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Population structure and diversity analysis of Indica and Japonica rice genotypes

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Abstract

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Eighty one rice genotypes were studied using 30 SSR markers and data obtained were analyzed using STRUCTURE software which classified the population into two sub groups or subpopulations, designated as subpopulation 1 and 2. Subpopulation 1 mostly comprised of 60 germplasms majority of which are *Indica* in origin, whereas subpopulation 2 mostly comprised of tropical and temperate *Japonica* subspecies. Cluster analysis was performed using UPGMA and Dendrogram was constructed which consisted of 4 clusters comprising of *Indica* accessions in majority and 3 clusters of tropical and temperate *Japonica* subspecies, thereby suggesting large diversity in the population.

Keywords: AMOVA; Cluster analysis; Dendrogram; Indica; Japonica; Population structure

Introduction

Rice is the second most consumed cereal and provides 20% calorie to 3.5 billion people across the globe. With the increase in population within next 25 years the global rice demand will enhance upto 852 million tons in 2035 (Khush, 2013). With rapid urbanization and depletion of resources increasing rice productivity under constrained conditions is the need for the future. In terms of rice yield a bottleneck situation has been reached due to years of selection resulting in depletion of genetic diversity in the gene pool. Such situation has resulted in limited improvement observed in case of various breeding approaches. Only viable process for enhancing diversity in the rice gene pool is by hybridization between genetically distant parents for increasing the probability of obtaining diverse recombinants created by new allelic combinations. At the same time hybridization between incompatible genetic backgrounds may result in sterility of the hybrids as observed in case of *Indica/Japonica* crosses. Reduced affinity of the two types of gametes towards each other as stated by Liu et al. (2004) may be held responsible for such spikelet sterility. Thus under present scenario the investigation targets at determining the nature of the genetic constitution, diversity and overall phylogenetic classification of a given population which can be later used in future breeding programs. Estimation of diversity and population structure can be done using SSR microsatellite markers. SSR markers are low cost and expresses high estimations of polymorphism (McCouch et al., 1997).Similar studies using SSR were conducted by Jin et al. (2010); Hesham et al. (2008); Sow et al. (2014); Das et al. (2013) and Choudhury et al. (2013). The present study aims at analyzing a population comprising of 81 rice germplasms using 30 SSR markers which were earlier tested for polymorphism to obtain elaborate results regarding the population structure and diversity.

Materials and Methods

Plant materials

For the present investigation 81 genotypes (Table 1) belonging to the three sub species of rice i.e. *Indica*, *Japonica* (tropical and temperate) were analyzed. The genotypes were collected from IRRI Philippines, University of Calcutta and Rice Research Station, West Bengal, India. Plants were grown in the *kharif* season of 2014-2015 at Rice Research Station, West Bengal, India.

DNA extraction and SSR analysis

Leaf samples of 81 genotypes were collected from 21-day-old seedlings and leaves were grounded using liquid nitrogen. The fine powder was allowed to thaw in 2 mL Eppendorf tubes and DNA was extracted using PROMEGA Plant DNA minikit. 30 rice microsatellite markers designated by RM covering over 12 chromosomes were initially chosen from Gramene web site (www.gramene.org) and tested for polymorphism.

PCR and Gel Electrophoresis

The Polymerase Chain reaction was conducted in a reaction solution of 25 µl containing template DNA, Primers, dNTPs, reaction buffer, $MgCl_2$ and Taq DNA polymerase. The PCR amplification was performed using a Eppendorf (Germany) thermo cycler. According to the cycle profile initial denaturation was performed at 94˚C for 5 minutes, followed by 36 cycles of 1 minute denaturation at 94˚C. 2 minutes of annealing at temperatures varying according to primers were performed. Finally 2 minutes and 5 min extension at 72˚C for the final product was performed. Amplified products were stored at -20˚C until further use.PCR products were subjected to electrophoresis in 2% Agarose gel in 1X TAE buffer at 120 volts. DNA bands were visualized under UV light using the Gel Documentation Unit (UVP, UK).

Table 1. List of genotypes constituting the population under investigation

Sl. No.	Genotype	Ancestry	Sl. No.	Genotype	Ancestry
1	KMR 3	*Indica*	49	Azucena	*Tropical japonica*
2	IR 10198R	*Indica*	50	Nipponbare	Temperate japonica
3	IR 40750R	*Indica*	51	CNR 33	*Indica*
4	IR 71604-4-1-4-4-4-2-2-2R	*Indica*	52	Pehkuh	-
5	IR 34686-1R	*Indica*	53	Deok Jeok jodo	*Temperate japonica*
6	IR 6876-1	*Indica*	54	Rathuwee	*Indica*
7	BR 827-35	*Indica*	55	Carolina gold sel	*Tropical japonica*
8	BR 1356	*Indica*	56	Double Carolina	*Temperate japonica*
9	BR 1356 Sel 2R	*Indica*	57	Trembese	*Tropical japonica*
10	NDR 97	*Indica*	58	Cenit	*Tropical japonica*
11	MTU 9992	*Indica*	59	Zhensan 2	*Temperate japonica*
12	DR 714-1-2R	*Indica*	60	I Geo Tze	*Indica*
13	Ajaya	*Indica*	61	Tchibanga	*Indica*
14	Pratiksha	*Indica*	62	Asse-y- pung	*Temperate japonica*
15	Sidhant	*Indica*	63	CNR 45	*Indica*
16	Gajapati	*Indica*	64	Milyang 46	*Indica*
17	Jajati	*Indica*	65	CNR 47	*Indica*
18	Purnendu	Indica	66	CNR 55-3	*Indica*
19	Gouri	*Indica*	67	CNR 55-6	*Indica*
20	MTU 1010	*Indica*	68	CNR 55-10	*Indica*
21	Konark	Indica	69	CNR 55-15	*Indica*
22	Lalat	*Indica*	70	CNR 57	*Indica*
23	Bhoi	*Indica*	71	CNR 77	*Indica*
24	Meher	*Indica*	72	Shatabdi	*Indica*
25	Surendra	*Indica*	73	CNR 93	*Indica*
26	PNR 546	*Indica*	74	CNR 98	*Indica*
27	IET 20144	*Indica*	75	CNR 102	*Indica*

Table 1. Continued

28	IET 19140	*Indica*	76	IR 8	*Indica*
29	N. Shankar	*Indica*	77	IR 64	*Indica*
30	Badami	*Indica*	78	CO 39	*Indica*
31	Nayanmoni	*Indica*	79	ARC -10086	*Temperate japonica*
32	Khandagiri	*Indica*	80	Minghui 63	-
33	Swarna Sub 1	*Indica*	81	Teqing	*Indica*
34	SwarnaDhan (IET 5656)	Indica			
35	Koimurali	Indica			
36	Basmati 370	*Indica*			
37	Local Basmati	*Indica*			
38	IR 36	*Indica*			
39	Dular	*Indica*			
40	Mahsuri	*Indica*			
41	Khitish	*Indica*			
42	Ratna	*Indica*			
43	Khanika	*Indica*			
44	Kerala Sundari	*Indica*			
45	Lemont	*Tropical japonica*			
46	Cuba 65	-			
47	ARC 10177	*Indica*			
48	Darmali	*Indica*			

Allele scoring

The size of intensely amplified DNA fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size DNA markers of a 50 base pairs (bp) DNA ladder, (Fermentus). Scoring was done on the basis of presence or absence of a specific size allele in a particular germplasm**.** The presence of the allele was denoted as 1 and absence as 0. And using the data a 1/0 matrix was constructed.

Data analysis

PIC value

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among the varieties, when tested using a particular marker. A *PIC* value of less than 0.25 indicates low polymorphism, a value between 0.25 and 0.5 indicates average polymorphism and a value higher than 0.5 indicates a highly polymorphic locus (Botstein et al., 1980).

Genetic diversity or expected heterozygosity ($H_{expected}$)

It is of major interest for estimating genetic variation in natural populations. High expected heterozygosity *($H_{expected}$)* indicates high genetic variability. The value of *He* ranges from zero (no heterozygosity) to nearly 1.0 (for a system with a large number of equally frequent alleles). Heterozygosity (expected) is calculated according to Nei (1973).

Population Structure and diversity analysis

Determination of the population structure was conducted using a model based approach utilizing the STRUCTURE version 2.3.4 software (Pritchard et al., 2000). The number of subpopulations into which the population under investigation can be classified (denoted by K) was calculated. The software uses a Bayesian model-based approach. For the purpose of the population structure analysis the SSR marker data was converted to 1 vs 0 matrix covering all possible polymorphisms. The analysis was conducted using the parameters like detection of admixture and allele frequency correlate model. The program was set with a Run length of 150,000 burning period length followed by 150,000 Marko Chain Monte Carlo (MCMC) replication. The k value ranged from 1 to 10. The optimum value for K was determined by plotting mean estimate of the log posterior probability of the data L (K) with respect to a specific K value. The maximum value of L (k) determines the optimum number of subpopulation present. The ad-hoc quantity ΔK proposed by Evanno et al. (2005) based on second order rate of change of the likelihood function with respect to K was estimated using Structure Harvester (Earl, 2012). ΔK showed a clear peak at the optimal K value.

Cluster Analysis using UPGMA

A dendrogram was constructed to determine genetic diversity among the genotypes by using UPGMA (un-weighted pair group method with arithmetic averages) clustering algorithm by software program DARwin 5.1.153 (Perrier & Bonnot, 2003).

Estimation of AMOVA

The presence or absence of molecular variance within and between the sub groups predicted by the STRUCTURE software was estimated using the software GenAlEx 6.5 (Peakall and Smouse, 2006).

Results and Discussion

The present investigation sheds a valuable light upon the genetic constitution of 81 rice genotypes studied using 30 SSR markers. The study revealed a total of 105 alleles across 30 SSR locus (Table 2) suggesting that a high polymorphism can be expected in the population. The PIC value is an important parameter which determines the effectiveness of a primer in estimating the genetic constitution of a given germplasm. There are several factors which influence the PIC value like population size, diversity, method of genotyping and location of primer on a chromosome etc. In terms of

Table 2. Details of 30 SSR primers along with their PIC value, number of alleles scored and expected heterozygosity or genetic diversity

Sl no.	Name	Chrm no.	Repeat Motif	BP range	No. of alleles	Genetic Diversity ($H_{expected}$)	Pic value
1	RM1	1	(GA)26	82-125	4	0.61	0.57
2	RM237	1	(CT)18	128-142	4	0.66	0.62
3	RM234	7	(CT)25	150-169	3	0.6	0.55
4	RM 201	9	(CT)17	155-171	5	0.71	0.64
5	RM206	11	(CT)21	150-200	4	0.6	0.53
6	RM 247	12	(CT)16	130-175	4	0.72	0.67
7	RM 335	4	(CTT)25	104-130	4	0.67	0.58
8	RM 205	9	(CT)25	122-135	3	0.77	0.7
9	RM 6100	10	(CGA)8	144-164	3	0.83	0.79
10	RM 490	1	(CT)13	95-105	4	0.69	0.58
11	RM 443	1	(GT)10	115-124	3	0.63	0.57
12	RM 171	10	(GATG)5	250-328	3	0.6	0.52
13	RM 1108	10	(AG)12	137-145	3	0.54	0.49
14	RM152	8	(GGC)10	108-167	4	0.69	0.6
15	RM 21	11	(GA)18	75-172	4	0.66	0.59
16	RM 263	2	(CT)34	105-239	4	0.6	0.56
17	RM 535	2	(AG)11	88-158	3	0.62	0.58
18	RM 3331	12	(CT)15	123-150	3	0.7	0.68
19	RM 6863	8	(TGC)9	178-195	2	0.52	0.43
20	RM 224	11	(AAG)8(AG)13	132-163	3	0.58	0.49
21	RM 6641	2	(GTA)14	140-172	3	0.66	0.57
22	RM 19	12	(ATC)10	222-270	3	0.58	0.5
23	RM 55	3	(GA)17	200-240	3	0.76	0.68
24	RM 296	9	(GA)10	119-146	3	0.87	0.8
25	RM 270	12	(GA)13	104-155	4	0.69	0.58
26	RM 154	2	(GA)21	158-200	4	0.72	0.69
27	RM 18384	5	(CTT)22	280-350	3	0.76	0.65
28	RM 1369	6	(AG)27	145-179	3	0.61	0.57
29	RM 20224	6	(CT)25	155-240	5	0.74	0.68
30	RM 542	7	(CT)22	133-205	4	0.81	0.77

PIC value RM296 showed the highest estimate of 0.80.The average PIC value was observed to be 0.60 which can be inferred to be high according to Botstein et al. (1980). Some other markers having considerably high PIC value included RM 6100 (0.79), RM 205 (0.70) and RM 542 (0.77). Thus the above mentioned markers can be suggested for utilization in future studies. Another important parameter explaining genetic variance is the genetic diversity (He). The mean estimate for genetic diversity of the population was found to be quite high at 0.67. The highest genetic diversity was expressed by RM 296 at 0.87 followed by RM 6100 at 0.83 and RM 542 at 0.81.

The STRUCTURE analysis was performed to determine the genetic constitution of the population under study. The log likelihood evinced by the structure analysis showed the optimum value to be 2 i.e. K = 2. The ad hoc value ΔK was also at ΔK = 2 (Figure 1) suggesting that the optimum number of subpopulations into which the population can be classified is 2 (designated here as Subpopulation 1 and 2). Sub

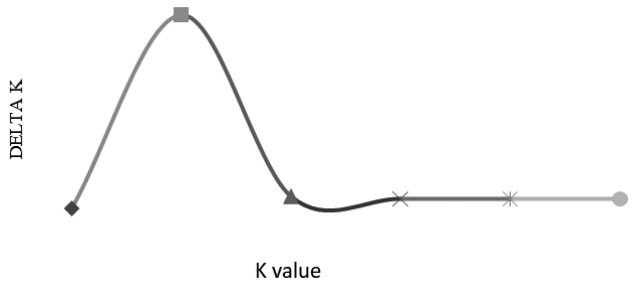

Fig. 1. Figure shows the optimum value for ΔK is at 2

population 1 consisted of 60 members whereas subpopulation 2 on the other hand had 15 members. 6 genotypes were designated as admixtures. The allotment of germplasms to a specific subpopulation was on the basis of their membership fraction. Accessions with the probability of ≥ 80% for a particular subgroup were designated under that sub group or subpopulation (Figure 2a, b). The assignment of germplasms were further confirmed using their inferred ancestry scores for a particular subgroup as predicted by STRUCTURE software in (Table 3). Other genotypes which did not score a value ≥80% for any subgroup and shared considerable ancestry with both the subgroups were stated as admixtures.

It can be clearly observed that almost all of the germplasms belonging to subpopulation 1 were *Indica* in origin, and further represented a major proportion of the population under study. Popular *Indica* varieties belonging to subpopulation 1 included varieties like Lalat, IR36, IR8, IR64, Khitish, Shatabdi etc. Subpopulation 2 mostly comprised of tropical and temperate *Japonica*. Popular varieties in subpopulation 2 included Nipponbare and Azucena.

Cluster Analysis

The cluster analysis revealed that the members of subpopulation 1 occupied four separate clusters on the upper branches of the dendrogram (Figure 3) i.e. cluster number 1, 2, 3 and 4. Thus, majority of the *Indica* genotypes were distributed across these four clusters (Table 4). The largest cluster consisting of *Indica* germplasms was Cluster number 3, which comprised of 25 members. Cluster 1 and 2 both consisted of 15 members while cluster 4 consisted of 10 members. Subpopulation 2 comprising of temperate and tropical

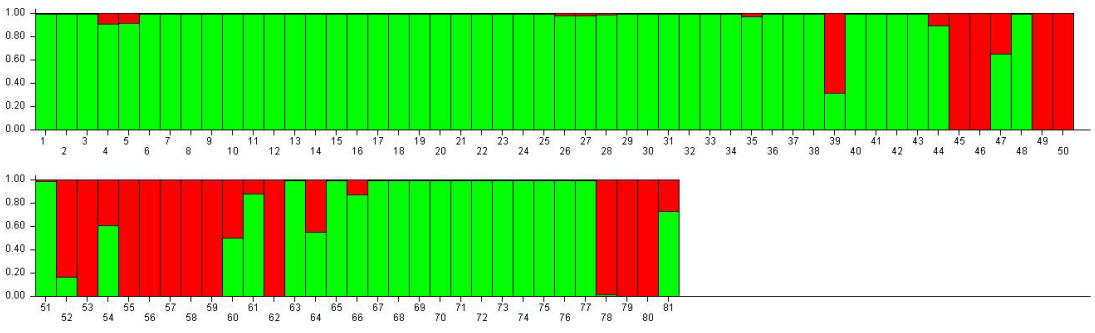

Fig. 2a. Population structure of 81 genotypes classified into two sub populations based on membership fraction. The green bars signify membership fraction corresponding to subpopulation 1 and red bars signify subpopulation 2

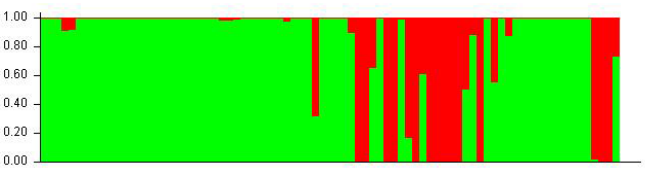

Fig. 2b. The structure of the population as a whole

Table 3. Inferred ancestry scores of genotypes determining their assignment to a particular subpopulation or as admixture

Genotype	SP 1	SP 2	IA	Genotype	SP 1	SP 2	IA
KMR 3	0.002	0.998	SP 1	Khitish	0.003	0.997	SP1
IR 10198R	0.005	0.995	SP 1	Ratna	0.003	0.997	SP1
IR 40750R	0.005	0.995	SP 1	Khanika	0.003	0.997	SP1
IR 71604-4-1-4-4-4-2-2-2R	0.091	0.909	SP 1	Kerala Sundari	0.102	0.898	SP1
IR 34686-1R	0.080	0.920	SP 1	Lemont	0.998	0.002	SP 2
IR 6876-1	0.003	0.997	SP 1	Cuba 65	0.997	0.003	SP 2
BR 827-35	0.002	0.998	SP 1	ARC 10177	0.346	0.654	Admix
BR 1356	0.002	0.998	SP1	Darmali	0.002	0.998	SP 1
BR 1356 Sel 2R	0.002	0.998	SP 1	Azucena	0.997	0.003	SP 2
NDR 97	0.002	0.998	SP 1	Nipponebare	0.998	0.002	SP 2
MTU 9992	0.002	0.998	SP 1	CNR 33	0.011	0.989	SP 1
DR 714-1-2R	0.002	0.998	SP 1	PehKuh	0.829	0.171	SP 2
Ajaya	0.002	0.998	SP 1	DeokJeok Jodo	0.998	0.002	SP 2
Pratiksha	0.002	0.998	SP 1	Rathuwee	0.388	0.612	Admix
Sidhant	0.002	0.998	SP 1	Carolina Gold (sel)	0.997	0.003	SP 2
Gajapati	0.002	0.998	SP 1	Double Carolina	0.998	0.002	SP 2
Jajati	0.002	0.998	SP 1	Trembese	0.996	0.004	SP 2
Purnendu	0.002	0.998	SP 1	Cenit	0.993	0.007	SP 2
Gouri	0.002	0.998	SP 1	I geo tze	0.495	0.505	SP 2
MTU 1010	0.002	0.998	SP 1	Tchibanga	0.116	0.884	Admix
Konark	0.002	0.998	SP 1	Asse-y- pung	0.996	0.004	SP 1
Lalat	0.002	0.998	SP 1	CNR 45	0.002	0.998	SP 2
Bhoi	0.002	0.998	SP 1	Milyang 46	0.446	0.554	SP 1
Meher	0.002	0.998	SP 1	CNR 47	0.002	0.998	Admix
Surendra	0.002	0.998	SP 1	CNR 55-3	0.127	0.873	SP 1
PNR 546	0.021	0.979	SP 1	CNR 55-6	0.002	0.998	SP 1
IET 20144	0.016	0.984	SP 1	CNR 55-10	0.002	0.998	SP 1
IET 19140	0.010	0.990	SP 1	CNR 55-15	0.002	0.998	SP 1
N. Shankar	0.002	0.998	SP 1	CNR 57	0.002	0.998	SP 1
Badami	0.002	0.998	SP 1	CNR 77	0.006	0.994	SP 1
Nayanmoni	0.003	0.997	SP 1	Shatabdi	0.002	0.998	SP 1
Khandagiri	0.002	0.998	SP 1	CNR 93	0.002	0.998	SP 1
Swarna Sub 1	0.002	0.998	SP 1	CNR 98	0.002	0.998	SP 1
SwarnaDhan (IET 5656)	0.002	0.998	SP 1	CNR 102	0.002	0.998	SP 1
Koimurali	0.026	0.974	SP 1	IR 8	0.002	0.998	SP 1
Basmoti 370	0.002	0.998	SP 1	IR 64	0.002	0.998	SP 1
Local Basmoti	0.002	0.998	SP 1	CO 39	0.980	0.020	SP 1
IR 36	0.002	0.998	SP 1	ARC -10086	0.997	0.003	SP 2
Dular	0.680	0.320	Admix	Minghui 63	0.994	0.006	SP 2
Mahsuri	0.003	0.997	SP1	Teqing	0.265	0.735	Admix

SP 1 – Sub Population 1, SP 2 – Sub Population 2, Admixture – Admix, IA - Inferred ancestry

japonica formed 3 separate clusters in the lower branches of the dendrogram, designated as Clusters 5, 6 and 7.

The dendrogram exhibited separate distribution of the *Indica* and *Japonica* genotypes which is in accord with the STRUCTURE analysis. The further distribution of *Indica* genotypes into four separate clusters indicated considerable variation among the *Indica* genotypes. Similar observation can be made about the *Japonica* types which were also classified into three separate clusters. In case of future breeding programs hybridization between genotypes belonging to separate clusters for both the sub species can ensure higher variability in terms of allelic constitution across multiple locus. But in order to perform an Indica/Japonica inter subspecific hybridization, presence of WC gene (wide compatible) is required for avoiding spikelet sterility. For this purpose the genotype Dular can be useful since WC gene has been reported in the variety by Kumar & Virmani (1992). In the current analysis, Dular was grouped with the *japonica* genotypes in cluster 7. On the basis of the Structure analysis, the variety was indicated as an admixture possessing genetic components from both sub-groups. The intermediate genetic constitution of Dular can be useful for performing three-way crosses between *Indica* and *Japonica* parents.

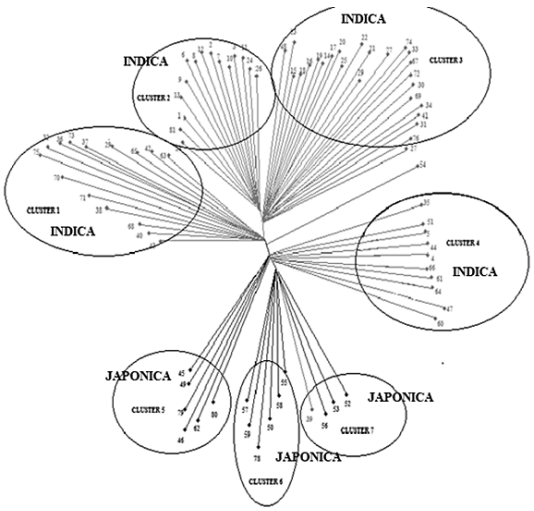

Fig. 3. Clustering Pattern of Genotypes through SSR Markers

Analysis of molecular variance

The AMOVA (Table 5) suggested that variation between the two subgroups was very high at 85%, whereas variation among the individuals belonging to the same subgroup was only 15%. This prediction is expected due to autogamous nature of rice. Thus, variation within a particular population will be naturally lower than variation between two sub-populations. Similar situations were also reported by Salem & Salam (2016), Nachimuthu et al. (2015).

Table 4. Distribution of eighty one genotypes across seven clusters

Cluster No.	Genotypes belonging to each cluster
1	43)Khanika ,40)Mahsuri ,68) CNR 55-10 , 38) IR 36 71) ,CNR 77,70) CNR 57,75) CNR 102, 32) Khandagiri,36) Basmati 370, 73) CNR 93, 37)Local Basmati , 29) N. Shankar,65)CNR 47, 42)Ratna, 63) CNR 45
2	28)IET 19140,81)Teqing,1)KMR 3,13)Ajaya , 9)BR 1356 Sel 2R ,6)IR 6876-1 ,8)BR 1356, 12)DR 714-1-2R ,2)IR 10198R ,7)BR 827-35,10) NDR 97 ,3)IR 40750R ,31)Nayanmoni, 24)Meher, 26)PNR 546
3	48)Darmali, 23)Bhoi, 15) Sidhant , 18) Purnendu, 16) Gajapati,19) Gouri, 14) Pratiksha 17) Jajati , 20) MTU 1010, 25) Surendra ,22) Lalat, 21)Konark, 29) N. Shankar,77)IR 64 74)CNR 98, 33) Swarna Sub 1, 67) CNR 55-6, 72) Shatabdi, 30) Badami , 69)CNR 55-15 34)SwarnaDhan (IET 5656), 41)Khitish, 31)Nayanmoni, 76) IR 8, 27)IET 20144
4	35) Koimurali, 51) CNR 33, 5) IR 34686-1R ,44) Kerala Sundari, 4)IR 71604-4-1-4-4-4-2-2-2R, 66) CNR 55-3, 61) Tchibanga, 64) Milyang 46, 47) ARC 10177, 60)I geo tze
5	45) Lemont, 49) Azucena, 79)ARC -10086, 46) Cuba 65, 62) Asse-y- pung, 80) Minghui 63
6	57) Trembese, 59) Zhensan 2,78) CO 39, 50) Nipponebare, 58) Cenit,55) Carolina gold (sel)
7	39)Dular, 56) double Carolina, 53) deok jeok jodo, 52) pehkuh

Table 5. Table showing Analysis of Molecular Variance (AMOVA)

Source	df	SS	MS	Est. Var.	Variation, %
Among Pops	1	367 088.443	367 088.443	15 185.743	85%
Within Pops	74	192 034.117	2630.604	2630.604	15%
Total	75	559 122.560		17 816.348	100%

Conclusion

The population structure and diversity analysis suggested that significant variability can be expected among the genotypes investigated. The molecular markers used for analyzing the population, successfully classified the genotypes based on their genetic background. Thus, the SSR markers used in the current study can be applied in future breeding programs for determining genetic diversity of a given population of variable rice genotypes. In the current study the population structure analysis classified the genotypes into two sub populations; arising from two varied ancestry i.e. *Indica and Japonica*. Thus, significant differences in genetic architecture can be expected among the genotypes belonging to the separate subgroups. Furthermore, classification of the genotypes representing two separate subgroups using the cluster analysis indicated that considerable variance can be expected even within each sub population. This diversity can be exploited in future breeding programs for obtaining desirable recombinants showing maximum allelic variations across all the chromosomes. Finally intermediate types like Dular which shared genetic constitution with both *indica* and *japonica* subtypes were detected. Such genotypes can be used for performing three-way inter sub-specific crosses which can produce fertile F_1 hybrids in future breeding programs. Such approaches can help in avoiding spikelet sterility in F_1 hybrids, which is otherwise expected in case of *indica* x *japonica* crosses.

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