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Analysis of genetic diversity and population structure in upland rice accessions of India

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Abstract
Rice is one of the important staple cereals which are consumed as a main part of diet by more than half of world's population. Rice production in India accounts to more than 40% of country's grain production. Rice is a major crop of Assam and it plays a major role in state's economy. A panel of 50 upland diverse rice cultivars collected from different parts of India were genotyped using SSR markers distributed across the 12 chromosomes in rice. The phenotyping using various yield traits were performed in rainout shelter as well as in open field conditions. A significant variation among the genotypes for all the characters under drought stress as well as in irrigated conditions study was obtained indicating the existence of considerable amount of variability among the genotypes selected for study. Grain yield is the most important character for selection under drought stress condition. Mannitol treatment may be an effective tool for selecting genotypes in drought stress. The log likelihood revealed by Structure showed the optimum subpopulations (K) value as 2 (K= 2), which indicated that the entire population can be grouped into two subgroups. The results clearly indicated that two subgroups were formed due to the different adaptation behaviour of cultivars to drought stress and yield attributes under drought. A total of forty-three cultivars were grouped in cluster I however, remaining seven cultivars were grouped in cluster II. The assessment of genetic diversity among the cultivars is pre requisite for any successful breeding programme.

Keywords: Rice, SSR, grain yield, drought stress

Introduction
Rice is the world's most important food crop and a primary source of food for about half of the world's population. It is the grain with the second-highest worldwide production, after maize. Majority of the rice produce comes from developing countries in Asia, Africa, and Latin America. In the absence of basic infrastructure such as irrigation facilities, many areas rely only on rainfall and are severely affected in cases of uneven rainfall incidences, leading to drought and/or flood.

India had approximately 43 million ha rice growing area nearly 60% of which were in Eastern India and only rainfed rice growing area of Eastern India accounts for 12.9 million ha (Keelery, 2020) [7]. Total production of Rice during 2018-19 is estimated at record 115.60 million tonnes. Production of rice has increased by 4.59 million tonnes than the production of 111.01 million tonnes in 2nd Advance Estimates of 2017-18. It is also higher by 7.80 million tonnes than the five years average production of 107.80 million tonnes (Ministry of Agriculture and Farmers Welfare, GOI, 2019).

Development of new high yielding rice varieties with enhanced disease resistance, tolerance to abiotic stresses, and specific quality characteristics is needed to ensure global food security (Collard *et al*., 2019) [2]. Climate change is impacting the Earth's surface and atmosphere, causing a reduction in rainfall and elevated temperatures (Lamaoui *et al*., 2018) [8] as well as increasing unpredictability and frequency of extreme weather events (Wassmann *et al*., 2009) [12]. Drought is one of the major abiotic stresses that results to a decline in rice production in the rainfed areas. This study estimates a broad overview of Simple Sequence Repeat (SSR) based genetic diversity present in 50 short duration varieties using 58 SSR markers. In this study, we determined the population structure of 50 high yielding short duration varieties. Further, we used the mixed linear model method in the TASSEL software to conduct association analysis.

Materials and Methods

Planting Material, Experimental Location and Experimental Design

The 50 upland rice cultivars were collected from different parts of India during *Kharif* 2016. The cultivars were screened in two different environment *i.e*., irrigated condition and rain out shelter. The field experiment was conducted in Regional Rice Research Station (RARS), Titabor, Assam. Seeds were sown in a nursery and 21-day-old seedlings were transplanted in Randomized block design with three replications. Data on various yield traits *i.e.* Days to 50 per cent Flowering (DTF), Plant Height (PH), Panicle number (PN), Harvest index (HI), Biomass yield (BY), Grain yield (GY) and Days to maturity (DTM) were scored using standard evaluation system for rice (SES) (IRRI, 1996) [6] from the trials conducted under two different hydrological conditions *viz*., artificial drought stress created in rainout shelter and irrigated (non-stress) conditions.

Evaluation of the genotypes for drought stress using Mannitol

Twenty five seeds of each fifty genotypes was germinated under three different treatments *viz.,* T_o- Non stress (Water), T_1- 1% mannitol (54mM) and T_2- 2% mannitol (108mM) to check their germination ability under mild (T_1) and severe (T_2) osmotic stress conditions. Seeds of each genotype were treated with above treatments in petridishes. All total 150 petridishes were arranged for 50 genotypes (50 petridishes/ treatment). Petridishes were checked daily and if the petridishes were dried than they were to be poured with treatment. The check genotypes *i.e.*, Kolong (stress susceptible) and IR-64drought (stress tolerant) were used. After 21 days of treating seeds, number of germinated and non- germinated seeds was counted for calculating germination percent along with root length and shoot length were measured. The spearman rank correlation was studied from mannitol treatment at mild and severe were compared with drought stress condition and irrigated condition separately.

Genotyping

Isolation of genomic DNA and PCR amplification

DNA extraction was done by using a modified Dellaporta *et al.,* 1983 protocol. For PCR analysis, each reaction mixture contained 1 µl of genomic DNA (100 ng), 0.5 µl of each primers (at a concentration of 10 pmole/µl), 2.5 µl of 10× PCR buffer, 0.75 µl of 50 mM $MgCl_2$, 0.25 µl of 2.5 mM dNTP mixture, 0.2 µl (1 unit) of 5 unit/µl Taq DNA polymerase and 19.3 µl of PCR-grade water. The temperature profile of the first PCR cycle was 94 °C for 5 mins, 55 °C for 1 min; followed by 35 cycles of 1 min at 95 °C, 1 min at 55-60 °C and 1 min at 72 °C. The final extension was at 72 °C for 5 min. PCR products were analysed on 3.5% agarose gel and visualized by ethidium bromide.

Population structure and cluster analysis

Population structure consisted of a Q matrix that describes the percent subpopulation parentage for each line in the analysis. These percentages were calculated by Structure2.3.3 software (Pritchard *et al*., 2000) [10]. The degree of genetic relationship among the studied rice genotypes as revealed by Jaccard's coefficient of similarity was represented through cluster analysis using the algorithm of 'Unweighted Pair Group Method with Arithmetic Average' (UPGMA) by feeding similarity matrix as input data. The graphical representation of genetic relationship among the genotypes was done in the form of dendrogram.

Results and Discussion

The 50 upland rice cultivars were evaluated for various yield component traits under drought stress and control conditions. Data of various traits were scored using standard evaluation system for rice (SES) (IRRI, 1996) [6] under two different hydrological conditions *viz*., artificial drought stress created in rainout shelter and irrigated (non-stress) conditions. The mean performance of various yield traits recorded under both drought stress and irrigated conditions are presented in Table 1.

Spearman rank correlation coefficients among different characters under irrigated and stress conditions

Spearman rank correlation coefficients among different characters under irrigated condition were compared with mannitol at mild (T_1), severe (T_2) and control (T_o) and presented in Table 2 and Table 3 respectively.

Allelic distribution of SSR markers

PCR amplifications were carried out using 60 SSR markers distributed uniformly on the 12 chromosomes (Table 4). Total of 130 alleles were detected using 60 SSR markers. The overall size of amplified products ranged from 100bp (RM286, RM256, RM287, RM31, RM212, RM5) to 700bp (RM169) (Fig. 1 and Fig. 2). The number of alleles per loci varied from 1 to 4 among the 60 markers with an average of 2.166 alleles per locus. This number is higher than the study conducted by Rathi *et al*. (2014) [11] which reported the average number of alleles 1.867 per locus using 120 SSR markers on a panel of 100 rice cultivars. On the other hand Yang *et al*. (1994) [13] reported 9.3 alleles per locus and Das *et al*. (2013) [3] reported 7.9 alleles per locus.

Population structure

The log likelihood revealed by Structure showed the optimum subpopulations (K) value as 2 (K= 2), which indicated that the entire population can be grouped into two subgroups (SG1 and SG2) (Table 5). Based on the membership fractions, the cultivars with the probability of ≥80% were assigned to corresponding subgroups while the cultivars showing <80% were categorized as admixture. Subgroup SG1 consisted of 07 cultivars, 33 cultivars including drought susceptible check (Kolong) were grouped in SG2 and ten cultivars were retained as admixture including IR-64 drought (drought tolerant check) (Fig. 3). The results clearly indicated that two subgroups were formed due to the different adaptation behaviour of cultivars to drought stress and yield attributes under drought.

Population structure of different rice germplasm was also reported by Ebana *et al*. (2008) [5], Agrama *et al*. (2010) [1]. Similarly, the population structure of two subgroups was observed by Zhang *et al*. (2009) [14] in a collection of 3024 rice landraces from China.

Cluster analysis

Both Cluster I and II were divided into 2 sub clusters at Jaccard's coefficient value of 0.46 and 0.76, respectively. On basis of similarity coefficient, these sub clusters were further divided into sub-sub clusters. A total of forty-three cultivars were grouped in cluster I however, remaining seven cultivars were grouped in cluster II (Fig. 4). The cultivars Kolong and

IR-64 drought were grouped in the cluster I. The assessment of genetic diversity among the cultivars is pre requisite for any successful breeding programme. It assists in selection of suitable parental cultivars for development of breeding population.

Table 1: Mean performance of agronomic traits under drought stress and irrigated conditions

Genotypes	Environment	Days to flowering	Plant height	Harvest Index	Biomass (g/plot)	Panicle number	Days to maturity	GY (kg/ha)
Genotype Mean	Normal	89.50	108.90	0.31	958.12	6.48	119.55	4128.33
	Stress	81.54	102.50	0.23	934.03	4.41	111.60	1996.37
Range (Min-Max)	Normal	76.00-104.67	81.40-139.37	0.22-0.52	479.66-1704.10	4.67-9.33	101.00-136.33	2776-.67-6016.67
	Stress	67.00-96.67	77.70-131.43	0.14-0.65	288.33-1795.00	2.33-7.00	93.33-129.00	611.67-3948.63
Kolong	Normal	76.33	100.33	0.33	918.67	6.33	105.33	3373.33
	Stress	68.67	93.00	0.26	906.67	4.33	97.67	2048.33
IR-64 drought	Normal	94.67	110.80	0.35	1490.60	7.33	126.33	4795.00
	Stress	85.67	104.53	0.28	1451.67	5.33	117.33	3948.63
C.D. 5%	Normal	2.28	11.03	3.80	72.46	0.93	2.94	515.15
	Stress	3.53	11.24	0.05	122.53	1.06	4.20	361.80

Table 2: Spearman rank correlation coefficients among different characters under irrigated condition

Irrigated condition												
	Root length			Shoot length			Germination			Vigour index		
	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
Days to flowering	-0.037	-0.200	-0.137	-0.033	-0.091	-0.096	-0.011	-0.103	-0.191	-0.097	-0.047	-0.053
Plant height	0.030	0.363**	0.205	0.191	0.481**	0.139	0.128	0.197	0.207	0.142	0.259	0.161
Grain yield	0.072	0.320*	0.343*	0.106	0.322*	0.427**	0.218	0.356*	0.391**	0.274	0.348*	0.396**
Harvesting Index	0.237	0.310*	0.356*	0.159	0.187	0.200	0.159	0.292*	0.395**	0.205	0.235	0.239
Biomass	-0.254	-0.044	0.146	-0.376**	-0.163	0.115	-0.109	-0.126	-0.135	-0.188	-0.043	0.062
Plot yield	0.070	0.010	0.074	-0.031	-0.040	0.132	-0.112	-0.223	-0.198	-0.061	-0.024	0.082
Panicle no.	-0.094	-0.122	0.241	-0.127	-0.112	0.244	0.015	0.065	0.122	0.055	0.047	0.231
Days of maturity	0.006	-0.153	-0.054	0.064	-0.041	-0.004	0.000	-0.088	-0.147	-0.031	-0.002	0.022

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

Table 3: Spearman rank correlation coefficients among different characters under drought stress condition

Drought stress condition												
	Root length			Shoot length			Germination			Vigour index		
	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
Days to flowering	-0.057	-0.192	-0.162	-0.013	-0.052	-0.118	-0.013	-0.087	-0.189	-0.104	-0.048	-0.077
Plant height	0.040	0.335*	0.179	0.205	0.463**	0.113	0.135	0.214	0.225	0.149	0.241	0.142
Grain yield	0.465**	0.484**	0.517**	0.219	0.469**	0.506**	0.151	0.382**	0.457**	0.266	0.321*	0.290*
Harvesting Index	0.448**	0.463**	0.488**	0.113	0.399**	0.444**	0.063	0.274	0.474**	0.188	0.305*	0.256
Biomass	-0.086	0.013	0.209	-0.272	-0.154	0.207	0.021	-0.109	-0.068	-0.069	-0.004	0.152
Plot yield	-0.116	-0.017	0.182	-0.270	-0.130	0.212	-0.019	-0.088	-0.117	-0.078	0.013	0.119
Panicle no.	-0.069	-0.090	0.300*	-0.138	-0.121	0.297*	0.069	0.082	0.164	0.084	0.088	0.271
Days of maturity	0.006	-0.153	-0.054	0.064	-0.041	-0.004	0.000	-0.088	-0.147	-0.031	-0.002	0.022

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

Table 4: Details of 60 SSR markers used for diversity analysis

S. No.	Marker	Chromosome No.	Min. Allele size	Max. Allele size	No. of alleles	S. No.	Marker	Chromosome No.	Min. Allele size	Max. Allele size	No. of alleles
1	RM495	1	200	215	2	31	RM31	5	100	135	2
2	RM3604	1	140	0	1	32	RM170	6	340	380	2
3	RM243	1	130	160	2	33	RM588	6	110	0	1
4	RM5348	1	125	0	1	34	RM343	6	190	195	2
5	RM9	1	200	250	2	35	RM162	6	170	0	1
6	RM129	1	190	200	2	36	RM5344	7	115	140	2
7	RM5	1	100	0	1	37	RM125	7	135	160	2
8	RM212	1	100	125	2	38	RM11	7	140	160	2
9	RM12146	1	105	115	2	39	RM182	7	220	270	2
10	RM5638	1	280	300	3	40	RM152	8	130	155	3
11	RM341	2	165	180	2	41	RM25	8	140	150	2
12	RM263	2	150	180	2	42	RM256	8	100	150	2
13	RM555	2	225	0	1	43	RM444	9	190	235	2
14	RM530	2	165	185	2	44	RM24334	9	190	0	1
15	RM16030	3	130	235	3	45	RM524	9	180	210	3
16	RM347	3	140	340	4	46	RM257	9	130	150	2
17	RM7332	3	130	280	4	47	RM242	9	195	230	3

18	RM545	3	210	230	2	48	RM474	10	240	260	2
19	RM517	3	250	270	2	49	RM496	10	150	180	2
20	RM1256	3	145	155	2	50	RM333	10	200	230	2
21	RM232	3	150	450	4	51	RM6327	11	180	205	3
22	RM1038	3	240	300	3	52	RM286	11	100	110	2
23	RM523	3	150	0	1	53	RM287	11	100	110	2
24	RM16278	4	150	170	2	54	RM209	11	140	170	2
25	RM261	4	130	145	2	55	RM206	11	140	180	3
26	RM16686	4	250	0	1	56	RM19	12	185	245	3
27	RM518	4	155	175	2	57	RM1261	12	185	300	4
28	RM592	5	220	300	2	58	RM28166	12	190	205	2
29	RM169	5	200	700	3	59	RM3331	12	105	165	3
30	RM164	5	150	170	2	60	RM28519	12	150	160	2

Table 5: Population structure of 50 rice cultivars

S. No.	Cultivars	Inferred ancestry		Structure group	S. No.	Cultivars	Inferred ancestry		Structure group
		Q1	Q2				Q1	Q2	
1	Luit	0.022	0.978	SG2	26	NLR 3276	0.013	0.987	SG2
2	Kopilee	0.012	0.988	SG2	27	NLR 3354	0.016	0.984	SG2
3	Kolong	0.009	0.991	SG2	28	NLR 4002	0.013	0.987	SG2
4	Disang	0.007	0.993	SG2	29	MDT 6	0.022	0.978	SG2
5	Lachit	0.005	0.995	SG2	30	MDT 10	0.250	0.750	AD
6	Chilarai	0.004	0.996	SG2	31	MTU 1010	0.012	0.988	SG2
7	Shraboni	0.006	0.994	SG2	32	NLR 33057	0.014	0.986	SG2
8	IR-64 drought	0.310	0.690	AD	33	Dhala Shaita	0.984	0.016	SG1
9	Dikhow	0.011	0.989	SG2	34	Binuhangin	0.605	0.395	AD
10	Numali	0.015	0.985	SG2	35	Dular	0.935	0.065	SG1
11	NLR 30491	0.012	0.988	SG2	36	ARC 10955	0.995	0.005	SG1
12	NLR 40024	0.006	0.994	SG2	37	Kali Aus	0.511	0.489	AD
13	NLR 34449	0.004	0.996	SG2	38	Uri	0.127	0.873	SG2
14	NLR 145	0.005	0.995	SG2	39	Dangar	0.424	0.576	AD
15	NLR 33671	0.006	0.994	SG2	40	Jabor Sail	0.465	0.535	AD
16	NLR 34242	0.049	0.951	SG2	41	Kalamkati	0.303	0.697	AD
17	NLR 40054	0.007	0.993	SG2	42	Aus 257	0.996	0.004	SG1
18	NLR 40058	0.032	0.968	SG2	43	Gul Murali	0.996	0.004	SG1
19	NLR 40065	0.014	0.986	SG2	44	Moshur	0.987	0.013	SG1
20	NLR 33358	0.040	0.960	SG2	45	Juma	0.953	0.047	SG1
21	NLR 3042	0.005	0.995	SG2	46	Dharia Boalia	0.387	0.613	AD
22	NLR 3242	0.021	0.979	SG2	47	Mikhudeb	0.349	0.651	AD
23	NLR 3217	0.026	0.974	SG2	48	Apo	0.089	0.911	SG2
24	NLR 3238	0.018	0.982	SG2	49	Vandana	0.306	0.694	AD
25	NLR 3083	0.029	0.971	SG2	50	Sahbhagi dhan	0.030	0.970	SG2

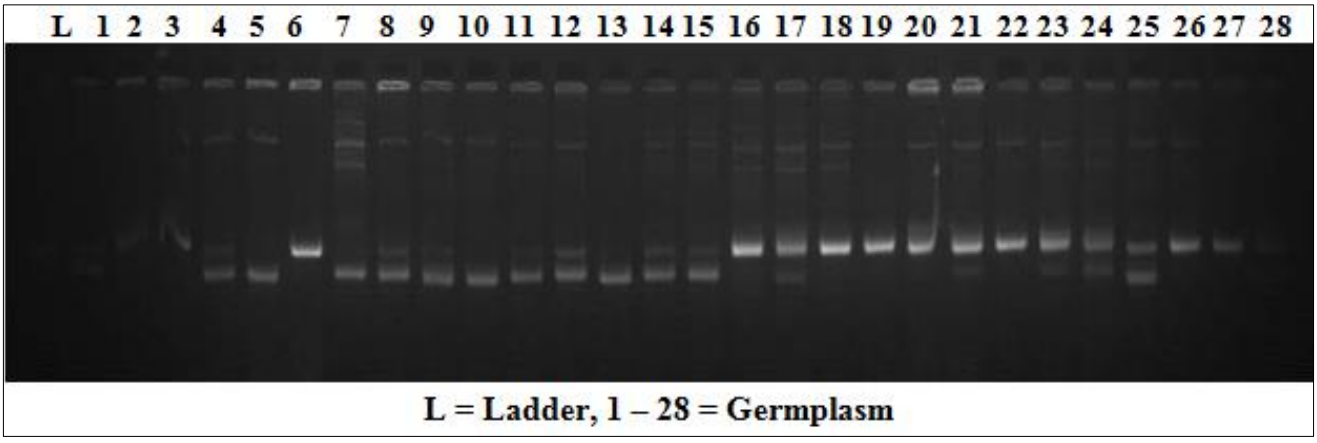

Fig 1: Separation of alleles and its variation of SSR marker RM5638 in rice cultivars

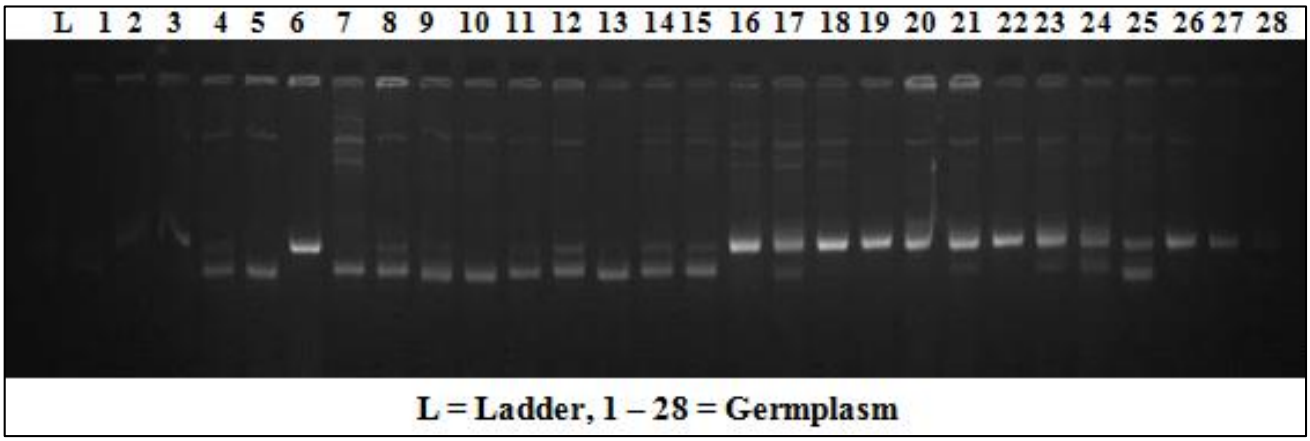

Fig 2: Separation of alleles and its variation of SSR marker RM9 in rice cultivars

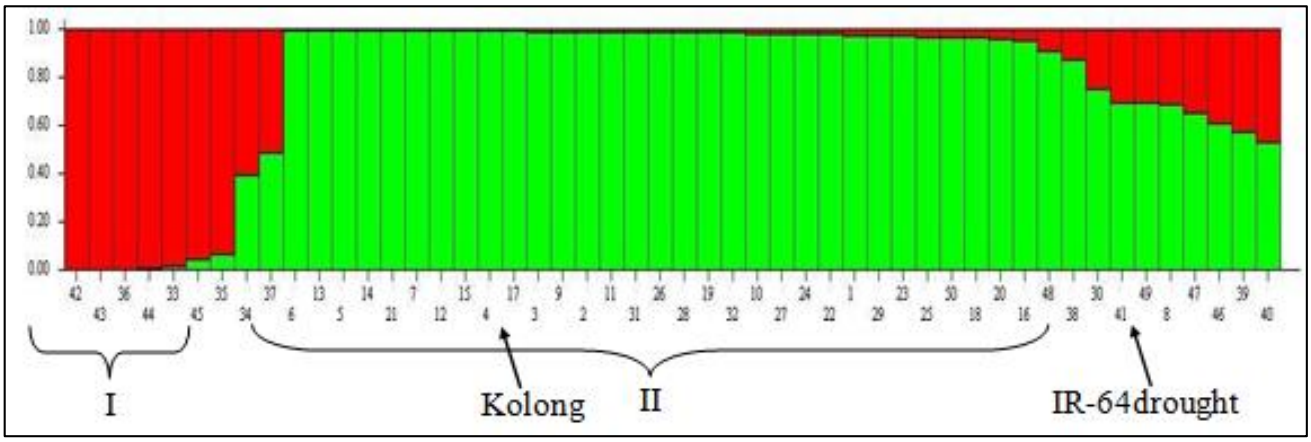

Fig 3: Population structure of 50 rice cultivar Check varieties: Kolong-3 and IR-64drought-8 Vertical bar expressed as % and each group is represented by red and green colour

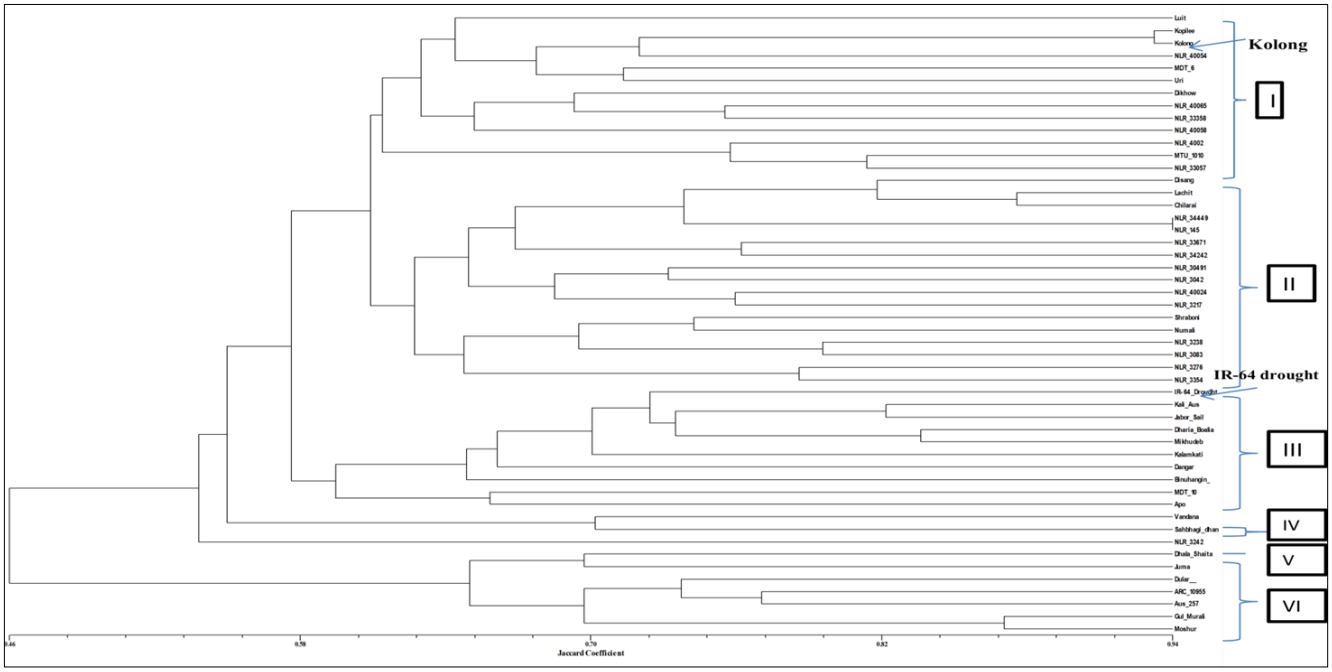

Fig 4: Cluster analysis of the 50 rice cultivar

Conclusion

In the present investigation, genotyping of the lines were carried out by using SSR markers distributed across the 12 chromosomes. Population structure of the 50 cultivars was analysed using the software STRUCTURE and it revealed that the log likelihood revealed by Structure showed the optimum subpopulations (K) value as 2 (K= 2), which indicated that the entire population can be grouped into two subgroups. Subgroup SG1 consisted of 07 cultivars, 33 cultivars including drought susceptible check (Kolong) was grouped in SG2 and ten cultivars were retained as admixture including IR-64 drought (drought tolerant check).

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