, Nayak DK, Mahender A, Meher J, Anandan A and Pandit E 2016. Population Structure, Genetic Diversity and Molecular Marker-Trait Association Analysis for High Temperature Stress Tolerance in Rice. PLoS ONE 11 (8): e0160027.

Pritchard JK, Stephens M and Donnelly P 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945-59

Rahman MS, Sohag, MKH and Rahman L 2010. Microsatellite based DNA fingerprinting of 28 local rice (*Oryza sativa* L.) varieties of Bangladesh. J Bangla Agri Univ 8, 7–17

Randi E and Lucchini V 2002. Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conservation Genetics 3: 29–43

Rao AN, Johnson DE, Sivaprasad B, Ladha JK and Mortimer AM 2007. Weed management in direct-seeded rice. Adv Agron 93:153–255

Ravi M, Geethanjali S, Sameeyafarheen F and Maheswaran M 2003. Molecular marler based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica 133: 243-252

Roy RN and Misra RV 2002. Economic and environmental impact of improved nitrogen management in Asian

rice-farming systems. In: Proceedings of the 20th Session of the International Rice Commission p. 23–6

Seetharam K, Thirumeni S and Paramasivam K 2009. Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. Afr J Biotechnol 8(10):2050–2059

Shah SM, Naveed SA and Arif M 2013. Genetic diversity in Basmati and non- Basmati rice varieties based on microsatellite markers. Pak J Bot. 45: 423–431

Singh N, Choudhury DR, Singh AK, Kumar S, Srinivasan KRK, Tyagi N, Singh K and Singh R 2013. Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. PLoS ONE 8(12): e84136

Singh H, Deshmukh R K , Singh A., Singh A K and Gaikwad K 2010. Highly variable SSR markers suitable for rice genotyping using agarose gels. Mol. Breed 25 359-364

Siwach P, Jain S, Saini N, Chowdhury VK and Jain RK 2004. Allelic diversity among Basmati and non-Basmati long-grain indica rice varieties using microsatellite markers. J Plant Biochem Biotechnol 13: 25-32

Sow M, Ndjiondjop MN, Sido A, Mariac C, Laing M and Bezançon G 2014. Genetic diversity, population structure and differentiation of rice species from Niger and their potential for rice genetic resources conservation and enhancement. Genet Resour Crop Evol 61(1): 199–213

Surapaneni M, Balakrishnan D, Mesapogu S, Krishnam Raju A, Rao YV and Neelamraju S 2016. Genetic characterization and population structure of Indian rice cultivars and wild genotypes using core set markers. 3 Biotech 6(1):95.

Tarang A and Hosseini M 2016. Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers. International & Iranian genetic congress.5: 1-6.

Upadhyay P, Neeraja CN, Kole C and Singh VK 2012. Population Structure and Genetic Diversity in Popular Rice Varieties of India as Evidenced from SSR Analysis. Biochem Genet 50(9-10):770-83.

Wang Z, Wang J, Bao Y, Wang F and Zhang H 2010. Quantitative trait loci analysis for rice seed vigor during the germination stage. J Zhejiang Univ-Sci B (Biomed and Biotechnol) 11(12):958-964

Xu Y and Crouch JH 2008. Marker-Assisted Selection in Plant Breeding: From Publications to Practice. Crop Sci. 48:391–407

Yan AO, Yong XU, Fen CX, An W, Fei T, Li-quin S and Qiao-quan L 2015. A genetic diversity assessment of starch quality traits in rice landraces from the Taihu Basin, China. J. Integrative Agri 15(3):493–501.

Yu SB, Xu WJ, Vijayakumar CH, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS and Li ZK 2003. Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program; Theor Appl Genet 108:131–140.

Zhang P, Li J, Li X, Liu X and Zhao X 2011. Population structure and genetic diversity in a rice core collection (*Oryza sativa* L.) investigated with SSR markers. PLoS ONE 6(12): e275

Zhang ZH, Qu XS, Wan S, Chen LH and Zhu YG 2005. Comparison of QTL controlling seedling vigor under different temperature conditions using recombinant inbred lines in rice (*Oryza sativa*). Ann Bot 95:423-429

Zhou L, Wang JK, Yi Q, Wang YZ, Zhu YG and Zhang ZH 2006. Quantitative trait loci for seedling vigor in rice under field conditions. Field Crops Res 100: 294–301