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Diversity Analysis For Drought Tolerance Rice Genotypes Using Microsatellite Markers

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Abstract

Using a panel of ten drought tolerance related microsatellite markers distributed amongst five chromosomes of rice, a set of 18 rice genotypes categorized into three drought tolerant groups.. Molecular characterization of the genotypes gives precise information about the extent of genetic diversity which helps in the development of an appropriate breeding program. In the present study, a largest amplicon was produced by marker RM18 and the smallest by RM136. A total of 82 allelic products were generated at 10 microsatellite loci with an average of 5.4 alleles per locus. The polymorphism information content values varied from 0.297 (RM87) to 0.902 (RM231) with an average 0.746. A similarity coefficient based dendrogram was constructed using un-weighted paired group method with arithmetic average (UPGMA) for genotypes classification. The first cluster accommodated genotypes, namely, RAU-1421-12-1-7-4-3, RAU-1415-3-5-7-6-9-5-3, RAU-1401-1-8-1-5, RAU-1428-4-3-2-7-26, RAU-1478-5-2-2-4-6, RAU-1477-9-7-22-5-7-3, RAU-1451-6-6-1-1-5-2, RAU-1416-4-2-5-2-2, RAU-1397-2-5-8-1-2-5-4 and RAU-1451-3-5-7-6-9-5-1. The second cluster accommodated genotypes, namely RAU1453-12, RAU1471-10, RAU 1463-16, RAU1428-31-5-4, RAU1426-43-2-5-7-2, RAU1451-35-7-6-9-5-1 and the last cluster accommodated genotypes RAU1397-25-8-1-2-5-4), Molecular marker based analysis confirmed the drought tolerant genotype correlated to the morpho-physiological parameters. The work showed the utility of microsatellite markers in ascertaining the status of rice plants with respect to drought stress tolerance.

Keywords: Drought tolerance, Microsatellite markers, Rice, Diversity.

Introduction

India is self-sufficient in rice, but due to a high population growth rate, the ongoing process of climate changes, rice production will be decline to the current levels of sufficiency. Rice is highly sensitive to drought. Drought affects virtually all aspects of rice growth in varying degrees all stages from germination to maturity. Drought is the most serious abiotic stress reducing rice production and cause yield instability even in rainfed lowland ecosystem of eastern India. In Asia, at least 23 million ha (about 20% of the total area) under rainfed rice is drought prone. A recent estimate on climate change predicts the water deficit to deteriorate further in years to come [Wassmann et al., 2009] and the intensity and frequency of drought are predicted to become

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worse [Bates et al., 2008]. Out of the total 20.7 million ha of rainfed rice area reported in India, approximately 16.2 million ha lie in eastern India [Singh et al., 2000], of which 6.3 million ha of upland area and 7.3 million ha of lowland area are highly drought-prone [Pandey et al., 2009]. From the beginning of the Green Revolution era in rice in 1965 till 2009, on 14 occasions, rice production in India failed to achieve the estimated production. On those 14 occasions, rice production was in fact lower than the previous year's production. Drought was the cause of reduced production on 11 occasions. Economic losses can be minimized with availability of drought tolerant cultivars with improved yield. Severe drought in the wet season during the reproductive stage not only had an adverse effect on rice production but also reduced the area sown under wheat, pulses, and oilseeds in the subsequent dry season because of the unavailability of sufficient moisture in the soil, thereby reducing the production of these crops.

The two most recent severe droughts (in 2002 and 2009) witnessed a significant reduction in rice as well as total food production. In 2002, 29% of the geographical area suffered from drought due to 19% annual rainfall deficiency. This year, rice production fell by 21.5 million tons. Similarly, in 2009, rice production decrease 10.02 million-ton. India is already one of the most water-stressed countries in the world, a situation which is going to degrade into outright water scarcity due to high population growth. The total flow of water in major rivers is declining and there is no additional water to be injected into the system. Some traditional rice varieties are still grown, partly due to their greater drought tolerance and yield stability, but these have low yield potential. Breeding of drought tolerant rice with improved yield suitable for rainfed areas is an efficient way to overcome this natural disaster. Tolerance to drought is genetically and physiologically complicated and inherited quantitatively. Application of molecular- marker aided selection technique for improvement of drought tolerance would accelerate breeding procedure by increasing selection efficiency. One of the most important aims in plant breeding is to determine the chromosomal regions related to drought tolerance.

Among various PCR based markers, SSR markers are more popular marker. Simple sequence repeats (SSR) also termed microsatellites are tandemly repeated DNA sequence motifs consist of 2-6 nucleotide core units widely dispersed in genomes of human as well as plants [Litt and Lutty, 1989]. Variations in the length of tandem repeats can be detected by amplification of the SSR-containing DNA stretch via PCR using primers designed from the sequences of flanking regions which are generally conserved among genotypes of the same species. The length of the amplified fragment will vary according to the number of repeats which can simply be measured by gel electrophoresis. In crop plants, length polymorphisms in SSRs was firstly reported in soybean, which led to application of this new PCR-based marker system to other plant genomes. SSRs are highly polymorphic, which is easy to score and use for genotyping. The distinguishing features of SSR loci include their high information content, abundance, even distribution across the genome, reproducibility, and locus specificity which undoubtedly made SSRs a marker of choice for genome mapping. The

potential of SSRs as co-dominant marker has been exploited in rice to investigate genetic diversity SSR markers are more popular in rice because they are highly informative, mostly monolocus, codominant, easily analyzed and cost effective. SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies evaluate genetic diversity among rice genotypes for salinity tolerance and abiotic stresses [Vanniarajan et al., 2012; Faridul Islam et al., 2012]. SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes from germplasms of various sources, even they can detect finer level of variation among closely related breeding lines within a same variety. Because of the low value of heritability of grain yield (GY) under stress and the lack of effective trait selection index related to drought tolerance, it is very important to find molecular marker associated with water stress tolerance in rice. Many SSR markers have been reported to be linked to drought tolerance traits or QTLs in rice such as yield under drought, maximum root length, relative spikelet fertility [Yue et al., 2006] basal root thickness and root dry weight. Carefully chosen set of SSR markers is playing an important role to identify gene(s) for drought tolerance that can be helpful for plant breeders to develop new cultivars, besides facilitating an unbiased assessment of genetic differentiation, an unambiguous description of rice cultivars and development of unique molecular profiles of rice genotypes. The present study focused on the informative and highly informative markers identified in this study could be utilized in further studies for association mapping and marker assisted selection for drought tolerance in Indian rice genotypes.

Materials And Methods

Plant Material And Genomic DNA Extraction

Eighteen rice genotypes were evaluated in this study. The list of rice genotypes is given in (Table- 1). The experimental materials consisted of 18 rice genotypes which were procured from the plant breeding and genetics department of Dr. Rajendra Prasad Central Agricultural University (Dr.R.P.C.A.U) PUSA, (Bihar) and Raipur. Genomic DNA was isolated from leaves of two weeks old rice plant using CTAB method as described by [Sagai-Maroof et al.,1984] with some modifications. The isolated DNA was stored at -20^{o}C in TE buffer until further use and the quality of extracted DNA samples was ascertained by agarose gel (0.8%) electrophoresis using 1kb DNA ladder (Gene ruler, Fermentas) as standard. The amount of DNA *in* the extracted DNA samples was determined by measuring the optical density at 260nm. Absorbance ratio (ratio of absorbance at 260 and 280 nm) was used as an indicator to check the purity of extracted DNA samples.

Amplification of genomic DNA

A panel of ten drought tolerance associated microsatellite primer pairs distributed among five chromosomes (1, 2, 6, 7 and 8) of rice genome was used for targeted amplification of the genomic DNA. The information pertaining to chromosomal positions, primer sequences, repeat motifs of the microsatellite sites flanked by these primer pairs was obtained from rice

genome database (http://www.gramene.org) and the most suitable annealing temperature for each of the primer pairs was experimentally determined (Table- 2). Polymerase chain reaction (PCR) based **a**mplification was carried out using 15 µl reaction mixture containing 5X PCR buffer, 1mM deoxyribonucleotide triphosphates (dNTPs), 10mM of MgCl2, 5µM of each forward and reverse primer, 1 unit Taq DNA polymerase (Fermentas) and 20 ng of template DNA. The PCR amplification was carried out with the help of a Thermocycler (Eppendorf) programmed to 1 cycle of 4 min at 94^{o}C as an initial strand separation step. This was followed by 35 cycles of 1 min at 94^{o}C for denaturation, 1 min for primer annealing with varying annealing temperature depending on the primer pairs used (54^{o}C-58^{o}C) and 2 min at 72^{o}C for primer extension. Finally 1 cycle of 10 min at 72^{o}C was used for final extension and then products generated from amplification reactions were stored at 4^{o}C till separation and resolution through electrophoresis.

The products of amplification reactions were separated using 2% Top vision Agarose (Fermentas) gels. Gels were stained in ethidium bromide solution. A 50 bp DNA ladder (Gene ruler, Fermentas) was used as size marker to determine the molecular size of amplified products. Electrophoresis was done at 100V for 90 minutes in 0.5X TBE buffer. After electrophoresis, the gels were documented by using a gel documentation system (Alpha Innotech).

Scoring Of Alleles And Data Analysis

The products of amplification reaction, which appeared in the form of bands on the gel, were analyzed by determining their position and size in relation to the position and size of the ladder. The different bands produced by each one of the ten primer pairs utilized for targeted amplification of specific region of genomic DNA in the present study were compared and classified into the two different categories of shared and unique bands. Allelic diversity and the suitability of the microsatellite sites based polymorphism for identification of polymorphic and informative markers to characterize and differentiate the experimental materials was assessed on the basis of comparison of polymorphism information content (PIC) of the primer pairs. The data on polymorphism information content (PIC) of the primer pairs was generated by calculating the value according to the formula given by [Anderson et al., 2006] as follows:

$$PIC_i = 1 - \sum_{j=1}^{k} P^2ij$$

Where, P_{ij} is the frequency of the j^{th} allele for i^{th} marker and summation extends over k alleles

The information regarding informativeness of the marker was obtained by computing the polymorphism per cent as follows:

PP = (Number of unique alleles/Total number of alleles) × 100

Clustering Of Entries

The polymorphism in respect of microsatellite sites was recorded on the basis of presence or absence of the bands in different entries used in the present study. All the entries used during molecular characterization were scored for the presence and absence of the microsatellite sites based bands. The data were entered into binary matrix as discrete variables and this data matrix was subjected to further analysis. Genetic similarities among entries were calculated on the basis of presence and absence of common bands. The

genetic associations among entries were analyzed by calculating the similarity coefficients [Dice, 1945] for pair-wise comparisons based on the proportions of shared bands produced by primers.

Similarity coefficient =2a/ (2a+b+c)

Where,

a = Number of bands between J^{th} and K^{th} genotypes

b = Number of bands present in J^{th} genotype but absent in K^{th} genotype

c = Number of bands absent in j^{th} genotype but present in K^{th} genotype

Cluster analysis was performed using the data on similarity coefficients. The method used for tree building in the cluster analysis involved sequential agglomerative hierarchical non-overlapping (SAHN) clustering based on similarity coefficients. The dendrogram based on similarity indices was obtained by un-weighted pair-group method using arithmetic mean (UPGMA). Analysis was performed with the help of NTSYS-pc software [Rohlf, 2000]. The nature of differentiation and divergence amongst drought tolerant, moderately drought tolerant and drought sensitive genotypes under evaluation in the present investigation was assessed by identifying the clusters at appropriate phenon levels.

Result and discussion

Number Of Alleles And Allelic Diversity

Ten drought tolerance associated microsatellite markers generated polymorphic bands in 18 genotypes were evaluated. The largest band size was produced by marker RM18 (161-417 bp) and the smallest by RM136 (99 -111 bp). A total of 82 allelic variants were detected at 10 primer binding sites with an average of 5.4 alleles per locus (Table-3). Similar results were observed in some earlier reports by [Pervaiz et al., 2010; Upadhyay et al., 2011; Rahman et al., 2012] who found an average of 4.4, 4.35 and 4.18 alleles per locus The number of loci ranged from one in the cases of markers RM85, RM136, RM163, RM212 and RM231. In the cases of remaining primer pairs, namely, RM3, RM18, RM72, RM87 and RM225, two loci were detected. The number of alleles per locus ranged from four in the cases of RM85 to thirteen in the cases of RM 225. A total of 15 loci were amplified and found polymorphic and average polymorphism percentage (PP) was 44.40. The PIC values revealing allele diversity and frequency among the entries varied from 0.297 in the case of marker RM87 to 0.902 in the case of RM231 with an average of 0.746.The markers having polymorphism information content (PIC) higher than the average value of 0.746 were RM231, RM225, RM163, RM212, RM72 and RM3. The polymorphism per cent was recorded to be the maximum (63.60) in the case of RM163 and the minimum (22.20) in the case of RM87. The markers RM231, RM72 and RM225 recorded higher polymorphism per cent than the average value of 44.40 (Table- 3). This result is consistent with the reported PIC value in previous works [Sajib et al., 2012]. According to the early reports on the PIC values ranged from a low of 0.24 to a high of 0.92 and averaged 0.61 [Jain et al., 2004], 0.19 to 0.90 with an average of 0.75 [Borba et al., 2009], which is markedly similar or comparable result in our study. [Upadhyay et al., 2011] also reported the average PIC value of 0.78.

Assessment Of Genetic Diversity

A similarity matrix based on the presence or absence of bands in respect of each of the

amplified products was used to establish the level of relatedness between the various rice varieties. The pair-wise estimates of similarity coefficients ranged from 0.00 to 0.46 (Table-4). A close perusal of the data on similarity coefficients clearly indicates that the magnitude of similarity coefficient between RAU-1477-9-7-22-5-7-3 and RAU-1451-66-1-1-5-2 (0.46) was the maximum amongst pair-wise combinations of genotypes under evaluation in the present study. Apparently therefore, the microsatellite based molecular profiles of these two genotypes generated by using 10 SSR primer pairs were most similar amongst the eighteen varieties under consideration in the present study. This was followed by the similarity revealed between the SSR based molecular profiles of RAU-1397-25-8-1-2-5-4 and RAU-1451-35 (0.45), RAU-1428-43 and RAU-1478-52 (0.44), RAU-1428-6 and RAU-1417-2(0.44), RAU-1428.31and RAU-1463 (0.43).

Interestingly, the SSR based molecular profiles did not reveal any similarity between RAU-1417-2 and RAU-1463-16, RAU-1417-2 and RAU-1453-12,RAU-1421-12 and Sahbhagi dhan, RAU-1401-18 and Sahbhagi Dhan, RAU-1416-4 and Sahbhagi Dhan. This inference was derived on the basis of the magnitude of similarity coefficient, which was equal to zero in all these cases. The estimates of similarity coefficients, as revealed by the SSR markers, indicated a considerably greater extent of variation among the rice varieties under evaluation in the present study and provided greater confidence for the assessment of genetic divergence and interrelationship. Similar inference has been derived in the studies conducted on the molecular marker based including SSR marker based divergence analysis in rice by earlier researchers[Pervaiz et al., 2010].

5.2.4 Cluster Analysis Based On Dendogram

The multivariate nature of SSR makers has the unambiguous advantage of discriminating genotypes more precisely. The cluster analysis revealed allelic richness of four clusters (Fig 2) for various sizes at a similarity coefficient level of 0.25. SSR analysis resulted in a more definitive separation of cluster of genotypes indicating a higher level of efficiency of SSR markers for the accurate determination of relationships between accessions that are too close [Ravi et al., 2003]. Grouping based on SSR markers, in general, agreed with the parental pedigree information providing indispensable information regarding the genetic diversity among the genotypes. Varieties and lines sharing the common ancestry were clustered in to the same group, indicating the efficiency of SSR markers in detecting the genetic diversity in rice. To estimate the genetic relatedness among the 18 rice genotypes, similarity analysis was done and dendogram showing the genetic relatedness among 18 rice genotypes was constructed.

Table 1**-** List of 18 rice genotypes studied in both drought and normal conditions

Sl. No.	Genotypes	Sources
1.	RAU-1428-6-7-3-6	RAU,PUSA
2.	RAU-1417-2-1-5-7-7	RAU,PUSA
3.	RAU-1421-12-1-7-4-3	RAU,PUSA
4.	RAU-1415-3-5-7-6-9-5-3	RAU,PUSA
5.	RAU-1401-1-8-1-5	RAU,PUSA
6.	RAU-1428-4-3-2-7-2-6	RAU,PUSA
7.	RAU-1478-5-2-2-4-6	RAU,PUSA
8.	RAU-1477-9-7-22-5-7-3	RAU,PUSA
9.	RAU-1451-6-6-1-1-5-2	RAU,PUSA
10.	RAU-1416-4-2-5-2-2	RAU,PUSA
11.	RAU-1397-2-5-8-1-2-5-4	RAU,PUSA
12.	RAU-1451-3-5-7-6-9-5-1	RAU,PUSA
13.	RAU-1426-4-3-2-5-7-2	RAU,PUSA
14.	RAU-1428-3-1-5-4	RAU,PUSA
15.	RAU-1463-16	RAU,PUSA
16.	RAU-1471-10	RAU,PUSA
17.	RAU-1453-12	RAU,PUSA
18.	Sahbhagi Dhan	IGKV, Raipur

Table 2- Sequences and analysis of microsatellite primer pairs used for the amplification of genomic DNA extracted from eighteen genotype.

SSR Loci	Chr. no.	Primer sequences	Repeat Motif	Annealing temp.
RM 3	6	**(F) ACACTGTAGCGGCCACTG**	**(GA)2GG(GA)25**	
		(R) CCTCCACTGCTCCACATCTT		**56**
RM 18	7	**(F) TTCCCTCTCATGAGCTCCAT**	**(GA)4AA(GA)(AG)16**	
		(R) GAGTGCCTGGCGCTGTAC		**56**
RM 72	8	**(F) CCGGCGATAAAACAATGAG**	**(TAT)5 C(ATT)15**	
		(R) GCATCGGTCCTAACTAAGGG		**55**
RM 85	2	(F) **CCAAAGATGAAACCTGGATTG**	**(TGG)5(TCT)12**	
		(R) GCACAAGGTGAGCAGTCC		**54**
RM 87	5	**(F) CCTCTCCGATACACCGTATG**	**(CTT)3T(CTT)11**	
		(R) GCGAAGGTACGAAAGGAAAG		**55**
RM 136	6	**(F)GAGAGCTCAGCTGCTGCCTCTAGC**	**(AGG)7**	
		(R) GAGGAGCGCCACGGTGTACGCC		**57**
RM 163	5	**(F) ATCCATGTGCGCCTTTATGAGGA**	**(GGAGA)4(GA)11C(GA)20**	
		(R) CGCTACCTCCTTCACTTACTAGT		**58**
RM 212	1	**(F) CCACTTTCAGCTACTACCAG**	**(CT)24**	
		(R) CACCCATTTGTCTCTCATTATG		**55**
RM 225	6	**(F) CCAGATTATTTCCTGAGGTC**	**(CT)16**	
		(R) CACTTGCATAGTTCTGCATTG		**52**
RM 231	1	**(F) TGCCCATATGGTCTGGATG**	**(CT)18**	
		(R) GAAAGTGGATCAGGAAGGC		**55**

Table 3- SSRs analysis used for the amplification of genomic DNA extracted from 18 rice genotypes.

Sl.No.	Primer	No. of locus	Size of alleles (bp)	No. of alleles	No. of unique alleles	No. of shared alleles	PP	PIC
1.	RM 3	2	132.61-156.82	08	4	4	50.00	0.794
2.	RM 18	2	161.36-416.67	09	4	5	44.40	0.658
3.	RM 72	2	265.38-325.00	12	6	6	50.00	0.855
4.	RM 85	1	115.52-124.14	04	1	3	25.00	0.667
5.	RM 87	2	123.08-188.64	09	2	7	22.20	0.297
6.	RM 136	1	99.31-110.71	05	2	3	40.00	0.673
7.	RM 163	1	156.67-190.00	11	7	4	63.60	0.877
8.	RM 212	1	119.57-152.17	09	4	5	44.40	0.859
9.	RM 225	2	104.35-150.00	13	6	7	46.15	0.88
10.	RM 231	1	141.00-181.58	12	7	5	58.33	0.902

PP: Polymorphism per cent; PIC: Polymorphism information content

Table 4- Ten SSR primer pairs based Dice's similarity coefficients among 18 rice entries used in the present study

	RAU 1428-6	**RAU 1417-2**	**RAU 1421-12**	**RAU 1415-35**	**RAU 1401-18**	**RAU 1428-43**	**RAU 1478-52**	**RAU 1477-9**	**RAU 1451-66**	**RAU 1416-4**	**RAU 1397-25**	**RAU 1451-35**	**RAU 1426-43**	**RAU 1428-31**	**RAU 1463-16**	**RAU 1471-10**	**RAU 1453-2**
RAU1417-2	0.54	1.00															
RAU1421-12	0.17	0.26	1.00														
RAU1415-35	0.08	0.17	0.25	1.00													
RAU1401-18	0.08	0.17	0.33	0.41	1.00												
RAU1428-43	0.17	0.08	0.16	0.16	0.33	1.00											
RAU1478-52	0.18	0.09	0.17	0.17	0.34	0.43	1.00										
RAU1477-9	0.09	0.00	0.08	0.26	0.17	0.34	0.27	1.00									
RAU1451-66	0.00	0.00	0.15	0.30	0.15	0.23	0.24	0.40	1.00								
RAU1416-4	0.08	0.00	0.16	0.33	0.08	0.16	0.08	0.34	0.30	1.00							
RAU1397-25	0.00	0.09	0.00	0.17	0.17	0.17	0.09	0.45	0.32	0.17	1.00						
RAU1451-35	0.09	0.00	0.08	0.34	0.17	0.08	0.09	0.54	0.32	0.34	0.45	1.00					
RAU1426-43	0.00	0.00	0.00	0.08	0.17	0.17	0.45	0.36	0.40	0.17	0.36	0.27	1.00				
RAU1428-31	.08	0.25	0.24	0.08	0.24	0.24	0.41	0.00	0.07	0.00	0.00	0.08	0.33	1.00			
RAU1463-16	0.00	0.00	0.07	0.00	0.07	0.07	0.16	0.16	0.21	0.07	0.24	0.32	0.32	0.44	1.00		
RAU1471-10	0.00	0.00	0.09	0.00	0.09	0.18	0.28	0.00	0.08	0.00	0.00	0.00	0.19	0.26	0.08	1.00	
RAU1453-12	0.09	0.00	0.26	0.08	0.08	0.17	0.27	0.09	0.16	0.08	0.00	0.27	0.09	0.25	0.24	0.28	1.00
Sahbhagidhan	0.09	0.09	0.00	0.00	0.00	0.09	0.19	0.00	0.08	0.00	0.00	0.09	0.19	0.26	0.16	0.20	0.28

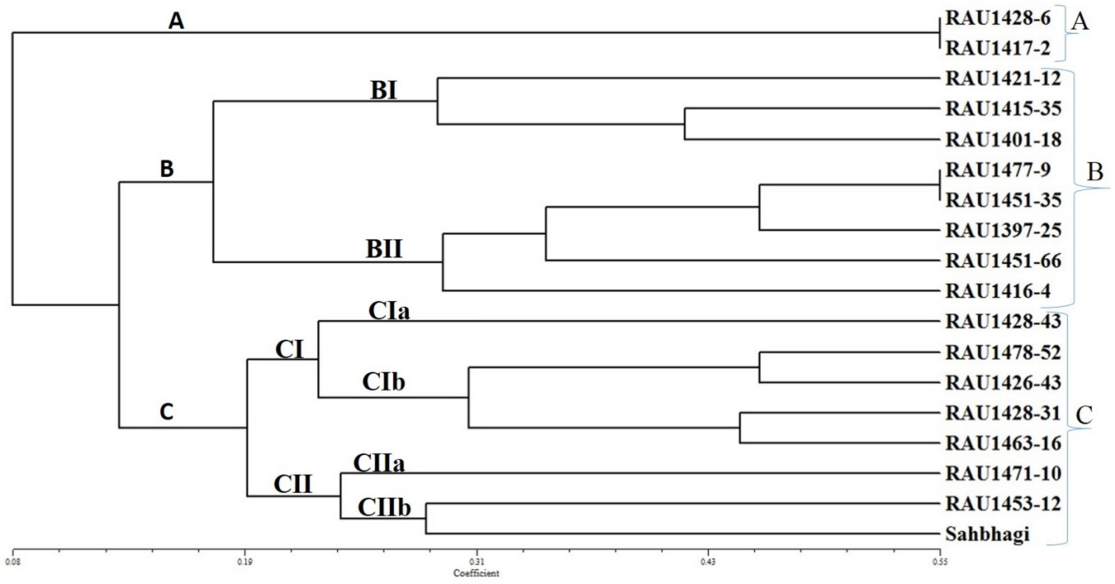

Fig.2. Dice similarity coefficient based Dendrogram among 18 rice genotypes evaluated using ten SSR markers. All genotypes were divided into three major cluster.

References

1. **Wassmann R., Jagadish S.V.K., Sumfleth K., Pathak H., Howell G., Ismail A., Serraj R., Redona E., Singh R.K. and Heuer S.** 2009. Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Advances in Agronomy,* **102**: 91-133.
2. **Bates B.C., Kundzewicz Z.W., Wu S., and Palutikof J.P., Eds.** 2008. Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change, IPCC. 210.
3. **Singh S. and Singh T.N.** 2000. Morphological, chemical and environmental factor affecting leaf rolling in rice during water stress. *Indian J. Plant Physiol*., **5**: 136-141.
4. **Pandey S. and Bhandari H.** 2009. Drought: economic costs and research implications. In: Seeraj R., Bennet J. and Hardy B.(eds.), Drought frontiers in rice: crop improvement for increased rainfed production. World Scientific publishing, Singapore, 3-17.
5. **Litt M. and Lutty J.A**. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucletide repeats within the cardiac muscle actin gene. *Am J. Genet.,* **44**: 397-401.
6. **Vanniarajan C., Vinod K.K. and Pereira A.** 2012. Molecular evaluation of genetic diversity and association studies in rice (*Oryza sativa,* L.). *J. Genet.,* **91**(1): 1-11.
7. **Faridul Islam A.S.M., Ali M.R., Gregorio G.B. and Rafiqul Islam M.** 2012. Genetic diversity analysis of stress tolerant rice (*Oryza sativa,* L.). *Afric. J. Biotechnol.*, **11**(85): 15123- 15129.
8. **Yue B., Xue W., Xiong L., Yu X., Luo l., Cui K., Jin D., Xing Y. and Zhang Q.** 2006. Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genet.,* **172**: 1213-1228.

10. **Anderson J.A., Chhurchill G.A., Autrique J.E., Tanksley S.D. and Sorrells M.E.** 1993. Optimizing parental selection for genetic linkage maps. *Genome*, **36**: 181-186.
11. **Dice L. R.** 1945. Measure of the amount of ecologic association between species. *Ecology*, **26**: 297-302.
12. **Rohlf F.J.** 2000. NTSYSpc: Numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Publications, New York, USA
13. **Cho Y.G., Ishii T., Temnykh S., Chen X., Lipovich L., McCouch S.R., Park W.D., Ayers N. and Cartinhour S.** 2000. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet*., **100**(5): 713-722.
14. **Zeng L., Kwon T.R., Liu X., Wilson C., Grieve C.M. and Gregorio G.B.** 2004. Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa,* L.) genotypes with different adaptations to saline soils. *Plant Sci.*, **166**: 1275–1285.
15. **Prathepha P.** 2012. Genetic diversity and population structure of wild rice, *Oryza rufipogon* from Northeastern Thailand and Laos. Australian Journal of *Crop Sci.*, **6**(4): 717-723.
16. **Pervaiz Z.H., Rabbani M.A., Khaliq I., Pearce S.R. and Malik S.A.** 2010. Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. *Electron. J. Biotechnol.*, **13**(3): 1-12.
17. **Upadhyay P., Singh V.K. and Neeraja C.N.** 2011. Identification of genotype specific alleles and molecular diversity assessment of popular rice (*Oryza sativa* L.) varieties of India. *Int. J. Plant Bred Gent.*, **5**(2): 130-140.
18. **Rahman M.M., Rasaul M.G., Hossain M.A., Iftekharuddaula K.M. and Hasegawa H.** 2012. Molecular Characterization and Genetic Diversity Analysis of Rice (*Oryza sativa* L.) Using SSR Markers. *J. Crop Imp.*, **26**(2): 244-257.
19. **Pervaiz Z.H., Rabbani M.A., Pearce S.R. and Malik S.A.** 2009. Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. *Afric. J. Biotechnol.*, **8**(21): 5641-5651.
20. **Nagy S., Poczai P., Cernak I., Gorji A.M., Hegedus G. and Taller J.** 2012. PICcalc: An Online Program to Calculate Polymorphic Information Content for Molecular Genetic Studies. *Biochem. Genet*., **50**: 670–672.
21. **Sajib A.M., Hossain M.M., Mosnaz A.T.M., Hossain H., Islam M.M., Ali M.S. and Prodhan S.H.** 2012. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landreces of rice (*Oryza sativa,* L.). *J. BioSci. Biotech*., **1(**2): 107-116.
22. **Jain S., Jain R.K. and McCouch S.R.** 2004. Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. *Theor. Appl. Genet.,* **109**(5): 965-977.
23. **Borba T.C.O., Brondani R.P.V., Rangel P.H.N. and Brondani C.** 2009. Microsatellite marker-mediated analysis

of the EMBRAPA rice core collection genetic diversity. *Genetica,* **137**(3): 293-304.

24. **Ravi M., Geethanjali S., Gameeyafarheen F. and Maheswaran M.** 2003. Molecular marker based genetic diversity analysis in rice (Oryza sativa L.) using RAPD and SSR markers. *Euphytica*, **133**(2):243-252.