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Physiological and molecular profiling of rice genotypes under drought stress

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ABSTRACT

Water availability is one of the major limiting factors that seriously influence rice production in the rainfed ecosystems and rice genotypes exhibit differential response to drought stress. With an objective to understand the physiological factors and the genomic loci that influence the tolerance to drought stress in rice, five weeks old seedlings of 38 rice genotypes were subjected to drought stress for 5, 7 and 9 days. Significant variation was observed for traits like shoot length, root length and tiller number plant^{-1} in all the treatments studied and 14 genotypes displayed higher levels of tolerance similar to controls. Among the physiological traits, high relative water content (> 75%) under severe drought stress, was recorded in 10 genotypes while 10 genotypes recorded higher levels of proline accumulation under stress. Eight genotypes and CR-143-2-2 (control) possessed high levels of tolerance to drought stress. In the molecular analysis with thirty microsatellite (SSR) markers linked to different drought tolerance QTLs, twelve markers confirmed the association of the markers with the associated drought tolerance traits in these tolerant genotypes.

Key words: *drought, rice, germplasm, microsatellite markers, relative water content, proline,*

The present challenge to plant breeders is the development of appropriate rice genotypes for the frequently occurring abiotic stresses like drought, salinity and submergence, the key factors that account for low productivity in rice (Wassmann *et al.* 2009). The changes in environmental factors expose the crops to various stresses during their vegetative and reproductive stages, resulting in significant changes in the crop behavior and reduction in grain yield (Guan *et al.* 2010). The native landraces, considered to be the important genetic resources are the base materials for the development of new varieties with incorporated tolerance to various biotic and abiotic stresses (Ram *et al.* 2007; Hanamaratti *et al.* 2008; Huang *et al.* 2010). For the proper utilization of genetic resources, identification of the superior alleles of the genes that govern the various traits is an important step in the crop improvement programmes.

Water deficit, one of the most important abiotic stresses, reduces both the growth and productivity of the crops, especially in arid and semiarid regions worldwide (Passioura 2007). The alterations in the plant architecture and developmental processes have been reported in terms of biochemical, physiological and morphological changes (Hasegawa *et al.* 2000; Parida and Das 2005). Pantuwan *et al.* (2002) described drastic changes in photosynthetic rate, accumulation of osmolytes, reduction of biomass, shoot growth, and yield due to drought stress in rice.

Genetic improvement of drought stress adaptation is one of the most major tasks of the future rice breeding program. Therefore, there is need to identify traits that confer drought tolerance in different rice genotypes that can give us novel insights about the genetic variability existing for abiotic stress tolerance in rice. Despite sustained efforts, the development of crops having drought tolerance using traditional breeding approaches, the advances are limited. Genetic diversity can play a significant role in sustainable development and food security, as it allows the selection of genotypes that can be used in plant breeding programs and utilization of indigenous and landraces with favourable

genes have been employed as donors to incorporate stress tolerance (Septiningsih *et al.* 2003; Thomson et al. 2007). Employing this strategy, drought tolerant crops have been developed in wheat (Fleury *et al.* 2010), rice (Leung 2008), pearl millet (Yadav *et al.* 2011) and maize (Tsonev *et al.* 2009).

Molecular markers are powerful tools in the assessment of genetic variation, in the revelation of genetic relationships within and among genotypes and have demonstrated the potential of plant genetic resources (Virk *et al.* 2000; Song *et al.* 2003 and Teixeira da Silva 2005). Simple Sequence Repeats (SSR) is an important tool for the many applications in the assessment of genetic variation and identification of germplasm (Ma *et al.* 2011), molecular map construction and gene mapping (Zhang *et al.* 2007; Ma *et al.* 2011), mutation studies (Wang *et al.*2009), maker assisted selection (Thomson 2009), construction of fingerprints (Xiao *et al.* 2006; Ma *et al.* 2011), genetic purity test (Ma *et al.* 2011), analysis of germplasm diversity (Zhou *et al.* 2003; Jin *et al.* 2010), association mapping (Jin *et al.* 2010), QTL mapping (Guo *et al.* 2010). and also to unravel the rice domestication events (Sweeney *et al.* 2007). The new biotechnological techniques, bioinformatics and statistical software can analyse effectively the genetic variation at both phenotypic and genotypic levels. Employment of these approaches along with morpho-physiological traits, can reveal differences among the genotypes and thus can provide a more direct, reliable and efficient tool for germplasm utilization. The present study is an attempt to identify rice genotypes having drought tolerance employing both physiological traits and molecular markers.

MATERIAL AND METHODS

Thirty eight rice genotypes which included both landraces and improved varieties of India along with three drought tolerant (CR-143-2-2, Vandana and N22) and two susceptible controls (IR20 and IR64) were evaluated in the study (Table 1).

The pot based experiment was conducted in a completely randomized design with four replications and five week old seedlings were subjected to water stress treatment for 5, 7 and 9 days. At the end of the drought stress treatment, observations were recorded on shoot length, root length and tiller number. Leaf samples were collected after stress and used for the estimation of proline, and relative water content. The SES scoring system (IRRI 1996) was followed to record drought scores.

After cutting the base of lamina, the leaves were sealed in plastic bags and transferred to the laboratory, quickly and fresh weight (FW) was recorded. Then the leaves were soaked in distilled water in test tubes for 4 h at room temperature (25°C) and low light, and turgid weights (TW) were estimated by blotting the leaves with blotting papers and recording the wt. Dry weights (DW) were obtained after oven drying of leaves for 72 h at 70°C. Relative water content (RWC) was calculated as per Bonnet *et al.* (2000).

Proline in the plant leaf tissues were extracted and analyzed as per standard protocol (Roy *et al.* 2009). Leaf samples (0.200gm), collected after drought stress, were grinded with 10 ml of 3% sulfosalicylic acid, the homogenate powder was filtered and 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent were added to 4 ml of filtrate. The mixture was shaken by hand and incubated at 95 °C for 1 h. The reaction was terminated by placing the container in an ice bath. The reaction mixture was mixed vigor-ously with 4 ml toluene and the upper toluene layer was measured at 520 nm using UV spectrophotometer (ELICO SL 159)

For molecular profiling, thirty three SSR markers related to drought tolerance were selected (Chandrababu *et al.* 2003; Akihiko *et al.* 2008; Kanakaraj *et al.* 2010; Li *et al* 2011; Temnykh *et al.* 2011) (Table 2). The markers were linked with several drought tolerance traits like proline content, deep root mass, leaf drying, relative water content, osmotic adjustment, basal root thickness, tiller number, deep root to shoot ratio, panicle length, canopy temperature, biomass and grain yield.

The markers that showed monomorphic banding pattern were excluded through pilot experiments. Genomic DNA was isolated from 30 day old seedlings as per CTAB method (Murray and Thompson 1980). The quantity and quality of DNA was determined by agarose gel (0.8%) electrophoresis with 1ìl of diluted genomic DNA samples and stained with ethidium bromide. After quantification, all the samples were diluted to 30ng $ìl^{-1}$ with 1XTriss EDTA for polymerase chain reaction (PCR).The PCR mix has a

Table 1. The list of the rice genotypes used in the study

Genotype	IC No	Source
CR-143-2-2 (DTC)	-	CRRI
Vandana (DTC)	-	CRRI
N22 (DTC)	IRGC6264	CRRI
IR64 (DSC)	282441	IRRI
IR20 (DSC)	75515	IRRI
BAM0008	124238	CTG
BAM0028	390641	CTG
BAM0046	390622	CTG
BAM0047	390317	CTG
BAM0050	390296	CTG
BAM0061	390755	CTG
BAM0083	390725	CTG
BAM0183	133973	CTG
BAM0234	134141	CTG
BAM0243	125211	CTG
BAM0245	390306	CTG
BAM0249	123847	CTG
BAM0251	124256	CTG
BAM0253	124389	CTG
BAM0256	124667	CTG
BAM0261	214169	CTG
BAM0271	7C123088	CTG
BAM0290	123518	CTG
BAM0295	125623	CTG
BAM0715	NAA	MGL
BAM0731	NAA	MGL
BAM0859	309044	AP
BAM0860	309045	AP
BAM0971	343980	AP
BAM1209	115996	AP
BAM1218	114698	AP
BAM1243	375802	AP
BAM2635	135574	ND
BAM2813	453800	ND
BAM3160	460480	ND
BAM3164	458839	ND
BAM3252	268284	ND
BAM3414	466823	ND
BAM3570	263982	ND
BAM3613	326202	ND
BAM3625	279827	ND
BAM4060	393076	ND
BAM4939	350548	ND

AP- Andhra Pradesh, CRRI-Central Rice Research Institute, CTG-Chhattisgarh, DTC-drought tolerant control, DSC-drought susceptible control, IC-Indigenous collection, IRRI- International Rice Research Institute, MGL- Meghalaya, ND- New Delhi, NAA-Name not available;

total volume of 10ìl containing 30 to 50ng of DNA template, 10pmol ìl^{-1} of each primer, 1.5mM $MgCl_2$, 2.5mM dNTPs, and 1 U of Taq polymerase. The PCR amplification conditions were one cycle at 94°C for 4 min; followed by 35 cycles at 94°C for 30 s, at 55°C for 30 s, and 72°C for 1 min; with a final extension at 72°C for 7 min (PTC-200 Thermo cycler; Bio-Rad, Germany). The PCR products were detected using a 1.5 % agarose gel electrophoresis and observations were recorded with a gel documentation system

The amplified products were scored for each SSR primer pairs based on the presence (1) or absence (0) of bands, generating a binary data matrix of 1 and 0 for each marker system. The data matrices were used to calculate genetic similarity based on Jaccard's similarity coefficients, and the dendrogram displaying relationships among 43 genotypes was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The computer package was NTSYSpc version 2.02(Applied Biostatistics Inc. USA. 1998).

RESULTS AND DISCUSSION

Significant variation was recorded for different morpho-physiological traits of the genotypes examined (Fig.1). In the 5 day treatment,13 genotypes showed high tolerance,14 genotypes are moderate tolerance, in the 7 day treatment, 12 genotypes showed high tolerance, 11 genotypes moderate tolerance while in the 9 day treatment, only 7 genotypes showed highly tolerance and 2 genotypes showed moderate tolerance (Fig. 2). On the overall basis, the 14 genotypes, BAM47, BAM50, BAM61, BAM251, BAM295, BAM731, BAM859, BAM2635, BAM3160, BAM3252, BAM3414, BAM3625, BAM3414, BAM4060 and N22 (tolerant control) displayed high levels of drought tolerance level.

Results indicated that high level of relative water content to the level of 96.93% (BAM245), 87.23% (N22), 80.28% (BAM3252) was recorded while in some genotypes like BAM1243 (43.39%) and BAM1209 (41.18%), lower levels of RWC was recorded (Fig.1). On overall basis, 8 genotypes BAM245, BAM295, BAM859, BAM3252, BAM3625, BAM4060 and N22 and CR-143-2-2 (tolerant controls) maintained highest RWC % (> 75%) in all the stress treatments.

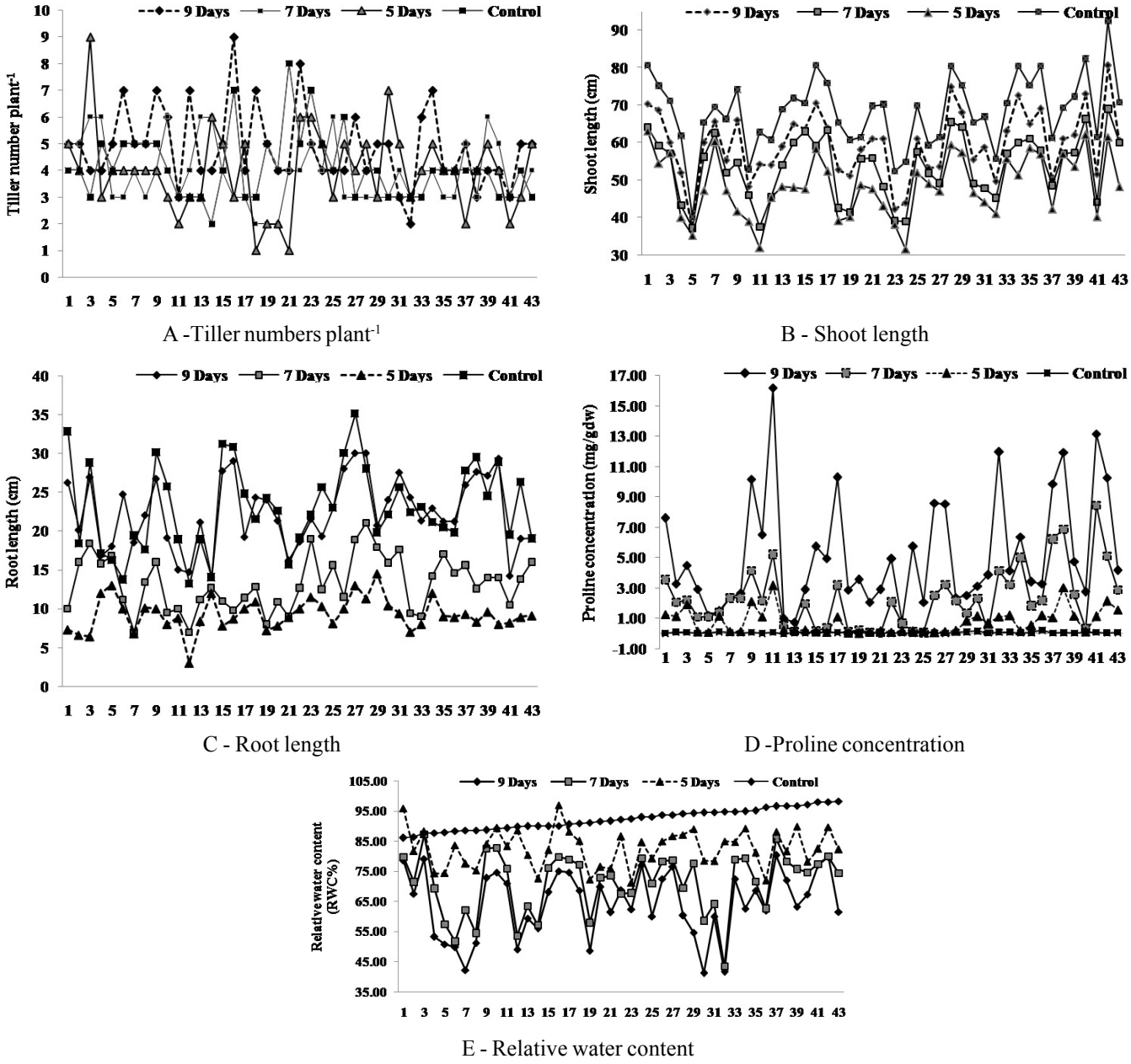

Fig.1. Morpho-physiological response of genotypes to drought stress

The proline content in the stress treatments varied from high levels like 3.15mg gdw^{-1} (BAM61), 8.45mg gdw^{-1} (BAM3625) and 16.19mg gdw^{-1} (BAM61) to lower levels 0.02mg gdw^{-1} (BAM253) and 0.05mg gdw^{-1} (BAM261), (Fig.1). From the study, 9 genotypes (BAM47, BAM61, BAM249, BAM731, BAM859, BAM1243, BAM3414, and BAM3625 and BAM4060) did accumulate high levels of proline levels under drought stress.

Out of the 33 SSR microsatellite markers employed, markers 12 markers showed polymorphism (Fig. 3) and the PIC values varied from 0.129 (RM250) to 0.493(RM545) (Fig 4). A dissimilarity matrix was used to determine the level of relatedness among the rice genotypes. The pair wise genetic dissimilarity of the genotypes (Table 3) indicated that the highest genetic dissimilarity was between the pairs IC343980-IC124389 (85.61%) and IC268284 and IC453800 (85.41%) while

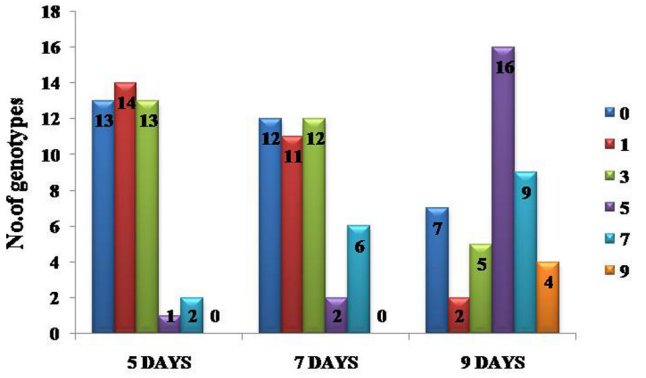

Fig. 2. Frequency distribution of 5, 7 and 9 days of drought stress rice genotypes with different tolerance score (in SES scale 0-9)

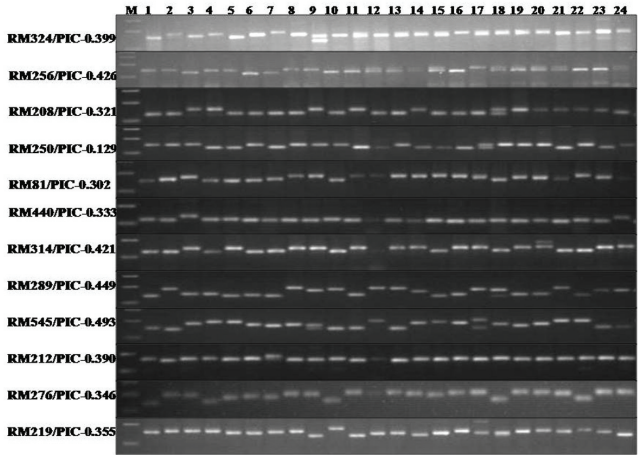

Fig. 3. PCR analysis with polymorphic 12 SSR Markers

the lowest genetic dissimilarity was between IC123518 and Vandana (0.04%) (Table 3).

The dendrogram generated from the SSR markers grouped the 43 rice genotypes into two distinct groups i.e. group-A and group-B. (Fig. 4). Group-A , having 42 genotypes, was further subdivided into nine subgroups with the subgroup AIV was the largest with 10 genotypes while Group B consisted of only one genotype which showed 21% genetic similarity with the other 42 genotypes.

Drought is one of the major abiotic stresses limiting grain yield in rice. The worldwide water shortage and uneven distribution of rainfall leads to serious situations that affect both the growth and development

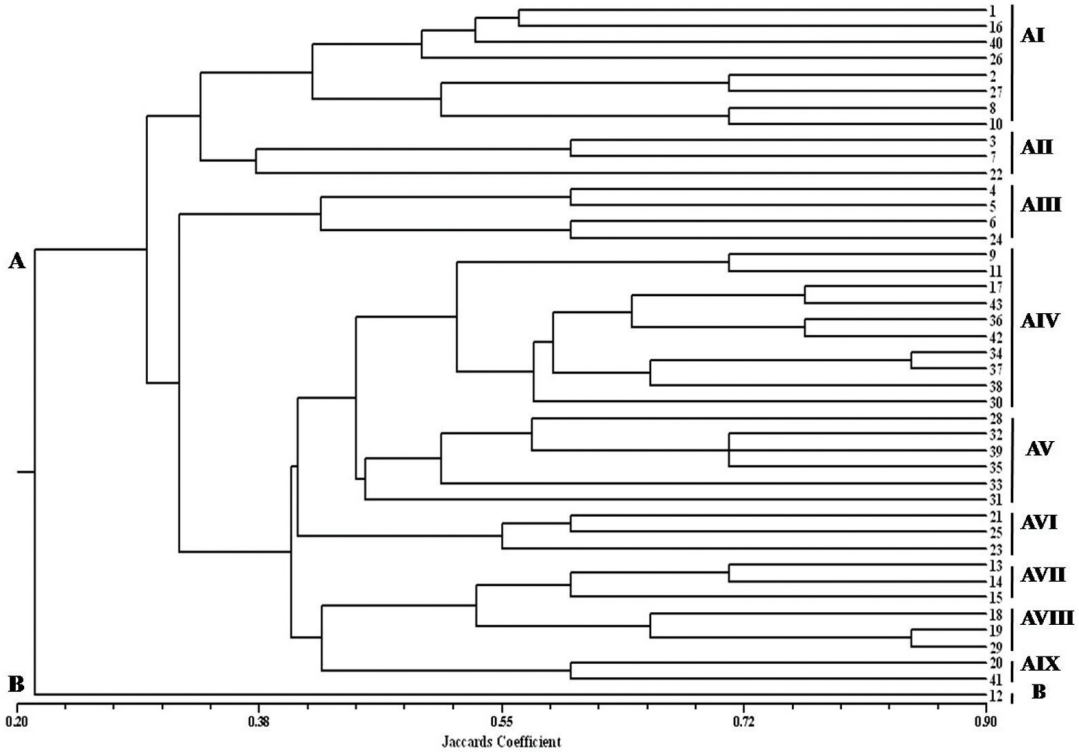

Fig..4. Dendrogram showing the genetic relationships among the genotypes

Table 2. The list of microsatellite markers employed in the study

Marker	Chr	Repeat motif	Primer sequence(Forward)	Primer sequence (Reverse)
RM208	2	(CT)17	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
RM212	1	(CT)24	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
RM81	3	(TCT)10	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC
RM545	3	(GA)30	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG
RM511	12	(GAC)7	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC
RM451	4	(GAT)8	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC
RM324	2	(CAT)21	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
RM317	4	(GC)4(GT)18	CATACTTACCAGTTCACCGCC	CTGGAGAGTGTCAGCTAGTTGA
RM315	1	(AT)4(GT)10	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
RM314	6	(GT)8(CG)3(GT)5	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC
RM289	5	G11(GA)16	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG
RM250	2	(CT)17	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
RM256	8	(CT)21	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
RM102	12	(GGC)7(CG)6	AACTTTCCCACCACCACCGCGG	AGCAGCAGCAAGCCAGCAAGCG
RM227	3	(CT)10	ACCTTTCGTCATAAAGACGAG	GATTGGAGAGAAAAGAAGCC
RM282	3	(GA)15	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG
RM85	3	(TGG)5(TCT)12	CCAAAGATGAAACCTGGATTG	CCAAAGATGAAACCTGGATTG
RM148	3	(TG)12	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG
RM127	4	(AGG)8	GTGGGATAGCTGCGTCGCGTCG	AGGCCAGGGTGTTGGCATGCTG
RM261	4	C9(CT)8	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC
RM125	7	(GCT)8	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
RM551	4	(AG)18	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
RM215	9	(CT)16	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
RM440	5	(CTT)22	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
RM253	6	(GA)25	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
RM219	9	(CT)17	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
RM229	11	(TC)11(CT)5C3(CT)5	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT
RM209	11	(CT)18	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC
RM126	8	(GA)7	CGCGTCCGCGATAAACACAGGG	TCGCACAGGTGAGGCCATGTCG
RM483	8	(AT)26	CTTCCACCATAAAACCGGAG	ACACCGGTGATCTTGTAGCC
RM276	6	(AG)8A3(GA)33	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
RM118	7	(GA)8	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG
RM127	4	(AGG)8	GTGGGATAGCTGCGTCGCGTCG	AGGCCAGGGTGTTGGCATGCTG

of plants (Luo and Zhang 2001). One of the major drawbacks of the rice improvement programs is lack of understanding of the genetic and molecular basis of drought tolerance in rice. The recent development of high-density linkage maps has provided the tools for dissecting the genetic basis underlying the complex traits, such as drought tolerance, into individual components and such efforts have led to the identification of Quantitative trait loci (QTLs) that are related to drought tolerance components like Osmotic adjustment (Zhang *et al.*, 2001; Robin *et al.* 2003), cell membrane stability (Tripathy *et al.* 2000), abscisic acid (ABA) content (Quarrie *et al.* 1997), stomatal regulation (Price *et al.* 1997), leaf water status, and root morphology (Courtois *et al.* 2000; Zheng *et al.* 2000; Zhang *et al.* 2001; Kamoshita *et al.* 2002; Price *et al.* 2002). The markers employed in the study are related to drought QTLs associated with various drought tolerance traits (Akihiko *et al.* 2008; Kanakaraj *et al.* 2010; Li *et al* 2011; Temnykh *et al.* 2011). The molecular markers as RM219, RM212 (McCouch *et al.* 2002, Boopathi, 2004 Bernier *et al.* 2007; Yue *et al.* 2006), RM440 and RM289 (Thomson *et al.*, 2003;Yun *et al.*,2013), RM545,RM81 (Shuxian *et al.*,2013

Table 3. Pairwise genetic distances of 43 rice genotypes obtained from SSR marker analysis.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
CR-143-2-2	1.00																				
Vandana	0.39	1.00																			
N22	0.25	0.26	1.00																		
IR64	0.32	0.20	0.09	1.00																	
IR20	0.47	0.26	0.14	0.60	1.00																
BAM0008	0.32	0.26	0.14	0.33	0.33	1.00															
BAM0028	0.19	0.50	0.60	0.09	0.20	0.26	1.00														
BAM0046	0.47	0.41	0.26	0.33	0.50	0.33	0.33	1.00													
BAM0047	0.25	0.50	0.14	0.33	0.41	0.26	0.33	0.60	1.00												
BAM0050	0.32	0.41	0.41	0.20	0.33	0.33	0.50	0.71	0.41	1.00											
BAM0061	0.25	0.33	0.20	0.20	0.26	0.26	0.33	0.41	0.71	0.41	1.00										
BAM0083	0.09	0.20	0.14	0.14	0.33	0.20	0.20	0.26	0.33	0.26	0.26	1.00									
BAM0183	0.19	0.20	0.33	0.20	0.33	0.20	0.41	0.33	0.50	0.26	0.50	0.33	1.00								
BAM0234	0.19	0.20	0.33	0.33	0.33	0.20	0.26	0.20	0.33	0.14	0.33	0.26	0.71	1.00							
BAM0243	0.19	0.20	0.41	0.26	0.14	0.20	0.33	0.33	0.33	0.26	0.33	0.14	0.60	0.60	1.00						
BAM0245	0.56	0.41	0.33	0.26	0.26	0.41	0.33	0.41	0.26	0.41	0.33	0.09	0.26	0.26	0.26	1.00					
BAM0249	0.25	0.33	0.20	0.41	0.41	0.41	0.33	0.60	0.71	0.41	0.50	0.26	0.50	0.33	0.33	0.33	1.00				
BAM0251	0.19	0.14	0.26	0.33	0.26	0.26	0.26	0.33	0.41	0.26	0.41	0.26	0.71	0.50	0.60	0.33	0.60	1.00			
BAM0253	0.19	0.14	0.41	0.26	0.33	0.33	0.41	0.33	0.41	0.33	0.50	0.26	0.71	0.50	0.41	0.41	0.60	0.71	1.00		
BAM0256	0.25	0.20	0.26	0.20	0.26	0.20	0.33	0.20	0.26	0.14	0.33	0.33	0.50	0.41	0.26	0.33	0.41	0.50	0.50	1.00	
BAM0261	0.32	0.14	0.20	0.33	0.26	0.41	0.26	0.33	0.26	0.26	0.33	0.14	0.41	0.26	0.50	0.26	0.41	0.60	0.41	0.41	1.00
BAM0271	0.47	0.26	0.41	0.09	0.20	0.26	0.33	0.33	0.20	0.33	0.20	0.20	0.33	0.20	0.33	0.33	0.26	0.33	0.26	0.26	0.41
BAM0290	0.25	0.04	0.33	0.41	0.33	0.26	0.20	0.26	0.26	0.26	0.41	0.14	0.33	0.33	0.41	0.14	0.33	0.41	0.41	0.26	0.50
BAM0295	0.39	0.26	0.20	0.41	0.60	0.60	0.26	0.41	0.41	0.41	0.41	0.26	0.33	0.33	0.20	0.33	0.50	0.33	0.41	0.26	0.33
BAM0715	0.32	0.09	0.26	0.26	0.50	0.26	0.26	0.33	0.33	0.26	0.41	0.33	0.60	0.41	0.33	0.14	0.41	0.50	0.50	0.50	0.60
BAM0731	0.44	0.39	0.47	0.19	0.32	0.25	0.47	0.32	0.14	0.47	0.25	0.19	0.19	0.19	0.14	0.56	0.19	0.19	0.32	0.39	0.25
BAM0859	0.39	0.71	0.20	0.33	0.41	0.33	0.41	0.60	0.50	0.60	0.33	0.20	0.26	0.26	0.26	0.41	0.41	0.20	0.20	0.14	0.20
BAM0860	0.25	0.33	0.41	0.09	0.14	0.26	0.50	0.26	0.20	0.26	0.20	0.20	0.26	0.14	0.20	0.26	0.33	0.26	0.33	0.41	0.33
BAM0971	0.14	0.14	0.41	0.20	0.26	0.26	0.41	0.26	0.33	0.33	0.41	0.33	0.60	0.41	0.33	0.33	0.50	0.60	0.85	0.41	0.33
BAM1209	0.32	0.20	0.20	0.50	0.33	0.20	0.14	0.50	0.50	0.33	0.50	0.14	0.33	0.33	0.33	0.33	0.60	0.41	0.41	0.33	0.33
BAM1218	0.47	0.26	0.14	0.33	0.33	0.33	0.20	0.50	0.33	0.41	0.33	0.14	0.20	0.09	0.20	0.33	0.41	0.26	0.26	0.20	0.50
BAM1243	0.25	0.33	0.41	0.20	0.26	0.26	0.50	0.41	0.33	0.41	0.33	0.20	0.41	0.26	0.33	0.26	0.50	0.41	0.50	0.41	0.50
BAM2635	0.25	0.33	0.50	0.26	0.14	0.26	0.33	0.41	0.33	0.41	0.26	0.09	0.33	0.33	0.50	0.33	0.50	0.41	0.41	0.20	0.33
BAM2813	0.47	0.20	0.09	0.50	0.50	0.33	0.14	0.50	0.50	0.33	0.50	0.14	0.33	0.20	0.20	0.33	0.60	0.41	0.41	0.33	0.50
BAM3160	0.25	0.20	0.26	0.33	0.41	0.26	0.33	0.41	0.50	0.26	0.50	0.26	0.60	0.41	0.33	0.26	0.71	0.60	0.71	0.60	0.50
BAM3164	0.19	0.14	0.33	0.33	0.20	0.33	0.26	0.33	0.41	0.33	0.60	0.14	0.41	0.41	0.41	0.33	0.60	0.50	0.60	0.41	0.41
BAM3252	0.39	0.20	0.14	0.41	0.41	0.41	0.20	0.41	0.50	0.26	0.50	0.14	0.41	0.26	0.26	0.26	0.71	0.50	0.50	0.41	0.60
BAM3414	0.32	0.14	0.26	0.33	0.33	0.50	0.26	0.33	0.41	0.33	0.50	0.14	0.50	0.33	0.33	0.41	0.60	0.60	0.71	0.33	0.50
BAM3570	0.39	0.33	0.26	0.26	0.26	0.41	0.33	0.60	0.50	0.41	0.50	0.14	0.41	0.26	0.41	0.41	0.71	0.50	0.50	0.41	0.60
BAM3613	0.56	0.33	0.33	0.33	0.60	0.26	0.26	0.60	0.33	0.41	0.26	0.26	0.41	0.41	0.33	0.50	0.33	0.33	0.41	0.26	0.26
BAM3625	0.25	0.20	0.26	0.20	0.26	0.14	0.26	0.20	0.26	0.14	0.26	0.33	0.41	0.33	0.20	0.26	0.41	0.41	0.50	0.60	0.26
BAM4060	0.26	0.21	0.21	0.28	0.28	0.44	0.28	0.35	0.44	0.35	0.64	0.21	0.44	0.35	0.28	0.44	0.64	0.53	0.64	0.53	0.44
BAM4939	0.20	0.28	0.28	0.28	0.28	0.44	0.44	0.44	0.53	0.44	0.53	0.21	0.44	0.28	0.28	0.44	0.77	0.53	0.64	0.44	0.35

Table 3 contd.

Contd. Table 3

Genotypes	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
CR-143-2-2																						
Vandana																						
IR64																						
IR20																						
BAM0008																						
BAM0028																						
BAM0046																						
BAM0047																						
BAM0050																						
BAM0061																						
BAM0083																						
BAM0183																						
BAM0234																						
BAM0243																						
BAM0245																						
BAM0249																						
BAM0251																						
BAM0253																						
BAM0256																						
BAM0261																						
BAM0271	1.00																					
BAM0290	0.26	1.00																				
BAM0295	0.33	0.50	1.00																			
BAM0715	0.41	0.60	0.50	1.00																		
BAM0731	0.25	0.19	0.25	0.25	1.00																	
BAM0859	0.20	0.09	0.33	0.14	0.39	1.00																
BAM0860	0.26	0.14	0.09	0.26	0.47	0.26	1.00															
BAM0971	0.33	0.33	0.33	0.41	0.32	0.20	0.33	1.00														
BAM1209	0.14	0.41	0.26	0.33	0.25	0.33	0.26	0.33	1.00													
BAM1218	0.41	0.26	0.26	0.33	0.32	0.41	0.33	0.33	0.50	1.00												
BAM1243	0.26	0.26	0.20	0.41	0.47	0.41	0.71	0.50	0.41	0.50	1.00											
BAM2635	0.33	0.26	0.20	0.20	0.25	0.41	0.41	0.41	0.50	0.41	0.60	1.00										
BAM2813	0.26	0.41	0.41	0.50	0.25	0.33	0.26	0.33	0.71	0.71	0.41	0.33	1.00									
BAM3160	0.20	0.41	0.33	0.60	0.32	0.26	0.50	0.60	0.60	0.41	0.71	0.41	0.60	1.00								
BAM3164	0.20	0.60	0.41	0.41	0.25	0.20	0.26	0.50	0.71	0.33	0.41	0.50	0.50	0.60	1.00							
BAM3252	0.33	0.50	0.50	0.60	0.19	0.26	0.33	0.41	0.60	0.60	0.50	0.41	0.85	0.71	0.60	1.00						
BAM3414	0.41	0.50	0.60	0.50	0.19	0.20	0.20	0.60	0.41	0.41	0.33	0.41	0.60	0.50	0.60	0.71	1.00					
BAM3570	0.33	0.33	0.33	0.41	0.32	0.41	0.50	0.41	0.60	0.60	0.71	0.60	0.60	0.71	0.60	0.71	0.50	1.00				
BAM3613	0.26	0.20	0.33	0.33	0.47	0.50	0.26	0.33	0.33	0.33	0.41	0.33	0.33	0.41	0.20	0.26	0.26	0.41	1.00			
BAM3625	0.26	0.20	0.20	0.33	0.32	0.20	0.41	0.60	0.33	0.33	0.50	0.33	0.33	0.60	0.33	0.41	0.33	0.41	0.33	1.00		
BAM4060	0.21	0.44	0.53	0.44	0.33	0.28	0.28	0.53	0.53	0.35	0.44	0.35	0.53	0.64	0.77	0.64	0.64	0.64	0.28	0.44	1.00	
BAM4939	0.28	0.35	0.53	0.35	0.26	0.28	0.28	0.53	0.44	0.28	0.35	0.35	0.44	0.53	0.64	0.53	0.64	0.53	0.21	0.35	0.69	1.00

Jonaliza *et al*., 2004), RM256 (Venuprasad *et al.* 2009), RM208,RM 324,RM250 (Zhou *et al*.,2011; Shalabh Dixit *et al* 2012; Isaac *et al*.,2011) and RM314 and RM276 (Bernier J *et al*.2007). In the present study, which was carried out with both tolerant and susceptible genotypes, the markers displayed polymorphism for some of the QTL related markers confirming the close relationship between the markers and the known QTLs. This can help in use of these markers in the marker assisted breeding prograrn to develop drought tolerant rice genotypes.

The UPGMA cluster analysis showed that all 43 rice genotypes could be easily distinguished based on the information generated by the 12 polymorphic SSR markers. The PIC values revealed that RM 256, RM314, RM289 and RM545 might be the best markers for identification of drought stress tolerance and diversity estimation of rice genotypes. Physiological, morphological, biochemical and molecular genetic diversity analysis in a large germplasm collection will be relevant for the successful implementation of the various breeding approaches for developing drought tolerant varieties in breeding programs.

Of the physiological traits, decreasing of relative water content is indicated that, loss of turgidity, which leads to stomatal closure and reduced photosynthetic rates (Lv *et al*. 2007). The performance of genotypes under water deficit condition, in which a sharp decline in RWC is expected, maintenance of a relatively high RWC during drought stress is an indicative of drought tolerance (Altinkut *et al*. 2001; Colom and Vazzana 2003). Under water-deficit stress conditions, proteins degrade and consequently the proline content increases faster than other amino acids in plants. Thus, proline accumulation can be used as a criterion for drought stress tolerance in plants (Shao *et al*. 2005; Gunes *et al*. 2005). Increasing of free proline concentration caused by water deficit in plants as earlier reported by many authors (Delauney and Verma 1993; Johari- pireivatlou *et al*. 2010). However, Tatar and Gevrek (2008) suggested that proline is mainly involved in protection against oxidative stress that osmotic adjustment in the drought stress condition. It has been also proven that proline has an important role in cellular membranes and stabilizing proteins at intra cellular level of plant cells in the presence of high levels of osmolytes (Farooq *et al*. 2009). In the present study, the genotypes (BAM47, BAM50, BAM61, BAM251, BAM295, BAM731, BAM859, BAM2635, BAM3160, BAM3252, BAM3414, BAM3625, BAM3414, BAM4060) having high levels of both RWC and proline accumulation under stress can be used in the breeding programs. The phenotypic characterization of genotypes under drought stress conditions revealed significant differences among the genotypes and the fourteen genotypes that showed highly tolerance can be employed as donors in the breeding programs.

In summary, it can be concluded that a combination of morphological, physiological and molecular approaches can help the researchers to select genotypes for complex traits like drought tolerance. In this context, as SSR markers can provide adequate power of resolution to discriminate between tolerant and susceptible genotypes, they can serve as a potential tool in both identification and characterization of different genotypes. This allows breeders to track genetic loci controlling drought tolerance traits in rice effectively, without having to measure the phenotype every time, thus reducing the need for extensive field testing over space and time

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