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Genetic diversity and population structure using linked SSR markers for heat stress tolerance in rice

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ABSTRACT

High temperature stress is an emerging threat to summer rice cultivation. In the present study, genetic diversity and population structure of 48 rice accessions of improved varieties and landraces were assessed by 35 microsatellite markers associated with high temperature stress tolerance. A total of 86 alleles were amplified with an average of 2.87 alleles per locus and their PIC values ranged from 0.0637 to 0.6799 with an average of 0.4105. The structure software grouped the total rice accession into two distinct sub-populations. The Fst values of the two populations were 0.0081 (population 1) and 0.3272 (population 2). The allele frequency divergence between two populations was 0.0814. The phylogenetic analysis grouped the genotypes into two major clusters and 3 sub-clusters. Clustering based on polymorphic high temperature stress tolerance linked SSR markers classified the total rice accessions mainly into 2 clusters.

Key words: *Linked SSR marker, heat tolerance, high temperature*

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the family *Poaceae* (Graminae) and subfamily *Oryzoidea* is a food crop consumed by most of the world population in every meal. The human population explosion demands more food grain to feed the population. To meet the demand, rice production needs to br increased by 0.6-0.9% annually until 2050 (Carriger and Vallee, 2007). In 2017 global paddy production is estimated to be 758.9 million tonnes (FAO, 2017).

Over the past 100 years, the global average temperature has increased by 0.6^0C and its rate will increase in future (Root et al., 2003; Meehl et al., 2005; Vuuren et al., 2008). Increasing temperature of earth surface temperature resulted in greenhouse effect and global warming. It was observed that yield of rice grain decreased by 10% for each 1^0C rise in temperature (Peng et al., 2004). During the flowering period high-temperature can damage pollen activity, pollen germination and pollen tube growth, which leads to sterility (Tsutomu et al., 2002; Jagadish et al., 2007). During grain filling high temperature causes spikelet sterility which results in loss of grain yield and quality (Ye et al., 2012).

Genetic diversity of a species is the total number of genetic characteristics present in its genetic makeup. Genetic variability helps in adapting to the changing environment. Due to more variation in the species some individuals have the allele which can adapt to the changing environment. Those individuals are more likely to survive to produce offspring bearing that allele and their population will be successful. Study of genetic diversity and exploration in rice germplasm contribute to increase in rice production in India. Superior traits like high yield, diseases resistance, and biotic-abiotic stress tolerance are dispersed in different species. Genetic diversity study helps to identify such alleles which contain the superior traits. Such quality traits can be used in a single background for crop improvement programme.

SSR markers are co-dominant and distributed throughout the genome and highly used in molecular

characterization (Salgotra et al., 2015; Pradhan et al., 2016; Pandit et al., 2017). SSR markers are highly polymorphic and multi allelic nature, thus even in less number of SSR marker can give a better genetic diversity (Mc Couch et al., 1997). Recent report suggested that there are significant studies were carried out to define a Simple Sequence Repeat (SSR) marker which is commonly used in genetic diversity studies in rice. It is reported that SSR markers were used for study of diversity in rice (Jin et al., 2010; Hesham et al., 2008; Sow et al., 2014; Das et al., 2013; Choudhury et al., 2013; Pradhan et al., 2016). Study of diversity and genetic relatedness of rice germplasm and its association mapping were studied (Nachimuthu et al., 2015; Pradhan et al., 2016; Pandit et al., 2017). Using SNP markers, genetic relationship and the degree of genetic diversity of temperate rice varieties were studied (Reig-Valiente et al., 2016). Hyper variable simple sequence (HvSSR) markers were used to assess the genetic diversity and genetic relationship in Indian rice varieties released over the years which shows huge amount of genetic diversity (Singh et al., 2016).

Genetic structure is important to know the genetic makeup of individuals within a population. Without knowing the genetic makeup of a population, targeted crop improvement programme will be very slow. Therefore, information about population structure is important for the plant breeders. Also, before using the population for association mapping, the structure of the population is important to constitute a proper panel with suitable individuals. Analysis of population structure of rice collected from a region is essential to study association mapping. The presence of subpopulations helps to identify invalid associations due to presence of unlinked markers with phenotypic variation (Buckler et al., 2002; Pradhan et al., 2016; Pandit et al., 2017). The population structure study was carried out within the rice core collection to differentiate of *indica* and *japonica* rice (Zhang et al., 2011). In another study association mapping (AM) for grain quality traits in Basmati germplasm were carried out using population structure analysis (Salgotra et al., 2015). Association mapping of agronomic traits in *Oryza sativa* were studied by Nachimuthu et al., 2015. The present investigation was carried out to study the genetic divergence and relatedness of the genotypes which can be utilised in the future crop improvement programme.

MATERIALS AND METHODS

Forty eight genotypes of rice consisting of released varieties and traditional cultivars including high temperature stress tolerant check N22 and susceptible check Satyakrishna were used in this study. The genotypes were sown in the petriplates in green house. The healthy leaves samples of 15 days old seedling were collected for genomic DNA extraction. Total genomic DNA was extracted after crushing in liquid nitrogen in microfuge tubes using CTAB extraction buffer (100mM Tris-HCl pH 8, 20mM EDTA pH 8, 1.3M NaCl, 2% CTAB) and chloroform-Isoamyl alcohol extraction followed by RNAase treatment and ethanol precipitation. Agarose gel electrophoresis was used to estimate DNA concentration and each sample was then diluted to approximately 30ng/ μ L. Thirty reported SSR markers distributed among different chromosome were selected according to high temperature stress tolerance. DNA amplification reaction was performed in 20 μ l aliquot containing 1.5mMTris HCl (pH 8.75), 50mM KCl, 2mM $MgCl_2$, 0.1% TrotonX-100, 200 μ m per each of dATP, dCTP, dTTP, dGTP, 4pmole of each forward and reverse primers (Table 2), 1 unit of taq polymerase and 30ng of genomic DNA. Amplification was done in a programmable thermal cycler (Veriti, Applied Bio Sciences). The reaction mixture was first denatured for 4 min at 94°C and then subjected to 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C; and then a final extension for 10 minutes at 72°C. Aliquots of 7 μ l of the products from PCR amplification were loaded in 4% agarose gel containing 0.8mg/ml ethidium bromide for electrophoresis in 1X TBE (pH 8.0). DNA ladder (50bp) was used for determination of size of the amplicons. The gel was run at 60 volts (2.5V/cm) for 4 hrs and photographed using a Gel-Doc System (SynGene). SSR alleles were scored sequentially from the largest to the smallest size band on their position relative to their ladder. Each fragment that was amplified using SSR markers was scored and data entered into a binary data matrix as discrete variables. The number of alleles, allele frequency, gene diversity, heterozygosity and polymorphic information index (PIC) were estimated using the program Power MarkerVer3 (Liu and Muse, 2005). The genotyping data were scored as 0 and 1 as per presence or absence of alleles. The analysis of data for possible population structure was done by using

STRUCTURE 2.3.4 software (Pritchard et al., 2000). The project was run with 150,000 burns-in followed by Markov Chain Monte Carlo (MCMC) replication with model parameter set of 'possibility of admixture and allele frequency correlated'. The K value varying from 1 to 10 was run 10 times for each K value. The maximal value of L (K) was used to identify exact number of sub population (Evanno et al., 2005). The Structure Harvester was used to determine the ΔK value as function of K which shows the clear peak at the optimal K value (Earl et al., 2012). The dendrogram was prepared using NTS- ys 2.1 software based on unweighted pair group method with arithmetic means (UPGMA) and principal coordinate analysis (PCO) also known as metrix multi dimensional scaling was done using Darwin 6.0 software which shows the genetic relationship and phylogeny among the genotypes (Pradhan et al., 2016; Das et al., 2013). The principal coordinate analysis interfered the diversity among the genotypes.

RESULTS AND DISCUSSION

In the present study, forty eight rice genotypes comprising of landraces and breeding lines were used for assessing the extent of genetic diversity and population structure for high temperature stress in the rice genotypes (Table 1). A total of 35 SSR primer distributed throughout the rice genome were used for genotyping, out of which 30 showed polymorphism and 5 (RM405, RM140, RM6132, RM6100, RM406) were monomorphic (Fig. 1). These monomorphic bands were excluded from the analysis. A total of 86 alleles were obtained with the 30 primers among the 48 genotypes. The number of alleles per locus generated by each marker varied from 2 to 4 with an average of 2.87 per locus(Table 3). Eleven markers detected four numbers of alleles, 12 markers showed three alleles and 7 markers produced two alleles. The major allele frequency per locus ranged from 0.333 (RM 7364) to 0.9659 (RM160) with an average of 0.6277. The highest number and effective number of alleles per locus were observed in the marker RM209 (0.812), RM 205 and RM 228 (0.833), RM525 (0.854), RM243 (0.875) and RM 160 (0.965). Similarly, the lowest number of alleles per locus was observed in the markers RM547 (0.468), RM336 (0.447), RM310 (0.437), RM242, RM 570 (0.4271) and RM7364 (0.333). The observed heterozygosity (Ho) ranged between 0.0 and 0.75(RM 249), with an average Ho of 0.0967. Among the 30 markers tested, 17 markers demonstrated the level of Ho more than zero, while 13 exhibited zero values. The expected heterozygocity or gene diversity (He) ranged from 0.208 (RM212, RM1209, RM566) to 0.75 (RM249) with an average of 0.0967. The mean Polymorphic information content (PIC) value was found to be 0.4105 with minimum value of 0.0637 (RM160) and maximum of 0.6799 (RM7364) (Table 3). The PIC value of the markers polymorphism within a population provides an estimate of the discriminating power of the marker (Nagy et al., 2012).

Genetic diversity gives valuable information about the genetic constitution of a genotype which can be used in the molecular breeding program. Population with high genetic variation contribute better progenies

Table 1. Genotypes used for genetic diversity and population structure study for heat stress

Sl. No.	Genotype name	Sl. No.	Genotype name	Sl. No.	Genotype name
1	Annapurna	17	IR88963-31-6-1-1	33	IET-21626
2	Satyakrishna	18	IR36	34	IR 888793-12-2-2-4
3	Wita-12	19	RP 5215-52-19-8-6-3-2-B	35	IR 88839-9-1-4-2
4	N22	20	CR Dhan-601	36	Swarna
5	IR 83142-B-36-B	21	CR3622-6-1-2-1-2	37	Naveen
6	Maudamani	22	CR3820-4-5-3-4-1	38	IR 88864-6-2-7-1
7	CR 2599-2	23	IR10C137	39	IR 88964-11-2-2-4
8	IR 10C 167	24	HHZ 11-DT7-SAL1-SAL 1	40	IR 78877-208-B-1-1/IR 78878-53-2-2-2
9	IR 10C108	25	IR 10C103	41	IR 83377-B-B-42-3
10	Dular	26	IR 84899-B-184-16-1-4-1	42	IR 87695-7-2-1-1
11	CR 2599-2	27	IR 84887-B-157-38-1-1-3	43	CRR 625-8-88-3-4
12	IR 83373-B-B-24-4	28	IR 84899-B-183-6-1-1-2	44	IR 88795-2-3-2-4
13	IR 88897-13-2-1-5	29	CR Dhan 305	45	Sahabhagi Dhan
14	IR8337-6-B-B -110-3	30	CR 2718-10-IR83927-B-B-279	46	Badshahbhog
15	IR83381-B-B-55-4	31	CR 1570-418-1-149-1	47	IR 64
16	IR88836-4-1-3-2	32	IR84899-B-179-1-1-1-1	48	CR 3826-8-3-2-1-1

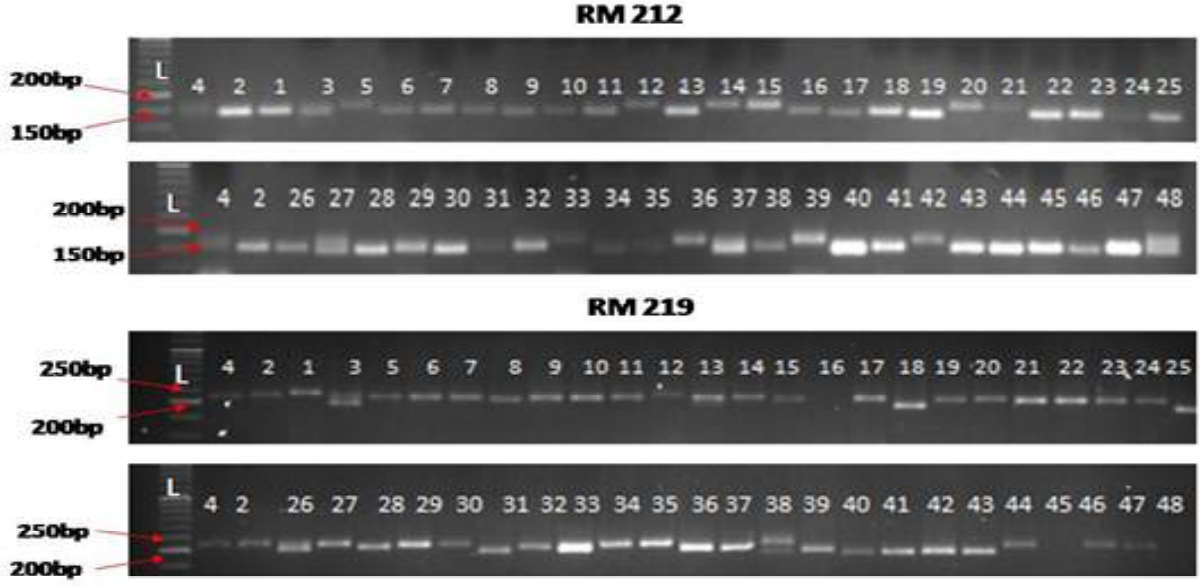


Fig. 1. Representative photos of the primer showing banding profile of primer RM 212 and RM 219 of 48 genotypes as listed in Table 1.L represents 50bp ladder.

in their segregating generations and serves as rich resource for crop improvement program. This study reveals the genetic diversity parameters and population structure among 48 genotypes of rice consisting of

Table 2. List of high temperature stress tolerance linked SSR markers selected along with their nucleotide sequence

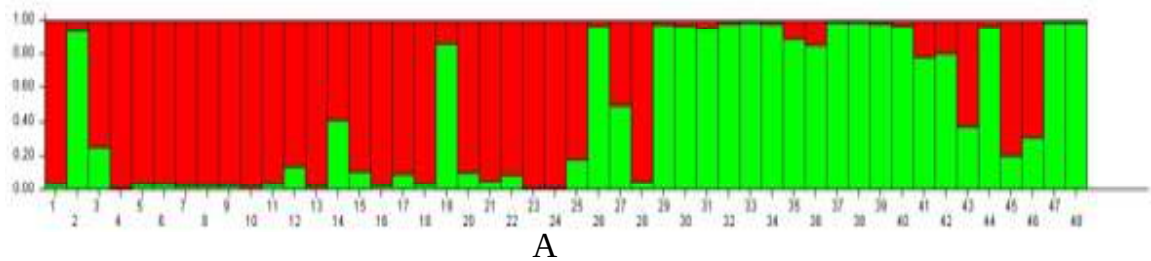
Sl.no.	Name of the marker	Chr.	Sequence of the Forward Primer	Sequence of the Reverse Primer	Expe- cted size(bp)	References
1	RM10346	1	GCTTGATCTGCCCTTGTTTCTTGG	AACTCGAGCGGCCTTCTCAGC	293	Liao et al., 2011
2	Indel 3	9	GGTTGCGACATTGGAGCCTTC	AATGCTTGGGTATGCTAGGTGAA		Wei et al., 2013
3	RM212	1	AAGGTCAAGGAAACAGGGACTGG	AGCCACGAATTCCACTTTCAGC	162	Jagadish et al., 2010
4	RM249	5	CAACTCCACTCCAGACTCAACTCC	GGTATGATGCCATGAAGGTCAGC	294	Buu et al., 2014
5	RM205	9	CCTAAGAGGAGCCATCTAACAACTGG	CTTGGATATACTGGCCCTTCACG	188	Jafar et al., 2008
6	RM 209	11	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC	134	Buu et al., 2014
7	RM 219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	202	Wei et al., 2013
8	RM225	6	TATGTGGTTGGCTTGCCTAGTGG	TGCCCATATGGTCTGGATGTGC	196	Xiao et al., 2011
9	RM228	10	TCTAACTCTGGCCATTAGTCCTTGG	AAGTAGACGAGGACGACGACAGG	193	Buu et al., 2014
10	RM234	7	TTCAGCCAAGAACAGAACAGTGG	CTTCTCTTCATCCTCCTCCTTGG	166	Jafar et al., 2008
11	RM242	9	AAACACATGCTGCTGACACTTGC	TTACTAGATTTACCACGGCCAACG	265	Ye et al., 2012.
12	RM247	12	AAGGCGAACTGTCCTAGTGAAGC	CAGGATGTTCTTGCCAAGTTGC	198	Antonio et al., 2005
13	RM 314	6	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC	118	Buu et al., 2014
14	RM336	7	GTATCTTACAGAGAAACGGCATCG	GGTTTGTTTCAGGTTCGTCTATCC	153	Argayoso et al.,2011
15	RM525	2	GTTGGTATTCCCTGATGAGTTTGC	GAGACAGAATCCTTACGCTGTGC	197	Zhang T et al., 2008
16	RM547	8	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC	283	Ye et al., 2012
17	RM566	9	AATATGGTGGCGCGTACATCC	TGATCGAGCCAACAACAACTGG	143	Ye et al., 2012
18	RM570	3	AGAAATGGTGAAAGATGGTGCTACCG	CTGAATGTTCTTCAACTCCCAGTGC	159	Chen et al., 2008
19	RM1209	7	AATGGAGCTCCTGACTCTAAAGC	TGCATCTCCTACAGAAACAAGG	179	Laio et al., 2011
20	RM3586	3	TCTTGATTGCTGGACCACATGC	TCGAGCTAGAAGACGACACACAGC	176	Zhang et al., 2008
21	RM7364	9	TTTCGTGGATGGAGGGAGTACG	TGGCGACTTATGAGCGTTTGTAGG	184	Wei et al., 2013
22	RM1089	5	AATAGGGCTTGAAATAAATTG	AATAGGGCTTGAAATAAATTG		Poli et al., 2013
23	RM310	8	GACTTGTGGTTGTTGCTTGTTGG	ACTGCCATATGCATTTCCCTAGC	176	Buu et al., 2014
24	RM127	4	CGAAGCTTTCGGTGGGATAGC	ACCTTGAGCGAGTCCTTGAACG	195	Buu et al., 2014
25	RM25181	10	AAAGAGCTTCCCTAATGGCTTCG	GAGAGAATGACCTCTCCCAAGACC	163	Buu et al., 2014
26	RM 160	9	CCCAAATCAGGAAAGTTTCTCAGC	AGTCATCCTTGGCTACCAGATGC		Buu et al., 2014
27	RM3351	5	GTCGAAACGTAGCCAGGCAATGG	CCATGGAAGGAATGGAGGTGAGG	139	Lee et al., 2005
28	RM6378	2	CTGATCATCTCATGCCTCCTACG	TCCATCTCCCAATATGACCAACC	167	Buu et al., 2014
29	RM174	2	ATAAGCGACGCCAAGACAAGTCG	GGAAGCAAGAAGGAAAGAGAGATGG	268	Buu et al., 2014
30	RM16686	4	GGCACTGCTTGCATATGGATCG	TGCCGGCGAACTTATCCTCTCC	94	Buu et al., 2014

Table 3. Details of SSR loci used for genotyping a set of 48 rice genotypes and their genetic diversity parameters.

Sl.no	Marker	Chr. No	SSR motif	Min.mol Wt	Max.mol. wt	No of alleles	Major allele frequency	Gene diversity	Heteroz-ygosity	PIC value
1	RM10346	1	AG(31)	225	310	4.0000	0.5938	0.5388	0.0208	0.4614
2	INDEL3	9	-	190	200	2.0000	0.7188	0.4043	0.4792	0.3226
3	RM212	1	AG(14)	160	180	3.0000	0.5729	0.5801	0.0208	0.5158
4	RM249	5	AG(17)	120	140	2.0000	0.5417	0.4965	0.7500	0.3733
5	RM205	9	AG(15)	100	140	3.0000	0.8333	0.2917	0.0000	0.2723
6	RM209	11	(CT)18	120	170	3.0000	0.8125	0.3168	0.0000	0.2864
7	RM219	9	(CT)17	200	240	2.0000	0.6250	0.4688	0.0000	0.3589
8	RM225	6	AG(14)	170	200	2.0000	0.6875	0.4297	0.0000	0.3374
9	RM228	10	AG(20)	100	150	2.0000	0.8333	0.2778	0.0000	0.2392
10	RM234	7	AG(13)	140	170	3.0000	0.8750	0.2231	0.0000	0.2058
11	RM242	9	AG(11)	200	250	3.0000	0.4271	0.6508	0.0417	0.5770
12	RM247	12	AG(28)	130	200	4.0000	0.5833	0.5443	0.0000	0.4647
13	RM314	6	(GT)8(CG)3(GT)5	100	120	2.0000	0.6042	0.4783	0.0000	0.3639
14	RM336	7	AAG(18)	125	190	3.0000	0.4479	0.6456	0.0833	0.5725
15	RM525	2	AAG(12)	170	220	3.0000	0.8542	0.2595	0.0000	0.2437
16	RM547	8	AAT(20)	200	300	3.0000	0.4688	0.5996	0.1875	0.5151
17	RM566	9	AG(15)	150	175	2.0000	0.5938	0.4824	0.0208	0.3661
18	RM570	3	AG(15)	210	250	3.0000	0.4271	0.6443	0.6667	0.5691
19	RM1209	7	AG(14)	160	170	2.0000	0.6354	0.4633	0.0208	0.3560
20	RM3586	3	AG(12)	120	150	3.0000	0.5417	0.5799	0.0833	0.5020
21	RM7364	9	AGAT9	300	340	4.0000	0.3333	0.7300	0.0000	0.6799
22	RM1089	5	AC(33)	190	240	4.0000	0.7188	0.4542	0.0625	0.4236
23	RM310	8	AC(22)	85	100	4.0000	0.4375	0.6899	0.0625	0.6374
24	RM127	4	AGG(8)	210	230	2.0000	0.7708	0.3533	0.1250	0.2909
25	RM25181	10	AAG(22)	130	170	4.0000	0.5625	0.6170	0.1458	0.5721
26	RM160	9	AAG(23)	140	160	2.0000	0.9659	0.0659	0.0227	0.0637
27	RM3351	5	AG(15)	170	180	2.0000	0.6000	0.4800	0.0000	0.3648
28	RM6378	2	AAG(19)	130	175	4.0000	0.6277	0.5380	0.1064	0.4834
29	RM174	2	AAG(7)	250	340	3.0000	0.5532	0.5559	0.0000	0.4695
30	RM16686	4	AGG(10)	80	100	3.0000	0.5854	0.5211	0.0000	0.4271
	Mean					2.8667	0.6277	0.4794	0.0967	0.4105

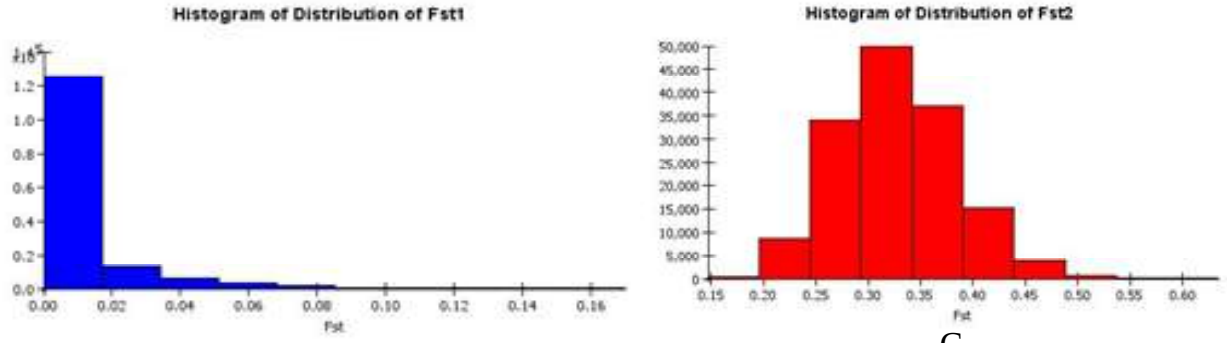
released variety and traditional cultivars were genotyped for high temperature stress tolerance using 30 SSR markers. All the 30 SSR markers linked to high temperature stress tolerance and related traits were previously reported (Table 2). The SSR markers showed high polymorphism using the 48 temperature stress tolerant genotypes. The SSR markers are earlier used in rice genetic diversity study (Das et al., 2013; Singh et al., 2013; He et al., 2014; Anandan et al., 2016a, b; Pradhan et al., 2016; Pandit et al., 2017). The 30 polymorphic SSR markers used for this study revealed a clear and consistent amplification profile.

The number of alleles per locus ranges from 2 to 4 with an average of 2.87 per locus. This value is comparable with Nachimuthu et al., 2015 value (3 alleles



A

Fig. 2. (A) Population structure of 48 genotypes using 30 heat tolerant linked SSR markers with name (K =2). which indicated that the entire population can be grouped into two sub-groups (SP1, SP2).



B C

Fig. 2. B and C. Population structure of a panel of 48 genotypes based on 30 molecular markers showing histogram of Fst_1 and Fst_2

/ locus) reported for agronomic trait in rice germplasm and lower than Cho, 2000 (5.5 alleles/locus) (Yu et al., 2003, (6.3 alleles/locus) (Das et al., 2013) which is 7.9 alleles / locus reported in rice landraces from Eastern and North Eastern States of India. The PIC value was found to be 0.4105 with minimum value of 0.0637 (RM160) and maximum of 0.6799 (RM7364). The previous publications has PIC values 0.48 (Ashfaq et al., 2012), 0.25 (Singh et al., 2013), 0.17 (Mahender et al., 2014), 0.33 (Anandan et al., 2016b), 0.30 (Pradhan et al., 2016), 0.38 (Tarang et al., 2016) and 0.454 (Pandit et al., 2017) reported in diverse set of rice germplasm. The observed heterozygosity (Ho) ranged between 0.0 and 0.75(RM 249), with an average Ho of 0.0967 which is very low similar to 0.18 reported by Nachimuthu et al., (2015).

In the present study, the STRUCTURE analysis categorized the population into two clusters with genotypes as pure and admixture type (Fig. 2A). The fixation index (Fst) values of two populations were 0.0081 and 0.3272 for population 1 and population 2 respectively (Fig. 2B & 2C). The allele frequency divergence between two populations was 0.0814. Recently Courtois et al., (2012), Das et al., (2013), Anandan et al., (2016), and Pradhan et al., (2016) have also reported varying number of sub-populations from different germplasm lines.

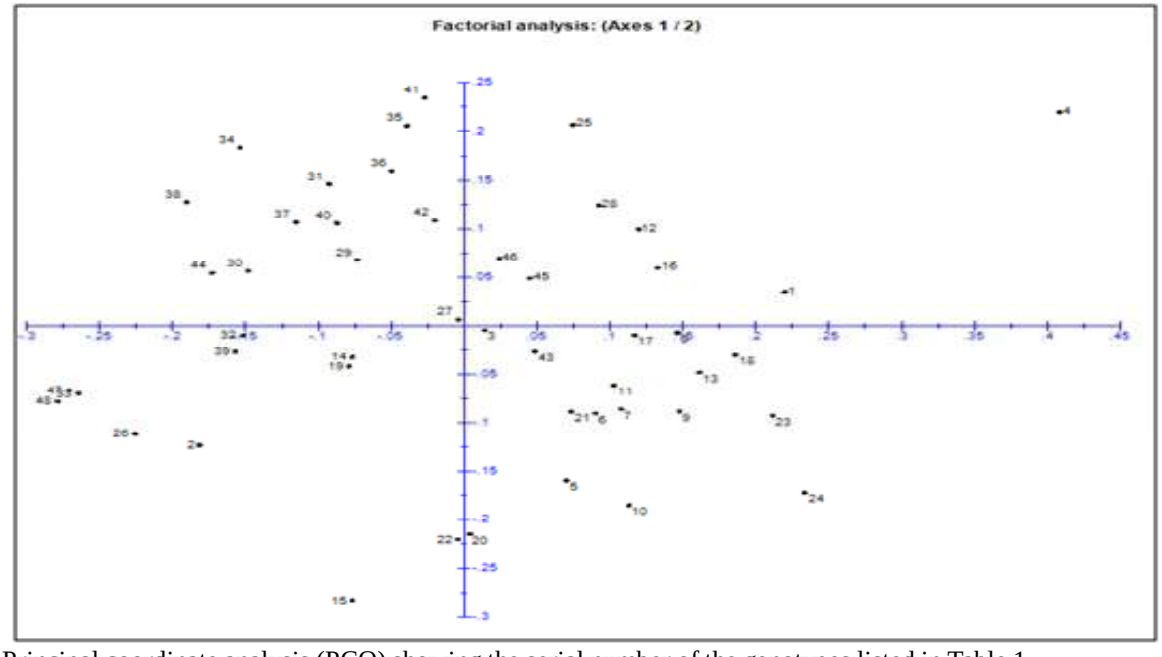


Fig. 3. Principal coordinate analysis (PCO) showing the serial number of the genotypes listed in Table 1.

Table 4. The population structure inferred ancestry values of individual genotype in each sub-population

Sl no	Genotypes	Inferred Ancestry		Structure Group
		Q1	Q2	
1	Annapurna	0.968	0.032	SP1
2	Satyakrisna	0.059	0.941	SP2
3	Wita-12	0.748	0.252	SP1,admix
4	N22	0.989	0.011	SP1
5	IR 83142-B-36-B	0.971	0.029	SP1
6	MAUDAMANI	0.968	0.032	SP1
7	CR2599-2	0.972	0.028	SP1
8	IR 10C 167	0.973	0.027	SP1
9	IR10C108	0.976	0.024	SP1
10	Dular	0.983	0.017	SP1
11	CR2599-2	0.967	0.033	SP1
12	IR-83373-B-B-24-4	0.863	0.137	SP1
13	IR-88897-13-2-1-5	0.981	0.019	SP1
14	IR8337-6-B-B -110-3	0.588	0.412	SP1,admix
15	IR83381-B-B-55-4	0.886	0.114	SP1
16	IR88836-4-1-3-2	0.978	0.022	SP1
17	IR88963-31-6-1-1	0.907	0.093	SP1
18	IR36	0.965	0.035	SP1
19	RP5215-52-19-8-6-3-2-B	0.137	0.863	SP2
20	CR Dhan-601	0.893	0.107	SP1
21	CR3622-6-1-2-1-2	0.946	0.054	SP1
22	CR3820-4-5-3-4-1	0.914	0.086	SP1
23	IR10C137	0.986	0.014	SP1
24	HHZ 11-DT7-SAL1-SAL 1	0.987	0.013	SP1
25	IR 10C103	0.819	0.181	SP1
26	IR 84899-B-184-16-1-4-1	0.034	0.966	SP2
27	IR 84887-B-157-38-1-1-3	0.506	0.494	SP1,admix
28	IR 84899-B-183-6-1-1-2	0.951	0.049	SP1
29	CR Dhan 305	0.027	0.973	SP2
30	CR 2718-10-IR83927-B-B-279	0.031	0.969	SP2
31	CR 1570-418-1-149-1	0.048	0.952	SP2
32	IR84899-B-179-1-1-1-1	0.017	0.983	SP2
33	IET-21626	0.012	0.988	SP2
34	IR888793-12-2-2-4	0.021	0.979	SP2
35	IR88839-9-1-4-2	0.112	0.888	SP1
36	Swarna	0.143	0.857	SP2
37	Naveen	0.014	0.986	SP2
38	IR88864-6-2-7-1	0.009	0.991	SP2
39	IR88964-11-2-2-4	0.019	0.981	SP2
40	IR78877-208-B-1-1/IR 78878-53-2-2-2	0.031	0.969	SP2
41	IR83377-B-B-42-3	0.216	0.784	SP2,admix
42	IR87695-7-2-1-1	0.196	0.804	SP2
43	CRR625-8-88-3-4	0.625	0.375	SP1,admix
44	IR88795-2-3-2-4	0.040	0.960	SP2
45	Sahabhagi Dhan	0.795	0.205	SP1,admix
46	Badshahbhog	0.688	0.312	SP1,admix
47	IR64	0.010	0.990	SP2
48	CR3826-8-3-2-1-1	0.010	0.990	SP2

The STRUCTURE analysis classified the Sub-population 1 (red color) composed of 29 genotypes grouped into one cluster and sub-population 2 consisted 19 genotyped grouped into cluster 2 (green color)(Fig. 2A). The sub-population 1 (SP1) posses 28 genotypes with 23 pure and 6 admixture types, 18 genotypes present in sub-population 2 (SP2) with one admixture. (Table 4).

The genotype data of 48 rice accessions were used to generate principal coordination analysis (PCO) to produce a set of uncorrelated axes to summarise the diversity in the groups. There are four different groups were produce (Fig. 3) comprised of the right upper most quadrant (1st) contained 8 genotype including N22 and Annapurna which are heat tolerant, similarly N22 and Annapurna present in single cluster were found in dendrogram. The left lower most (3rd) quadrant contained 11 genotype including Satyakrishna (Suscptible check) which revealed 3rd quadrant possessed susceptible genotype in one group and first quadrant contain tolerant genotype in one group. Similar trend has been reported earlier by Pradhan et al., 2016.

Cluster analysis is a useful way of knowing the genetic relationship among individuals. It was carried out to examine genetic distance and the dissimilarity matrix-using UPGMA method. Clustering based on polymorphic high temperature stress tolerance linked SSR markers classified the total 48 rice genotypes broadly into 3 distinct groups. Recently, Pradhan et al. (2016) has classified the high temperature stress tolerance germplasm into 3 groups. Earlier, Kumbhar et al. (2015) classified 50 rice genotypes comprising landraces, local selections, and improved varieties into 5 clusters and 11 sub-clusters using SSR and ISSR markers. The first cluster constituted two genotypes, N22 which is established heat tolerant (Pradhan et al., 2016) and Annapurna. The first subgroup constituted of IR83381-B-B-55-4 and Dular which are high tolerant to heat stress (Pradhan et al., 2016). Satyakrisna (Susceptible Check), IR 64, Badshahbhog, IR84899-B-179-1-1-1-1 were group together as single cluster and IR 10C103 formed a separate cluster showed relatedness to susceptible line Satyakrishna (Fig. 4).

CONCLUSION

The genotypic diversity study revealed that there were two types of sub-populations for high temperature stress tolerance present in the population. Each sub-population were derived from a common ancestor. The heat

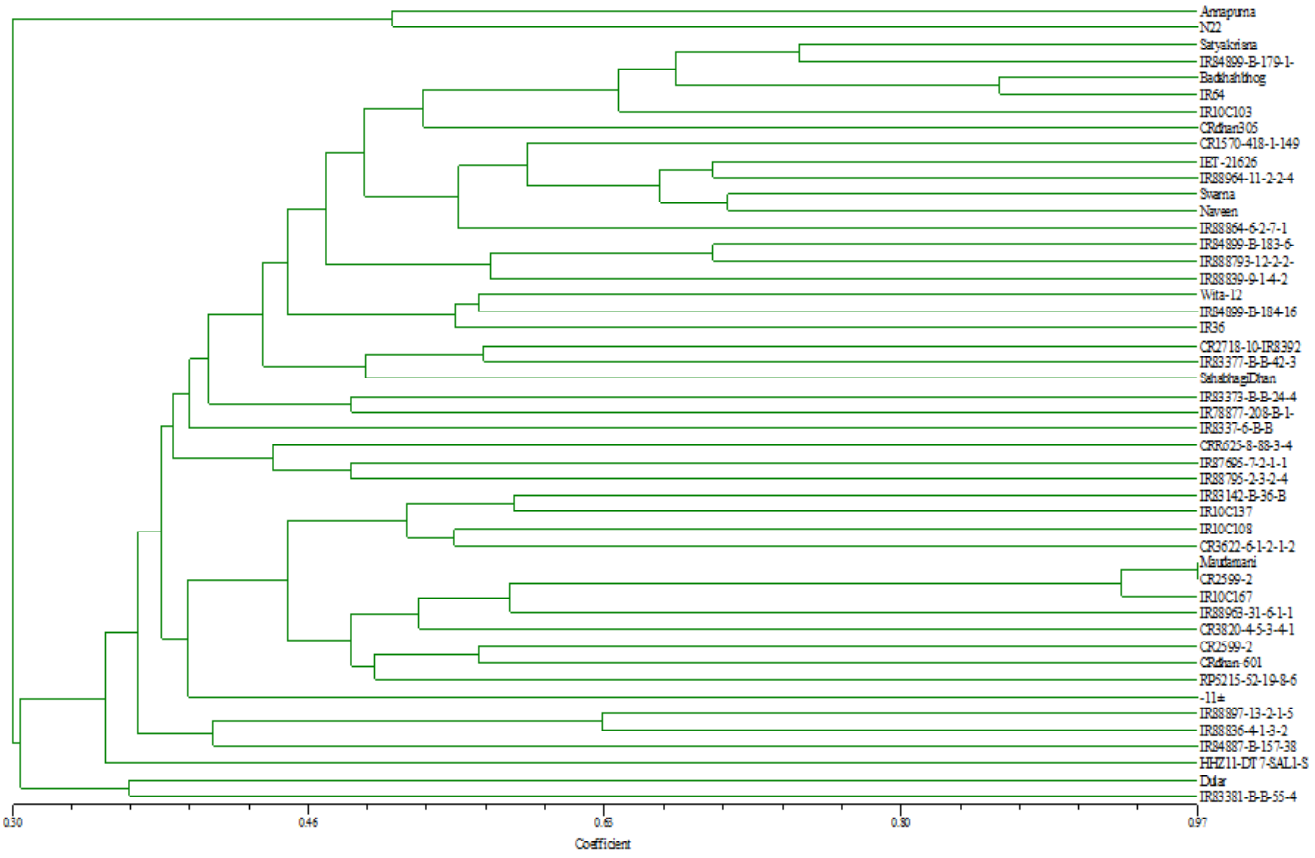


Fig. 4. Dendrogram derived from UPGMA cluster analysis of 48 rice genotypes using 30 SSR markers linked to heat tolerance

tolerant linked SSR markers showed high polymorphism and helps to construct the dendrogram which differentiate the susceptible and tolerant rice accessions of improved varieties and landraces used in the studied by NTSys 2.1. The principal coordinate analysis was done by Darwin 6 depicted the diversity among the genotypes which can be used in the marker assisted breeding program to produce heat tolerant genotype.

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