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MOLECULAR AND AGRONOMIC CHARACTERIZATION OF SELECTED RICE GERMPLASM UNDER NORMAL AND WATER STRESS CONDITIONS

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Diversity in uncharacterized rice germplasm grown under normal and water stress conditions was measured by various traits i.e. seed and root shoot traits (seed length, seed width, seed thickness, seed length width ratio, 1000 grain weight, shoot length, root length, shoot fresh weight, root fresh weight, root shoot ratio, root numbers and root thickness). Genotypic differences among the rice accession were determined by variances and association analysis of the desirable traits to set the selection criteria of the genotypes under water stress condition. The trait association along with the entire set of germplasm provided the information of the diversity that responsible for the high yield production of the crop in water stress environment. Some important traits showed positive significant correlation i.e., fresh root weight, shoot thickness and root numbers showed positive significant association (r=0.2530**, r=0.2891**, r=0.4626**, r=0.4515**, r=0.5781**, r=0.7164**, r=0.0603**, r= 0.5570**, r=0.5824**) with shoot length, root length, fresh root shoot weight. The seed thickness has positive correlation (r= 0.104**, r=0.246**) with seed length and seed width. The genotypes with longer roots (KSK-282, KSK-234, PS2) performed well under water stress condition were recommended for further development of drought tolerant germplasm. Twelve polymorphic SSR markers were used to assess molecular diversity of the selected germplasm. The average PIC values (0.464), polymorphism% (93.75), polymorphic alleles (3.58) and total number of alleles (3.83) were determined among the genotypes. The PIC value and number of alleles observed indicated moderate molecular diversity in the selected germplasm. The genotypes with better agro-morphological traits and long roots could be very useful for the development of new breeding populations for adverse environments.

Keywords: Rice, traits, germplasm, correlation, drought, SSR

INTRODUCTION

Rice is a global food grain crop among cereals for its unique characteristics and genetics. In rain fed production areas of rice, a big limiting factor is drought which affects dehydration tolerance, stomatal flow and osmotic adjustment. About 23 million hectares of rainfed rice is affected by drought worldwide (Serraj *et al*., 2011). To develop rice varieties with better drought tolerance is the best strategy to increase rice production in poor irrigation systems (Subbarao *et al*., 2005; Yang *et al*., 2018).

Physical appearance of rice and morphology is determined by grain length and shape and these factors appeal much to the farmers under different environments ultimately contributing the yield potential of the crop. On the other hand, selection and screening of genotypes rely on rice root shoot traits under various environments (Kamoshita *et al*., 2008; Wang *et al*., 2018).

Due to change in environmental conditions i.e. drought, floods and change in patterns of precipitations that affects water availability and food security worldwide (Davis, 2007). Under limited water conditions the breeders prior to the rice germplasm which have higher use of water efficiency. By increasing water scarcity rice production is endangered. In the case of irrigated rice to produce 1Kg of biomass 500L water is needed (Jodo, 1995; Cho and Oki, 2012; Rahman *et al*. 2017).

However, under irrigation ecosystem for irrigation actual water requirement is 3000-5000L to harvest 1Kg of rice (IRRI, 2001; Hoekstra, 2008). The stress response at seedling stage may be more pronounced than at reproductive and flowering stage (Pantuwan *et al*., 2002a; Pantuwan *et al*., 2002b). Yield improvement and direct selection of drought tolerant rice under stress is to facilitate the new breeding strategies for future progress in this field (Chang *et al*., 1982; Hossain and Fischer, 1995; Fischer *et al*., 2003).

Rice genotypes under water stress greatly differ among themselves on the basis of seed and root morphological traits (Ashfaq *et al*., 2012a; Ashfaq *et al*., 2012b). In anaerobic ecosystem low ability to extract water from soil layer and less deep rooting are associated with each other (Lilley and Fukai, 1994). In the present study some rice varieties showed more desirable characteristics under water stress condition and can be utilized for the genetic improvement of rice crop.

Herein, the selected rice germplasm was characterized based on agronomic traits and SSR markers. The genotypes with longer roots (KSK-282, KSK-234, PS2) and performing well under water stress condition were recommended for further development of drought tolerant germplasm. The SSR markers revealed moderate genetic diversity in the selected germplasm.

MATERIALS AND METHODS

***Plant material*:** The study was conducted on 30 rice accessions (Table 1). Various seed and agronomic traits were also studied of these genotypes.

***Seed Morphological traits*:** Various seed morphological traits were measured to study the seed morphology (to determine the seed size and shape), 1000-grain weight and yield potential of the crop. The seed morphology of each genotype was shown in the Fig 2.

The seed length was measured in millimeter separately five seeds of each genotype with digital Vernier Caliper in replicated form. The seed width of each grain was measured and recorded the observation separately.

The trait was measured with Vernier Caliper and recorded the observation in millimeter.

Seed length width ratio can be determined by using the following formula.

$$\text{Seed length width ratio} = \frac{\text{Seed length (mm)}}{\text{Seed width (mm)}}$$

The grain weight of each genotype was measured with electronic weighing balance in grams.

Table 1. Names, accession number and origin of rice genotypes used in the experiment.

No.	Variety name	Origin	Accession No.	Taxon
1.	W1193	Brazil	GSOR 311702	*Oryza rufipogon*
2.	CB-38	Pakistan	4439	
3.	Bas-385	Pakistan	Approved variety	*Oryza sativa*
4.	Bas-515	Pakistan	Approved variety	*Oryza sativa*
5.	Pk-386	Pakistan	Approved variety	*Oryza sativa*
6.	IR-64	Philippines, Luzon	GSOR 311793	*Oryza sativa*
7.	Daggar-303	-	0141	*Oryza sativa*
8.	IR-6	Philippines, IRRI	Approved variety	*Oryza sativa*
9.	Jhona-145	-	0125	*Oryza sativa*
10.	Sathi Bas	Pakistan, Punjab	GSOR 311134	*Oryza sativa*
11.	Bas-370	Pakistan	Approved variety	*Oryza sativa*
12.	Super basmati	Pakistan	Approved variety	*Oryza sativa*
13.	Shaheen Bas	Pakistan	Approved variety	*Oryza sativa*
14.	85HB013R	USA	-	*Oryza sativa*
15.	Basmati-Pak	Pakistan	Approved variety	*Oryza sativa*
16.	Dhankasarwala	Pakistan	0120	*Oryza sativa*
17.	Sonoaitor-145	-	0104	*Oryza sativa*
18.	Bas-198	Pakistan	Approved variety	*Oryza sativa*
19.	XIANGZHAOXIAN NO-15	China	GSOR 311447	*Oryza sativa*
20.	Gulfmont	United States, Texas	PI 502967	*Oryza sativa*
21.	KSK-133	Pakistan	Approved variety	*Oryza sativa*
22.	$R_4$8703196	USA	-	*Oryza sativa*
23.	CB-36	Pakistan	Breeding line	*Oryza sativa*
24.	B53R3540	Thailand	GSOR 310024	*Oryza sativa*
25.	45-5-17	El Salvador	GSOR 310267	*Oryza sativa*
26.	KSK-282	Pakistan	Approved variety	*Oryza sativa*
27.	Bas-2000	Pakistan	Approved variety	*Oryza sativa*
28.	KSK-434	Pakistan	Land race	*Oryza sativa*
29.	PS-2	Pakistan	Approved variety	*Oryza sativa*
30.	45-5-18	El Salvador	GSOR 310268	*Oryza sativa*

On the basis of above traits 10 different rice genotypes i.e. IR-64, Super Basmati, Bas Pak, KSK-434, CB-38, 45-5-18, KSK-282, Daggar-303, PS-2, R$_4$8703196 were screened out for further root shoot traits study under various water levels at seedling stage.

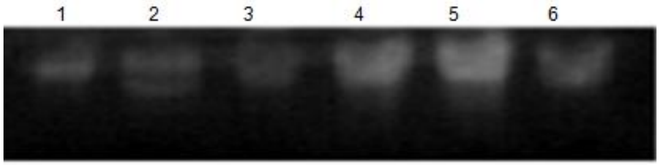

Figure 1. PCR quality DNA band of rice genotypes 1 = CB-38; 2= 45-518; 3= KSK-282:4= Daggar-303; 5= PS-2; 6= IR-64

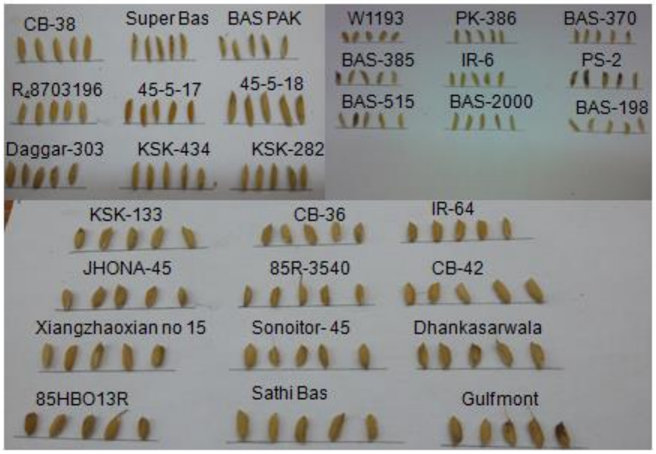

Figure 2. Seed morphological diversity of various indigenous and exotic rice lines

Growth Conditions**:** At different water levels i.e. normal, canal water and water stress condition rice genotypes grown with respect to their analysis on the basis of various traits. The seeds of all the genotypes were sown in the polythene bags. Each polythene bag has length 12 cm and width 8cm. A quantity of 500 gm sand was filled in each bag for growth of rice genotypes. To maintain the moisture content before sowing and germination of rice seeds in the polythene bags were soaked with water. Different root shoots traits were measured to determine the diversity and their performance. The root length was measured in centimeter after uprooting and washing the roots of each seedling plant The shoot length of each seedling plant measured with appropriate scale in centimeter. The root fresh weight of each seedling plant was measured in grams by using electronic balance. The shoot fresh weight was also measured in grams by using the electronic balance. The root shoot ratio was taken by dividing the root fresh weight over shoot fresh weight and measured in grams. It is calculated by following.

$$\text{Root shoot ratio} = \frac{\text{Root fresh weight (gm)}}{\text{Shoot fresh weight (gm)}}$$

Root thickness was also measured with Verniar Callipers of each genotype both under normal and water stress condition. Shoot thickness was also measured with Verniar Callipers of each genotype both under different water condition. Root numbers of each genotype was counted manually.

Genomic DNA Extraction**:** At seedling stage DNA was extracted from fresh leaves of each rice genotype using the CTAB method (Muray and Thompson, 1980). Nano Drop (ND 1000 Spectrophotometer) was used to determine purity, quality and quantity of extracted DNA at 260 and 280 nm. The DNA extractions of some randomly selected genotypes were shown in the Fig 1. The extracted DNA samples (with concentration of 40 ng/ul) were diluted using ddH$_2$O for further PCR analysis.

Statistical Analysis**:** Primers of different polymorphism extent were used for further PCR analysis to determine the band size of each genotype for further screening and selection purpose. The power marker (software package) (Liu and Muse, 2005) was used to determine the molecular characteristics of genotypes. PCR techniques were used for genotyping the rice material (Panuad *et al*., 1996) by using 12 different SSRs primers in-vitro conditions for genetic diversity determination of the among the genotypes/germplasm. The analysis of variance and correlation was carried out through SAS version 9.2.

RESULTS

Variance analysis (two way) designated that the significant ($P<0.01$ and 0.05) effects of all agronomic traits were mentioned in (Table 2 and 4). The traits showed major variation in all the genotypes studied that associated with their

Table 2. Analysis of variance of different seed morphological traits of various rice genotypes and their mean square values.

Source of variation	D.F	SL	SW	ST	L/W	TGW
Genotypes	29	2.890**	0.177**	0.058**	1.492**	72.191**
Replications	2	0.204	0.003	0.005	0.002	16.072
Error	58	0.109	0.003	0.004	0.096	18.449

Level of significance p<0.05=* and p<0.01=**; SL= seed length, SW = seed width, ST = seed thickness, L/W= length/ width ratio and 1000 grain weight

Table 3. Pearson's Correlation among different seed morphological traits of rice.

Traits	SL	SW	ST	L/W	1000GW
SL	1.000				
SW	-0.061*	1.000			
ST	0.104**	0.246**	1.000		
L/W	0.661**	-0.729**	-0.109**	1.000	
1000GW	0.154**	0.118**	0.059*	0.007^{NS}	1.000

Level of significance $p<0.05$=* and $p<0.01$=**

SL= seed length, SW = seed width, ST = seed thickness, L/W= length/ width ratio and 1000 grain weight

Table 4. Factorial analysis of variance of root shoots traits under different water levels at seedling stage.

SOV	D.F	Traits studied under different water levels							
		RL	SL	FSW	FRW	ST	RT	RN	R/S
Genotypes	9	11.35**	138.05**	0.160**	0.18**	0.470**	0.62**	109.35**	3.88**
Replication	2	12.32	19.20	0.001	0.07	0.030	0.04	8.05	1.43
Treatment	2	1.33	8.23	0.010	0.37	0.004	0.06	202.90	0.16
G x T	18	5.57	44.09	0.020	0.20	0.230	0.27	85.02	1.69
Error	40	4.13	14.29	0.009	0.05	0.080	0.05	42.69	1.01
CV		21.54	20.63	44.62	124.43	23.24	30.86	30.02	44.80

morphological diversity. Highly significant variation associated with high genetic diversity in relation their diverse traits studied. A good number of rice lines were selected on SSR and root morphological traits basis i.e. CB-38, PS-2, KSK-282 and KSK-434 that showed greatest genetic diversity Fig. 3 and Fig. 4.

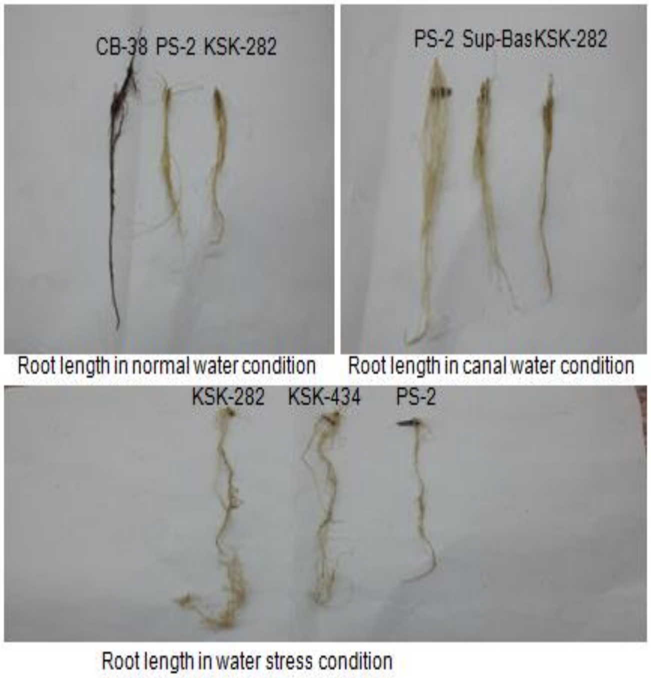

Figure 3. Performance of various rice genotypes under different water conditions

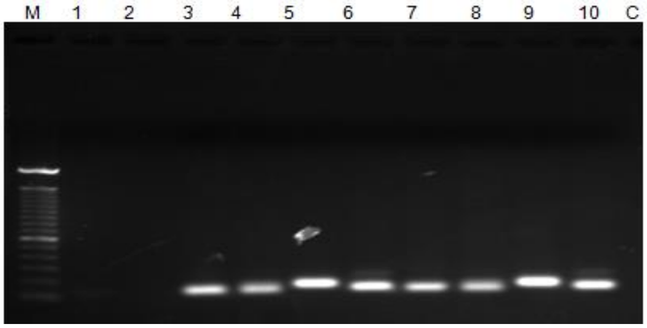

Figure 4. Polymorphic bands for marker RM-208 among l0 rice genotypes M= DNA maker; Lane 1= IR-64; Lane 2= Sup-Bas; Lane 3= Bas-Pak; Lane 4= KSK-434: Lane 5= CB-38, Lane 6= 45-5-18, Lane7= KSK-282; Lane 8= Daggar-303: Lane 9= PS-2; Lane 10=R$_4$8703196; C=negative control.

Pearson correlation analysis showed significant positive, significant negative and non-significant correlation in some seed morphological and the desirable root shoot traits at various water level conditions. Most of the traits showed positive association with each other and considering important morphological tool for the selection of high yielding diverse genotypes. Simple correlation of all these seed morphological and root shoot traits were shown in the (Table 3 and Table 5). Our emphasis was on long root penetration under water stress condition and their association with various significant desirable traits for selection and screening of the genotypes. Some of the traits e.g. root numbers, root thickness, root shoot ratio and fresh root weight positively correlated with root length (r=0.0603**, r=0.5318**, r=0.6374**, r=0.2891**).

Table 5. Association of various root shoot traits under different water conditions.

Traits	Association study of traits under normal water condition							
	SL	**RL**	**FSW**	**FRW**	**ST**	**RT**	**RN**	**R/S**
SL	1.0000							
RL	0.1165^{NS}	1.0000						
FSW	0.5823**	-0.0568^{NS}	1.0000					
FRW	-0.0831^{NS}	0.0257^{NS}	0.0160^{NS}	1.0000				
ST	0.3589**	-0.0367^{NS}	0.8217**	-0.2743**	1.0000			
RT	-0.2570**	0.2861**	-0.1120^{NS}	0.0812^{NS}	0.1028^{NS}	1.0000		
RN	0.6104**	0.0757^{NS}	0.7906**	-0.0300^{NS}	0.6312**	-0.0832^{NS}	1.0000	
R/S	-0.1098^{NS}	0.1750^{NS}	-0.0935^{NS}	0.0007^{NS}	-0.0146^{NS}	0.2790**	-0.0035^{NS}	1.0000
	Association study of traits under canal water condition							
SL	1.0000							
RL	0.0025^{NS}	1.0000						
FSW	0.6651**	-0.0965^{NS}	1.0000					
FRW	0.4415**	0.5167*	0.4049**	1.0000				
ST	0.5393**	0.0005^{NS}	0.4931**	0.3400**	1.0000			
RT	0.3035*	0.2873*	0.2668*	0.4011**	0.4791**	1.0000		
RN	0.7250**	-0.1312^{NS}	0.6978**	0.3203**	0.5617**	0.3108**	1.0000	
R/S	0.0277^{NS}	0.6377**	-0.0877^{NS}	0.5719**	0.2385*	0.5017**	-0.0343^{NS}	1.0000
	Association study of traits under water stress condition							
SL	1.0000							
RL	0.1352^{NS}	1.0000						
FSW	0.6855**	-0.0259^{NS}	1.0000					
FRW	0.2530**	0.2891**	0.1210^{NS}	1.0000				
ST	0.4626**	0.0896^{NS}	0.4515**	0.5781**	1.0000			
RT	0.0002^{NS}	0.5318**	0.0011^{NS}	0.3085**	0.1466^{NS}	1.0000		
RN	0.7164**	0.0603**	0.5570**	0.5824***	0.7031**	0.0396^{NS}	1.0000	
R/S	0.0867^{NS}	0.6374**	-0.0499^{NS}	0.3408**	0.2678**	0.3723**	0.2188*	1.0000

Table 6. Characteristics of the SSR used and their chromosomes location, product size, number of polymorphic alleles, and PIC values calculated for a set of 10 diverse rice genotypes.

SSR marker	Sequence	Chromosomes location	Product Size(bp)	Total no. of alleles	No. of polymer-phic alleles	% polymer-phism	PIC
RM449	F:TTGGGAGGTGTTGATAAGGC R:ACCACCAGCGTCTCTCTCTC	1	140	3	3	100	0.264
RM208	F:TCTGCAAGCCTTGTCTGATG R:TAAGTCGATCATTGTGTGGACC	2	183	4	3	75	0.643
RM282	F:CTGTGTCGAAAGGCTGCAC R:CAGTCCTGTGTTGCAGCAAG	3	140	3	2	75	0.244
RM127	F: GTGGGATAGCTGCGTCGCGTCG R: AGGCCAGGGTGTTGGCATGCTG	4	215	4	3	75	0.615
RM267	F:TGCAGACATAGAGAAGGAAGTG R:AGCAACAGCACAACTTGATG	5	160	4	4	100	0.286
RM204	F:GTGACTGACTTGGTCATAGGG R:GCTAGCCATGCTCTCGTACC	6	179	5	5	100	0.650
RM248	F: TCCTTGTGAAATCTGGTCCC R: GTAGCCTAGCATGGTGCATG	7	112	5	5	100	0.723
RM544	F:TGTGAGCCTGAGCAATAACG R:GAAGCGTGTGATATCGCATG	8	262	4	4	100	0.569
RM215	F:CAAAATGGAGCAGCAAGAGC R:TGAGCACCTCCTTCTCTGTAG	9	163	3	3	100	0.460
RM184	F: ATCCCATTCGCCAAAACCGGCC R:TGACACTTGGAGAGCGGTGTGG	10	220	3	3	100	0.330
RM254	F:AGCCCCGAATAAATCCACCT R:CTGGAGGAGCATTTGGTAGC	11	170	6	6	100	0.590
RM277	F: CGGTCAAATCATCACCTGAC R:CAAGGCTTGCAAGGGAAG	12	130	2	2	100	0.249
	Means			3.83	3.58	93.75	0.469

A set 12 SSR that covered almost all rice chromosomes were used for the study for genetic diversity. The average PIC values (0.464), polymorphism% (93.75), polymorphic alleles (3.58) and total number of alleles (3.83) were determined among the genotypes. The PIC value and number of alleles observed indicated moderate molecular diversity in the selected germplasm.

The rice lines under study showed variation on the basis of their genotypic differences as shown in the Fig 4. On the other hand, the polymorphic traits of diverse germplasms were shown in the Table 6 representing the greatest genetic diversity of the given genotypes.

DISCUSSION

The study was carried out to evaluate and screen out the fine genotypes on the basis of morphological as well as root shoots traits at different water levels for further screening and selection of the genotypes to start up a new breeding research program for the development of new plant material which will be useful different environmental conditions (Ashfaq *et al*., 2014). The maximum seed length was observed in 45-5-18 (12.22 mm) and 7.63 mm minimum in 85 HBO13R, respectively, in the entire set of the germplasm material. On the other hand, Daggar-303 had longest seed width 2.67 mm and super basmati had shortest 1.7mm respectively. More seed thickness was observed in Dhan kasarwala 2.3mm and less in Super basmati 1.6mm while L/W ratio was observed in super basmati 5.97 mm and shortest in *Oryza rufipogan* 2.9 mm. The maximum 1000 grain weight had Basmati Pak 37.61 mm and smallest of B53R3540 18.30 mm in all the entire rice germplasm lines under study (Ashfaq *et al*., 2013). Such types of qualitative and quantitative traits determine the crop growth rate, germination, vigor, quality and yield potential of the crop for its evaluation, selection process and enhancement. Furthermore, it will be very helpful for early selection and breeding processes to the scientific communities for increasing and development of new variable plants.

Grain morphology and other desirable traits had a great impact for the determination of shape, quality and increasing the yield potential of the crop. Further it can be used for the screening and classification of rice lines for future breeding programs. The field experiment was done to study the genetic diversity and screening the drought tolerant varieties of rice. Our results indicated that all the traits showed significant differences among rice genotypes. Some genotypes had long root system as compared to other genotypes of rice. All the genotypes were tested under different environmental conditions at seedling stage. KSK-282 had maximum root length as compared to other rice genotypes under water stress condition; PS-2 showed maximum root length in canal water condition and CB-38 had long root system under normal water condition (Ashfaq *et al*., 2014). Such kinds of information are very useful for breeding programs especially on the aspect of drought resistance in rice. Effect of root length on other traits under normal and water deficient condition was observed by Sanusan *et al*. (2010) and Clark *et al*. (2002).

The significant correlation among different traits showed that most of the traits are interlinked with each other for contributing the yield potential of the crop. But the main purpose of this study was to screen out and the performance of rice genotypes under water stress condition on the basis of various desirable root shoot traits. In stress condition many traits are correlated with each other and they showed significant differences among themselves. Fresh root weight is highly positively correlated with shoot length and root length (r=0.2530; r=0.2891). Similar study of root shoot traits was also reported by Wang *et al*. (2009) and Efisue *et al*. (2009). Root length is highly positively correlated with shoot length, fresh shoot weight, fresh root weight and shoot thickness respectively (r=0.7164; r=0.5570; r=0.5824; r=0.7031). On the other hand, root numbers is highly positively correlated with root length, fresh root weight, shoot thickness, shoot width and root thickness respectively (r=0.6374; r=0.3408, r=0.2678; r=0.3723; r=0.2188) (Ashfaq *et al*., 2017).

The associations between the markers and traits depends upon strength of linkage disequilibrium (LD) between the markers and genetic variation among the germplasm for further molecular mapping studies to locate and identify specific QTL that may be useful and fruitful for controlling the plant traits (Ashfaq *et al*., 2014). Such mapping studies in which we determine the association between marker traits are called Linkage disequilibrium mapping or association mapping that will lead to facilitate gene discovery, measurement of disequilibrium distance between chromosomes, sequence diversity and measurement of genomic regions that linked to a specific phenotypic trait (Mackay and Powell, 2006; Saeed *et al*., 2014). The microsatellite markers are the markers of choice that showed strong linkage disequilibrium between markers and traits for association studies and identification of new QTL. The associations between morphological traits and microsatellites markers were detected by using Power Marker software (Liu and Muse, 2005).

The genotypes which perform better under drought condition could be used in germplasm enhancement and variety development program. All these traits especially root shoot traits will be used for selection, screening, gene identification, genetic diversity and new gene discovery responsible for the yield potential of rice genotypes under drought condition. Furthermore, gene identification, gene modification and genetics of each rice germplasm line under different environmental conditions are still under progress for the determination of gene specificity that responsible for specific traits (Fen *et al*., 2015). Crop plant species have thousands of genes; some genes are still unknown regarding their functions and location. On the other hand, this may be the involvement of different factors for the creation genetic variability. More work is required in this field to understand the complexity of the genes and their proper function for the better utilization and improvement of rice crop (Sandu *at al*., 2017).

***Conclusions*:** The genotypes i.e. CB-38, PS-2, KSK-282, Super Basmati, and KSK-434 outperformed on the basis of longest root penetration in the soil and various morphological traits and will be used for the development of homozygous drought tolerant plant population with high yield potential in the next growing season. The genotype KSK-282 and PS-2 had better genetic potential and performed well under various water conditions. This type of preliminary screening is useful for the desirable traits with desirable genotypes and having adaptation potential for surviving in any wider environmental conditions. This will also help the breeders to design crosses to develop rice lines/cultivars with improved drought tolerance.

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