ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

DNA Fingerprinting and Half Diallel Analysis of Some Rice Genotypes under Water Deficit Conditions

[1]Eldessouky, E. I. Sara, [1]Heiba, S. A. A, [1]A. A. El-Mouhamady*, and [2]Y. M. Abdel-Tawab

[1]Genetics and Cytology Department, Division of Genetic Engineering and Biotechnology, National Research Centre (NRC), Dokki, Giza, Egypt.
[2]Departments of Seed Technology, Field Crops Research Institute, Agricultural Research Centre (ARC), Giza, Egypt.

ABSTRACT

Six promising lines of rice i.e. GZ5890-26-3-3-1, GZ5310-20-2-1, GZ6378-30-1-1-3-1, GZ5721-19-1-1, Giza178 and GZ6532-32-2-1-1 in addition to their crosses (without reciprocals) were evaluated under control and water deficit conditions at Sakha Research Station, Agricultural Research Center during 2014 and 2015. Data showed that eight genotypes were the best for all studied traits under control and water stress conditions. To evaluate the genetic diversity of the six lines, inter simple sequence repeats (ISSR) technique was used. Seven different primers were employed giving a total of 52 reproducible amplification products, 37 of them (71.15%) being polymorphism. Cluster analysis divided the genotypes into two distinct groups. The first group included GZ5890-26-3-3-1 and GZ6378-30-1-1-3-1, while the second group included the rest of the genotypes.

Keywords: Rice, Water stress, Yield components, AMOVA and ISSR Markers.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Rice is one of the most important crops in the world. Water is considered the most important limiting factor for increasing agriculture productivity all over the world. Improving the ability of plant rice to confront and withstand adverse environmental conditions such as water stress is considering the biggest jump in recent years using programs of plant breeding and methods of biotechnology (Liu *et al.*, 2010; Condon *et al.,* 2004 and Harris *et al.*, 2007).

Kumar *et al.,* (2008) increased the tolerance of some rice lines for drought by early maturity and reduced the waste in yield under water stress conditions.

Esselman *et al.,* (1999) revealed a number of PCR-based DNA markers such as RAPD and ISSR that have been widely used to investigate clonal diversity and population genetic structure because they overcome the limitations of allozyme markers.

Tsumura *et al.,*(1996) found that ISSR produce more reliable and reproducible bands compared with RAPD because of the higher annealing temperature and longer sequence of ISSR primers.

Intensive collection and molecular marker based studies on wild rice species have been conducted in most of east, southern and other African countries (Kiambi *et al.,* 2005; Ren *et al.,* 2003). The genetic diversity of rice cultivars in Taiwan is very narrow, as determined by pedigree analysis or molecular marker assay, because of the high selection pressure for good grain quality and repeated use of a few germplasm in breeding programs (LIN *et al.*, 2012).

The main objectives of the present study were evaluating six lines of rice and their crosses without reciprocal under control and drought conditions and to estimate the genetic diversity of the parents using ISSR markers.

MATERIALS AND METHODS

The present investigation was carried out at Sakha research station, Field Crops Research Institute, Agricultural Research Center (ARC) and National Research Centre (NRC), Dokki, Giza during the period from 2014 to 2016.

The plant materials are presented in Table (1). They were kindly provided by The Rice Research and training center. Seeds were grown in three planting dates with ten day intervals to overcome the differences in flowering time among the parents for crossing among them in 2014.

Table (1): The code, name and Characterization for drought tolerance of six rice genotypes.

Code	Name	Characterization
P1	GZ5890-26-3-3-1	Sensitive for drought
P2	GZ5310-20-2-1	Sensitive for drought
P3	GZ6378-30-1-1-3-1	Middle tolerant for drought
P4	GZ5721-19-1-1	Middle tolerant for drought
P5	Giza 178	High tolerant for drought
P6	GZ6532-32-2-1-1	High tolerant for drought

Parents and their crosses without reciprocals (using half diallel cross scheme) were grown under two levels of irrigation with three replicates for each level in a randomized complete block design .The first level was control irrigation conditions of continuous flooding, while the second level was flash irrigation every fifteen days without any standing water and drought treatment was applied two weeks after transplanting till harvesting in season 2015.

The two levels of irrigation were isolated from each other using spatial isolation system to avoid water infiltration from the control irrigation to drought experiment.

Plant height, heading date, Number of filled grains/panicle, 1000-grain weight, Number of panicles/plant, Grain yield/plant, Maximum Root Length and No. of Roots/plant traits were the most important measurements vegetative and physiological calculated for all genotypes under all conditions in 2015.

Data was subject to analysis of variance using plot means. General and specific Combining abilities (GCA & SCA) were calculated according to Griffing (1956) method II, model I (fixed effects). The relative importance (RI) of general and specific combining abilities on progeny performance (i.e., the ratio between additives vs. total genetic variance components) was estimated according to Betran *et al*. (2003). Broad-sense heritability (Hb = VG/VP), narrow-sense heritability (Hn= VA/VP) and High-parent heterosis were estimated according to Falconar & Mackey (1996).

DNA extraction

DNA was extracted from 100 mg of young leaves for each line using Bio basic kits protocol.

ISSR-PCR analysis

Seven primers of ISSR were selected. Their code and sequences are shown in Table (2). PCR reactions were carried out using a Master Cycler Gradient PCR (Eppendorf, Germany) 25µl reaction mixture containing 20ng genomic DNA, 1x PCR buffer, 0.2 mM of each dNTPs, 2.5 mM MgCl2, 0.5 units Taq DNA polymerase (Promega, USA) and 10 pmol of ISSR primer. The thermal cycling condition was as follows: an initial denaturation period of 2 minutes at 94 ºC was followed by 38 cycles of 30 seconds at 94 ˚C, 30 seconds at 42˚C, 1 minute at 72˚C, and then 10 minutes at 72˚C for final extension. The amplification products were separated on a 1.5% agarose gel and 1kb DNA ladder (Promega, USA) was ran simultaneously. The agarose gel was documented by using the UV-gel image acquisition camera (Geliance 200, Perkin Elmer). This program was modified according to Gezahegn *et al.,* (2010).

ISSR data analysis

The DNA bands produced at different loci were determined and named for each DNA sample. Banding profiles generated were converted into a binary data matrices on the basis of present (1) or absent (0) of bands. Data scoring is based on several criteria: (1) locus is assumed as independent or non-allelic, (2) there is no bias in scoring monomorphic fragments versus polymorphic fragments and (3) the similarity of fragment size is assumed to be the indicator of homology. Genetic data analysis was performed by using POPGENE version 1.32 software by assuming Hardy-Weinberg, gel analyzers3 protocol and SPSS analysis.

Table (2): Code and sequences of seven ISSR primers.

NO	Code name	Sequences
1	HB-14	5' CTC CTCCTC GC 3'
2	B-17898	5' CAC ACA CAC ACA GT 3'
3	B-17899	5' CAC ACA CAC ACA GG 3'
4	HB -12	5' CAC CACCAC GC 3'
5	A-814	5' CTC TCT CTC TCT CTC TG 3'
6	A-17898	5' CAC ACA CAC ACA AC 3'
7	B-844	5' CTC TCT CTC TCT CTC TGC 3'

RESULTS AND DISCUSSION

Mean Performance

The mean performance data of the parents and their crosses (without reciprocals) is presented in Table (3). The results show that P5 and P6 in addition to the crosses (P1 X P5, P1 X P6, P4 X P5, P4 X P6 and P5 X P6) had the lowest values for heading date under control and stress conditions. The values ranged from 83.6 to 91.4, and they ranged from 73.21 to 83.05 under control and stress conditions, respectively. So, these genotypes are considered early maturity as compared with the rest of the genotypes. Regarding to the plant height trait, the results show that the parent (P5) and the cross (P4 x P5) had the lowest values (93.09 and

89.6, respectively) under the control conditions, while the parent (P6) and the cross (P1 x P5) had the lowest values (65 and 61.33, respectively) under the stress conditions. Regarding to the rest of the traits, the results in Table (3) show that the parents p5 and p6 and the crosses (P1 x P5, P1 x P6, P4 x P5) were the best for the control and drought stress conditions. From these results, we can conclude that the genotypes (P5, P6, P1 x P5, P1 x P6, P4 x P5, P4 x P6, and P5 x P6) were the best ones under both conditions in all studied traits for drought tolerance. These results are in agreement with Ray et al., (2015).

It is noteworthy to indicate that the mean performances of some genotypes favored their respectively GCA effects. Such cases included the parents; (P3, P5 and P6) for all studied traits under control and drought conditions. These findings indicate that the intrinsic performance of these genotypes gave a good index of their general combining ability. Therefore, selection for improving such traits could be practiced either on the basis of mean performance or GCA effects. So, it can be summarized the most important results in the fact that these genotypes have shown advanced and moral results in the light of its assessment for the qualities of the physiological, morphological, yield and yield components traits under water stress conditions.

Variation and Interaction

Mean squares of half diallel analysis for all studied traits are detected in Table (4).The results revealed that mean squares of all genotypes were highly significant for all traits under control and water stress conditions. The data showed that the estimates of general and specific combining ability effects (GCA & SCA) were highly significant for all traits, which point to the importance of both additive and non-additive genetic variances. The GCA/SCA ratio was less than one for all traits. This reveals that non-additive type of gene action is more importance in the inheritance of these traits under all conditions. Therefore, the selection will be effective using bulk method not pedigree method. These findings were in agreement with those reported by Sathya and Jebaraj (2015).

Estimates of variance components

The closer the relative importance (RI) ratio is to one the greater the chances of predicting progeny performance based on GCA (Zhang and Kang 1997). It is evident from data presented in Table (5) that all the studied traits were influenced by environmental variation, and that non-additive genetic variance (which is reflected in low RI values) is a large portion of total genetic variance. These results indicate that these traits are greatly influenced by environmental conditions. The results showed that high heritability (Hb) estimates (over 97 %) were observed for all the studied traits. This indicated that the environment had large effects on these traits and selection of these traits would be more effective as compared to others. Nuruzzaman *et al*., (2002), Pradhan *et al.,* (2006) and Saleem *et al.,* (2010) reported similar results.

Heterosis over better-parent

The estimates of heterosis over better-parents for all crosses under control and water stress conditions are presents in Table (6). The results revealed that the cross (P1 X P2) under drought conditions and the crosses (P1 X P5, P1X P6, P4 X P5 and P4 X P6) under control and water stress conditions were significant and negatively highly significant for heading date trait of heterosis over better-parent. The cross (P1 X P4) under all conditions, the crosses (P1 X P6, P2 X P4, P4 X P5 and P4 X P6) under control conditions, and the crosses (P1 X P5 and P2 X P4) under drought conditions were negatively significant and highly significant for plant height trait over better-parent, respectively.

On the other hand, the crosses (P1 X P4 and P4 X P6) under drought conditions, the crosses (P2 X P3 and P5 X P6) under control conditions, and the crosses (P1 X p5, P1 X P6, P2X P4 and P3 X P4) under all conditions were positively significant and highly significant of heterosis over better-parent for number of filled grains/per panicle trait, respectively.

For 1000-grain weight trait, the crosses (P3 X P5, P4 X P6 P1 X P6, P4 X P5 and P5 X P6) under control and drought conditions were positively significant and highly significant of heterosis over better-parent, respectively.

ISSN: 0975-8585

Table (3):- Mean performances of the 6 parents and their crosses for the traits under control and drought stress conditions.

Genotypes	H.D		P.H		No. of F.G/P		1000-G.W		No. of .P/P		G.Y/P		M.R.L		No. of R/P	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
P1	122.12	127.66	123.8	117.4	130.6	98.5	21.06	12.8	21.3	12.5	31.4	20.8	23.6	18.77	210.4	186.8
P2	120.45	124.33	132.0	112.54	143.7	120.2	18.4	10.60	20.5	14.0	28.5	17.6	30.5	22.4	214.6	155.45
P3	101.03	97.80	120.0	110.8	150.0	110.56	26.3	18.4	22.4	16.0	36.0	25.7	27.9	22.6	277.8	250.4
P4	110.0	94.45	136.4	114.19	167.7	100.0	24.2	16.7	19.6	10.5	35.0	26.8	32.3	19.9	300.04	288.9
P5	91.40	81.60	93.09	77.33	288.4	240.0	28.01	26.8	32.3	28.0	40.3	33.7	40.8	33.2	370.8	355.4
P6	90.18	83.05	104.0	65.0	255.4	214.2	30.0	25.45	31.4	27.7	44.7	38.4	43.5	38.4	355.4	320.04
P1 x P2	126.52	114.63	128.6	120.0	133.65	96.4	17.45	12.11	17.4	11.05	25.4	19.5	21.4	15.7	199.45	166.34
P1 x P3	132.24	121.60	136.0	127.23	110.7	88.9	20.0	10.3	20.0	13.7	30.0	22.4	25.3	12.4	233.0	210.4
P1 x P4	124.80	117.87	105.5	100.0	125.18	112.0	20.6	14.8	23.6	14.64	28.4	20.10	33.8	21.7	199.34	120.32
P1 x P5	85.12	77.45	91.12	61.33	320.4	280.2	37.0	32.12	36.5	31.8	51.2	43.7	54.3	44.5	460.0	452.3
P1 x P6	87.33	75.0	92.67	72.64	288.2	260.34	38.7	28.13	40.0	33.4	53.6	47.3	60.4	53.2	444.7	426.10
P2 x P3	130.45	128.18	138.0	127.7	198.6	87.4	16.5	9.80	24.6	11.7	33.4	18.6	31.5	23.4	299.4	210.23
P2 x P4	125.60	115.29	120.4	100.0	210.0	167.13	19.3	13.4	25.4	18.9	26.5	15.6	35.6	12.7	305.3	112.66
P2 x P5	132.67	126.55	116.9	112.4	176.4	66.18	22.13	13.43	22.0	15.6	22.3	12.11	27.3	10.5	233.12	210.17
P2 x P6	118.30	112.00	125.6	119.3	141.6	56.34	26.34	19.5	24.3	13.61	25.9	21.4	26.4	15.3	236.5	220.87
P3 x p4	119.37	124.41	124.4	115.3	194.3	112.3	21.0	14.3	18.3	10.3	34.0	19.6	36.5	30.0	333.7	167.5
P3 x p5	128.87	131.45	113.6	104.7	200.0	123.6	23.2	18.4	26.4	13.0	31.45	11.5	22.60	11.7	323.8	288.4
P3 x p6	121.00	118.11	129.4	120.5	166.04	100.7	24.7	17.3	20.8	15.0	30.5	14.5	21.8	12.8	309.45	256.7
P4 x p5	83.60	73.21	89.60	80.32	267.3	210.5	32.5	28.6	40.8	32.6	60.0	51.0	61.4	50.45	430.4	415.3
P4 x p6	86.23	80.55	95.0	81.0	244.5	220.3	39.5	31.4	37.23	33.07	56.7	42.3	59.6	48.7	500.14	488.5
P5 x p6	85.0	80.04	100.0	82.03	310.3	230.0	36.7	29.5	35.04	29.7	58.4	47.6	72.4	50.3	477.8	400.3
LSD 0.05	1.29	1.69	2.96	2.07	1.38	1.68	2.21	4.94	1.71	2.22	1.55	1.95	2.12	3.09	1.26	2.07
LSD 0.01	1.73	2.26	3.96	2.77	1.84	2.25	2.96	6.61	2.29	2.98	2.07	2.61	2.84	4.14	1.69	2.77

P1: GZ5890-26-3-3-1, P2: GZ5310-20-2-1, P3: GZ6378-30-1-1-3-1, P4: GZ5721-19-1-1, P5: Giza 178, P6: GZ6532-32-2-1-1, C: control, T: Treatment, H.D: heading date, P.H: plant height , No .of F.G/P: Number of filled grains/panicle , 1000-G.W: 1000.grain weight, No. of P/P: Number of panicles per plant , G.Y/P: grain yield / plant, M.R.L : Maximum root length , No. of R/P : Number of roots/plant.

ISSN: 0975-8585

Table (4):- Mean Squares of 6- parent diallel crosses in F1 for the studied traits under control and drought conditions.

S.O.V	df	H.D		P.H		NO. of F.G/P		1000-G.W		NO .o f P /P		G.Y / P		M.R.L		No .of R / P	
		C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Replication	2	2.56	3.12	6.78	24.02	5.42	7.44	1.47	3.55	3.78	13.67	55.78	38.55	1.59	23.45	46.05	67.43
Genotypes	20	234.34**	674.09**	509.34**	234.78**	778.99**	816.23**	634.12**	787.56**	126.22**	150.0**	234.54**	567.0**	189.06**	745.9**	433.6**	504.67**
GCA	5	215.67**	279.09**	219.88**	188.33**	388.21**	276.98**	132.25**	251.40**	54.67**	56.0**	341.90**	222.44**	222.88**	320.11**	978.88**	250.12**
SCA	15	307.8**	333.55**	263.65**	378.45**	423.0**	388.63**	266.91**	455.13**	64.79**	88.56**	590.63**	390.34**	230.34**	343.45**	1023.0**	343.18**
Error	40	1.36	1.78	3.11	2.18	1.45	1.77	2.33	5.19	1.80	2.34	1.63	2.05	2.23	3.25	1.33	2.18
GCA/SCA		0.70	0.83	0.84	0.49	0.91	0.71	0.50	0.55	0.84	0.63	0.58	0.57	0.96	0.93	0.95	0.73

Table (5): The relative importance (RI) of additive Vs total genetic variance and the heritability estimates of 6- parent diallel crosses in F1 for the studied traits under control and drought conditions.

Variance Components	H.D		P.H		NO. of F.G/P		1000-G.W		NO .o f P /P		G.Y / P		M.R.L		No .of R / P	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
RI	0.26	0.29	0.29	0.20	0.31	0.26	0.20	0.21	0.30	0.24	0.22	0.22	0.33	0.32	0.32	0.27
Hn	25.82	29.36	29.13	19.74	31.37	26.15	19.57	21.29	28.98	23.25	22.36	22.02	32.39	31.57	32.33	26.54
Hb	99.67	99.62	99.16	99.54	99.76	99.66	99.30	99.10	98.03	97.97	99.79	99.59	99.35	99.35	99.91	99.53

RI = $2\ \delta^2_{GCA}/ (2\ \delta^2_{GCA} + \delta^2_{SCA})$, **Hn** = $2\ \delta^2_{GCA}/ (2\ \delta^2_{GCA} + \delta^2_{SCA}+ \delta^2_{e})*100$ and Hb = $((2\ \delta^2_{GCA}+ \delta^2_{SCA})/ (2\ \delta^2_{GCA} + \delta^2_{SCA}+ \delta^2_{e}))*100$.

ISSN: 0975-8585

Table (6):- Estimates of heterosis over better-parents of 6- parent diallel crosses in F1 for all studied traits under control and drought conditions.

Crosses	H.D		P.H		No. of F. G / P		1000-G.W	
	C	T	C	T	C	T	C	T
P1 x p2	5.03**	-7.80**	3.87**	6.62**	-6.99**	-19.80**	-17.14**	-5.35
P1 x p3	30.89**	24.33**	13.33**	14.82**	-26.20**	-19.59**	-23.95**	-44.02**
P1 x p4	13.45**	24.79**	-14.78**	-12.42**	-25.35**	12.0**	-14.87**	-11.37
P1 x p5	-6.87**	-5.08**	-2.11	-20.69**	11.09**	16.75**	32.09**	19.85*
P1 x p6	-3.16**	-9.69**	-10.89**	11.75**	12.84**	21.54**	29.0**	10.53
P2 x p3	29.12**	31.06**	15.0**	15.25**	32.4**	-27.28**	-37.26**	-46.73**
P2 x p4	14.18**	22.06**	-8.78**	-11.14**	25.22**	39.04**	-20.24**	-19.76
P2 x p5	45.15**	55.08**	25.57**	45.35**	-38.83**	-72.42**	-20.99**	-49.88**
P2 x p6	31.18**	34.85**	20.76**	83.53**	-44.55**	-73.69**	-12.20**	-23.37*
P3 x p4	18.15**	31.72**	3.66**	4.06**	15.86**	1.57**	-20.15**	-22.28
P3 x p5	40.99**	61.09**	22.03**	35.39**	-30.65**	-48.5**	-17.17**	-31.34**
P3 x p6	34.17**	42.21**	24.42**	85.38**	-34.98**	-52.98**	-17.66**	-32.02**
P4 x p5	-8.53**	-10.28**	-3.74*	3.86**	-7.31**	-12.29**	16.02**	6.71
P4 x p6	-4.38**	-3.01**	-8.65**	24.61**	-4.26**	2.84**	31.66**	23.37*
P5 x p6	-5.74**	-1.91	7.42**	26.20**	7.59**	-4.16**	22.33**	10.07
LSD0.05	1.29	1.69	2.96	2.07	1.38	1.68	2.21	4.94
LSD0.01	1.73	2.26	3.96	2.77	1.84	2.25	2.96	6.61
Crosses	**No. of P / P**		**G.Y / P**		**M.R.L**		**No. of R/ P**	
	C	T	C	T	C	T	C	T
P1 x p2	-18.3**	-21.07*	-19.11**	-6.25	-29.83**	-29.91**	-7.05**	-10.95**
P1 x p3	-10.71**	-14.37*	-4.45**	-12.84**	-9.31*	-45.13**	-16.12**	-15.97**
P1 x p4	10.79**	17.12	-18.85**	-25.0**	4.64	9.04	-33.56**	-58.35**
P1 x p5	13.0**	13.57**	27.04**	29.67**	33.08**	34.03**	24.05**	27.26**
P1 x p6	27.38**	19.28**	19.91**	23.17**	38.85**	38.54**	25.12**	33.13**
P2 x p3	9.82*	-26.87**	-7.22**	-27.62**	3.27	-43.80	7.77**	-16.04**
P2 x p4	23.9**	35.0**	-24.28**	-41.79**	10.21**	-43.30**	1.75**	-61.0**
P2 x p5	-31.88**	-44.28**	-44.66**	-64.06**	-33.08**	-68.37**	-37.13**	-40.86**
P2 x p6	-22.61**	-50.86**	-42.05**	-44.27**	-39.31**	-60.15**	-33.45**	-30.98**
P3 x p4	-18.30**	-35.62**	-5.55*	-26.86**	13.00**	32.74**	11.21**	-42.02**
P3 x p5	-18.26**	-53.57**	-21.96**	-65.87**	-44.60**	-64.75**	-12.67**	-18.85**
P3 x p6	-33.75**	-45.84**	-31.76**	-62.23**	-49.88**	-66.66**	-12.92**	-19.79**
P4 x p5	26.31**	16.42**	48.88**	51.33**	50.49**	51.95**	16.07**	16.85**
P4 x p6	18.56**	19.38**	26.84**	10.15**	37.01**	26.82**	40.72**	52.63**
P5 x p6	8.48**	6.07	30.64**	23.95**	66.43**	30.98**	28.85**	12.63**
LSD0.05	1.71	2.22	1.55	1.95	2.12	3.09	1.26	2.07
LSD0.01	2.29	2.98	2.07	2.61	2.84	4.14	1.69	2.77

Regarding to the number of panicles/plant, the crosses P1 X P4, P2 X P3 and P5 X P6 under control conditions and the crosses P1 X P5, P1 X P6, P2 X P4, P4 X P5 and P4 X P6 under all conditions were positively significant and highly significant of heterosis over better-parent, respectively.

With respect to grain yield/plant, maximum root length and no. of roots/plant traits, the crosses P1 X P5, P1 X P6, P4 X P5, P4 X P6 and P5 X P6 under control and water stress conditions were positively significant and highly significant of heterosis over better-parent. With respect to maximum Root Length trait, the cross P2 X P4 under control conditions and the cross P3 X P4 under both conditions were positively significant and highly significant of heterosis over better-parent, respectively. Regarding to the number of roots/plant trait, the crosses P2 X P3, P2 X P4 and P3 X P4 under control conditions were positively significant and highly significant of heterosis over better-parent.

From the previous data, we can conclude that the crosses P1 X P5, P1 X P6, P4 X P5, P4 X P6 and P5 X P6 under both conditions were the best genotypes and we can use them in the rice improving program. These results were in agreement with those reported by Sathya and Jebaraj (2015).

General and specific combining ability effects

The estimates of general combining ability (GCA) are presented in Table (7). The results revealed that the parents P3, P5 and P6 had negative and high significant values of general combining ability effects for heading date and plant height traits under control and water stress conditions, while the same parents showed positive and high significant values of general combining ability effects under both conditions for the other traits. This means the importance of additive and additive X additive types of gene actions in the inheritance of these traits for drought tolerance. Similar results were obtained by Sathya and Jebaraj (2015).

The estimates of specific combining ability (SCA) are presented in Table (8). The data showed that the crosses P1 X P5, P1X P6, P4 X P5, P4 X P6, and P5 X P6 had negative significant and high significant values of SCA effects for heading date and plant height traits under control and drought conditions, while the same crosses had positive significant and high significant values of SCA effects for the rest of the traits under both conditions. This indicate that the dominance, dominance X dominance and additive X dominance of gene interaction played a large role in the inheritance of these traits and selection will be effective with bulk method for drought tolerance. These results were in agreement with those reported by Raju *et al.,* (2014), Hasan *et al.,* (2015) and Sathya and Jebaraj (2015).

Generally, the parents P3, P5 and P6 are considered good combiners because they had high GCA effects under control and drought conditions. The crosses P1 X P5, P1X P6, P4 X P5, P4 X P6, and P5 X P6 are considered the best crosses because they had high SCA effects under control and drought conditions. So, these genotypes could be used in breeding programs for improving rice.

ISSN: 0975-8585

Table (7):- Estimates of General Combining Ability Effects of 6- parent diallel crosses in F1 for all studied traits under control and drought conditions.

Parents	H.D		P.H		No. of F.G/P		1000-G.W		No. of P / P		G.Y/P		M.R.L		No. of R/P	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
P1	11.34**	4.18**	10.23**	9.57**	-15.6**	-5.33**	-14.67**	-7.80**	-9.14**	-4.12**	-16.77**	-8.45**	-6.18**	-11.29**	-19.88**	-2.99**
P2	0.43	3.77**	8.45**	10.0**	-11.72**	-7.38**	-12.87**	-14.75**	-5.34**	-13.89**	-9.37**	-5.13**	-3.35**	-8.34**	-11.79**	-18.78**
P3	-8.34**	-5.13**	-17.78**	-3.27**	13.45**	4.33**	23.8**	19.01**	14.8**	7.11**	25.76**	2.55**	19.60**	26.65**	34.56**	17.33**
P4	7.707**	14.06**	12.97**	6.45**	-10.8**	-10.78**	-10.68**	-12.93**	-13.93**	-8.23**	-20.38**	-7.74**	-26.85**	-30.87**	-29.78**	-25.63**
P5	-4.67**	-10.11**	-4.76**	-7.21**	20.67**	8.46**	11.88**	5.67**	7.74**	3.34**	13.63**	4.87**	11.36**	14.38**	12.62**	8.38**
P6	-6.08**	-6.77**	-9.11**	-15.54**	4.0**	10.7**	2.54**	10.8**	5.87**	15.79**	7.13**	13.9**	5.42**	9.47**	14.27**	21.69**
LSD 0.05	0.78	0.94	1.55	1.56	1.35	1.38	1.12	1.28	1.44	1.24	2.55	1.55	1.68	1.35	1.39	2.14
LSD 0.01	1.36	1.44	2.23	2.65	1.77	1.86	1.49	1.72	2.24	1.98	3.78	2.49	2.67	2.18	1.52	2.67

Table (8):- Estimates of specific Combining Ability Effects of 6- parent diallel crosses in F1for all studied traits under control and drought conditions.

Crosses	H.D		P.H		No. of F.G/P		1000-G.W		No. of P/P		G.Y/P		M.R.L		No. R /P	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
P1 x p2	4.5**	4.6**	5.1**	6.85**	-10.7**	-8.34**	-11.8**	-11.76**	-7.11**	-20.18**	-0.33	-14.8**	-5.16**	-18.4**	-20.0**	-17.98**
P1 x p3	7.8**	10.0**	6.8**	15.87**	-8.45**	-13.7**	-5.99**	-24.7**	-5.78**	-15.16**	-44.5**	-22.14**	-4.35*	-0.55	-9.55**	-23.07**
P1 x p4	5.0**	3.17**	10.0**	26.88**	-7.44**	-12.5**	-3.18*	-3.41**	-10.2**	-13.27**	-0.78	-9.74**	-8.87**	-0.34	-8.45**	-10.55**
P1 x p5	-6.4**	-12.0**	-5.7**	-14.8**	34.8**	26.8**	30.5**	24.7**	15.3**	32.08**	55.44**	14.66**	23.88**	5.8**	7.34**	34.21**
P1 x p6	-5.12**	-11.6**	-4.8**	-11.0**	30.66**	30.0**	27.8**	41.05**	27.6**	22.3**	26.07**	12.0**	12.66**	19.0**	12.6**	27.8**
P2 x p3	10.7**	4.7**	7.11**	13.0**	-5.78**	-5.36**	-9.56**	-15.6**	-3.5*	-4.5**	-28.66**	-30.8**	-4.06*	-1.88	-11.03**	-5.46**
P4 x p4	9.4**	8.0**	5.9**	16.8**	-4.65**	-3.89*	-15.7**	-13.5**	-4.02*	-8.33**	-6.34**	-9.2**	-10.67**	-4.11*	-13.6**	-20.40**
P2 x p5	3.78**	7.3**	4.87**	5.60**	-3.78**	-4.43*	-13.4**	-5.67	-3.25*	-10.0**	-30.65	-7.56**	-7.6**	-6.7**	-9.51**	-12.67**
P2 x p6	5.89**	13.7**	7.7**	4.88*	-34.05**	-11.4**	-10.0**	-20.4**	-6.34**	-15.77**	-12.5**	-4.5*	-40.88**	-35.12**	-13.44**	-3.5**
P3 x p4	12.0**	6.0**	5.16**	5.13**	-39.34**	-7.17**	-37.3**	-12.0**	-4.22**	-7.97**	-5.88*	-0.94	-14.67**	-0.38	-10.56**	-8.4**
P3 x p5	4.78**	3.67**	4.89**	10.8**	-17.57**	-34.05*	-3.80**	-8.93**	-38.14**	-4.66**	-5.0	-5.78**	-0.78	-13.54**	-3.5**	-10.0**
P3 x p6	5.16**	10.57**	20.96**	8.39**	-9.43**	-7.86**	-33.94**	-4.0**	-30.51**	-5.34**	-0.54	-1.22	-11.94**	-12.95**	-2.5**	-5.03**
P4 x p5	-17.6**	-20.7**	-11.88**	-45.9**	24.8**	18.7**	24.77**	16.5**	13.0**	12.6**	23.89**	27.88**	34.0**	14.77**	23.5**	4.5**
P4 x p6	-19.0**	-18.3**	-34.0**	-22.7**	23.8**	20.7**	33.0**	20.06**	18.5**	20.4**	11.23**	36.28**	16.44**	23.8**	40.3**	14.87**
P5 x p6	-20.89**	-9.11**	-15.0**	-19.8**	27.13**	12.5**	28.6**	17.66**	38.67**	17.8**	18.55**	15.86**	22.0**	30.6**	18.4**	35.68**
LSD0.05	2.45	2.40	3.44	4.61	2.67	3.7	2.54	1.97	3.18	2.68	5.77	3.79	3.88	3.55	1.78	2.88
LSD0.01	3.36	3.05	4.52	5.43	3.25	4.88	3.78	2.65	4.06	3.15	7.14	5.18	5.06	4.48	2.45	3.33

ISSN: 0975-8585

ISSR analysis

Seven ISSR primers were used to study the relationship among six rice genotypes. The results of ISSR analysis are presented in Fig. (1) and Table (9).

The present study confirms that ISSR method is efficient to discriminate among rice genotypes. The comparative analysis of Asian cultivated rice showed that the cultivars retained only 10 - 20% of the diversity in the wild species (Zhu *et al.,* 2007). Liu and Burke (2006) showed that in the wild sunflower (*Helianthus annus* L.), only 40-50% of the diversity are maintained cultivars. Moreover, cultivated maize maintained approximately 80% of the diversity found in its wild ancestor (Wright and Gaut, 2005). The evaluation of ISSR polymorphisms of sorghum indicated that land races retained 86% of the diversity observed in the wild (Casa *et al.,* 2005). The seven primers gave a total of 52 bands with an average of 7.43 / primer. Primer A-814 revealed the highest number of bands (13 bands), while primer HB-12 gave the lowest number of bands (5 bands). The highest polymorphism (100%) was revealed by the primer A- 814, while the lowest polymorphism (50%) was revealed by the primer HB-14.

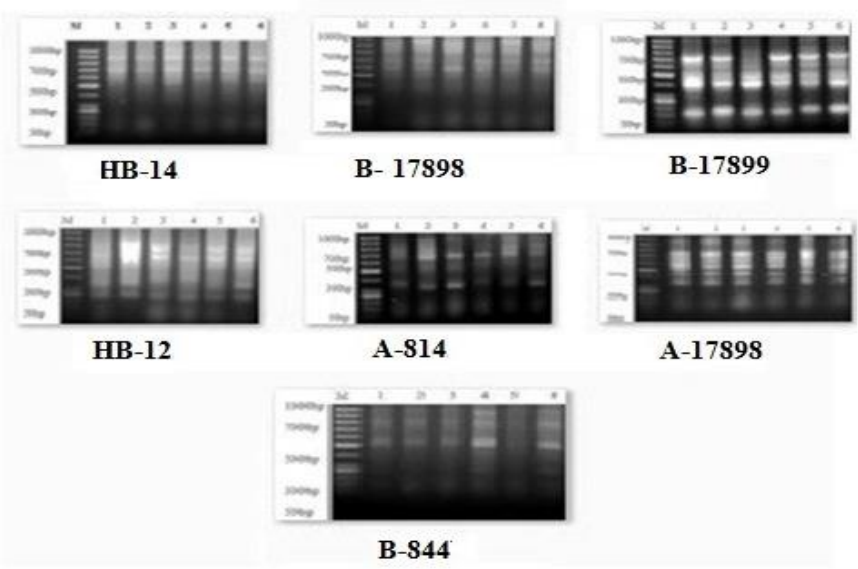

Fig. (1): Banding patterns of six rice genotypes using seven ISSR primers (HB-14, B-17898, B-17899, HB-12, A-814, A-17898 and B-844); M= 100bp Ladder.

Table (9): Total number, monomorphic, polymorphic bands and percentage of polymorphism.

Primer name	Total bands	Monomorphic bands	Polymorphic bands	% polymorphism
HB-14	6	3	3	50%
B-17898	5	2	3	60%
B-17899	9	3	6	66.667%
HB -12	5	2	3	60%
A-814	13	0	13	100%
A-17898	8	3	5	62.5%
B-844	6	2	4	66.667%
Average	7.43	2.14	5.29	71.15%
Total bands	52(100%)	15(28.85%)	37(71.15%)	

On the other hand, the seven ISSR Primers revealed one negative and 13 positive marker (Table 10). The negative marker was revealed by primer B-17898, while the positive markers were revealed by the rest of the primers (Table 10). 14 markers among six rice genotypes for drought stress using seven ISSR primers could be applied to genetic linkage analysis, quantitative trait loci (QTL) mapping, and marker assisted selection (MAS) to improve rice breeding efficiency for drought tolerance and can be exploited in DNA fingerprinting to variety identification. These results are in agreement with (Chuang *et al.*, 2011).

ISSN: 0975-8585

Table (10): Negative and positive markers of six rice genotypes using ISSR primers

ISSR primer	MS(bp)	P1	P2	P3	P4	P5	P6	MT(N or P)
HB-14	1334	+	-	+	-	-	+	
	1106	+	+	+	+	+	+	
	816	+	-	+	-	-	-	
	696	+	+	+	+	+	+	
	534	+	+	+	+	+	+	
B-17898	1190	-	+	-	-	+	-	
	1091	-	-	+	-	-	+	
	909	+	+	+	+	+	+	
	642	+	+	-	+	+	+	N(P3)
	518	+	+	+	+	+	+	
B-17899	805	-	-	-	-	-	+	P(P6)
	764	-	-	-	-	+	-	P(P5)
	703	+	+	-	+	-	-	
	557	-	+	-	-	+	-	
	439	+	+	+	+	+	+	
	375	+	+	+	+	+	+	
	318	-	+	-	+	+	+	
	300	+	-	+	-	-	-	
	120	+	+	+	+	+	+	
HB -12	1061	-	-	+	-	-	-	P(P3)
	775	-	+	+	-	+	+	
	585	+	+	+	+	+	+	
	242	-	+	-	-	-	+	
	147	+	+	+	+	+	+	
A-814	1216	-	+	-	-	-	-	P(P2)
	1061	-	-	-	-	-	+	P(P6)
	924	+	-	-	-	-	-	P(P1)
	760	-	-	-	-	+	+	
	712	-	-	+	+	-	-	
	687	-	+	-	-	-	-	P(P2)
	679	+	-	-	-	-	-	P(P1)
	533	-	-	-	+	-	+	
	451	-	+	+	-	-	-	
	294	-	-	-	+	-	-	P(P4)
	257	+	-	-	-	-	+	
	254	-	-	-	-	+	-	P(P5)
	238	-	+	+	-	-	-	
A-17898	1067	-	+	+	-	+	+	
	729	+	+	+	+	+	+	
	628	+	-	-	+	+	+	
	589	-	+	+	-	-	-	
	511	+	+	+	+	+	+	
	391	-	-	-	+	-	+	
	364	+	+	+	-	+	-	
	296	+	+	+	+	+	+	
B-844	1133	-	-	-	+	-	+	
	1048	+	-	-	-	-	-	P(P1)
	869	+	+	+	+	+	+	
	626	-	-	-	+	-	-	P(P4)
	524	-	-	-	+	-	-	P(P4)
	471	+	+	+	+	+	+	
Range	120:1334(bp)	-	-	-	-	-	-	-
Total	-	17	20	16	18	17	20	1(N) +13(P)

MS: molecular size (bp), MT: marker type, N: negative, P: positive.

Similarity and dissimilarity

Genetic diversity is the key determinant of germplasm utilization in crop improvement. Population with high level of genetic variation is the valuable resource for broadening the genetic base in any breeding program. The proximity matrix evaluated of relations among six rice genotypes. The high level of similarity was 100% between P2 and P5, while the lowest similarity was 24.2% between P1 and P2 (Table11). Fernández *et al.,* (2002) and Muthusamy *et al.*, (2008) found that the similarities among 16 barley cultivars using ISSRs were greater than the similarities observed using RAPD markers. These results may be due to highly polymorphic, abundant nature of the microsatellites due to slippage in DNA replication. On the other hand, the genetic variations observed in some of the landraces were very narrow because it might have resulted during the long

cultivation history of the species, as an adaptation to the local agro climatic conditions and may be due to narrow genetic base (Seehalak *et al.,* 2006).

Table 11: Similarity matrix of six rice genotypes.

Case	Matrix file input					
	P1	P2	P3	P4	P5	P6
P1	1.000					
P2	.242	1.000				
P3	.649	.695	1.000			
P4	.418	.299	.000	1.000		
P5	.418	1.000	.357	.481	1.000	
P6	.357	.412	.302	.759	.759	1.000

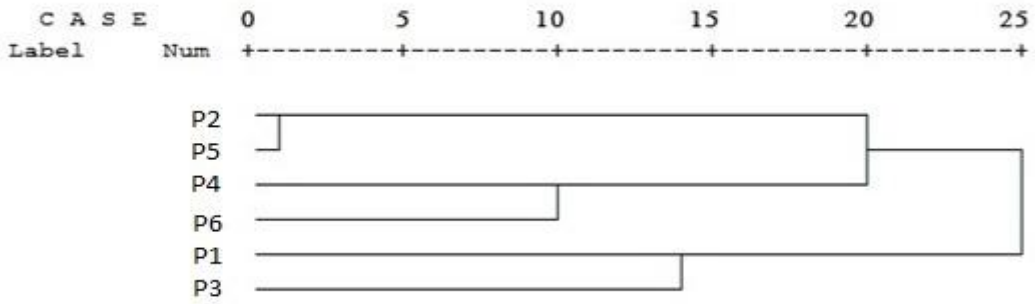

Fig. (2): Genetic distances among six rice genotypes (P1:P6).

CONCLUSION

The final results revealed that the genotypes (P3, P5, P6, P1 x P5, P1 x P6, P4 x P5, P4 x P6 and P5 x P6) were the best genotypes under control and water stress conditions for all studied traits. ISSR technique is an efficient method to differentiate among the parents. Therefore, these genotypes could be used in the rice breeding programs to improve rice productivity under water deficit conditions.

REFERENCES

[1] Betran, F.J.; Beek, D.; Banziger, M. and Edmeades, G.O. (2003). Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop Sci., 43:807-817.

[2] Casa AM, Mitchell SE, Hamblin MT, Sun H, Bowers JE, Paterson AH, Aquadro CF, Kresovich S (2005). Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. Theor. Appl. Genet. 111: 23-30.

[3] Chuang, H.-Y., H.-S.Lur, K.-K.Hwu, and M.-C. Chang. (2011). Authentication of domestic Taiwan rice varieties based on fingerprinting analysis of microsatellite DNA markers. Bot. Stud. 52: 393-405.

[4] Condon AG, Richards RA, Rebetzke GJ and Farquhar GD. (2004).Breeding for high water-use efficiency. J Exp Bot 5:2447–2460.

[5] Esselman EJ, Jianqiang L, Crawford DJ, Winduss JL, Wolfe AD. (1999). Clonal diversity in the rare Calamagrostisporteri ssp. insperata (*Poaceae*): comparative results for allozymes and random ampli-®ed polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Molecular Ecology 8: 443-451.

[6] Falconar DS, Mackay TFC.1996. Introduction to Quantitative Genetics. Chapman and Hall. London,U.K.

[7] Fernandez, M.E.; Figueiras, A.M. and Benito, C. (2002).The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theoretical and Applied Genetics. Vol. 104(5): 845-851.

[8] Gezahegn G.,Kassahun T. and Endashaw B. (2010).Inter Simple Sequence Repeat (ISSR) analysis of wild and cultivated rice species from Ethiopia. African Journal of Biotechnology Vol. 9(32: 5048-5059.

[9] Griffing, B., 1956.Concept of general and specific combining ability in relation toodiallel crossing system.Aust. J. 3io. Sci., 9: 463-493.

[10] Harris K, Subudhi PK, Borrell A, Jordan D, Rosenow D, Nguyen H, Klein P, Klein R and Mullet J .(2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. Journal of Experimental Botany, Vol. 58: 327–338.

[11] Hasan MJ, Kulsum MU, Hossain E, Hossain MM, Rahman MM, Rahmat NMF (2015).Combining ability analysis for identifying elite parents for heterotic rice hybrids. Acad. J. Agric. Res. 3(5):070-075.

[12] Kiambi DK, Ford-Lloyd BV, Jackson MT, Guarino L, Maxted N, Newbury HJ (2005).Collection of wild rice (Oryza L.) in east and Southern Africa in response to genetic erosion. PGR Newsletter, 142: 10-20.

[13] Kumar, A, Bernier, J, Verulkar, S, Lafitte, HR, Atlin, GN (2008). Breeding for drought tolerance: Direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. Field Crops Research 107: 221-31.

[14] Lin, H.-Y.; Wu Y.-P.; Hour, A.-L. ; Ho, S. –W.; Wei, F.-J. ; Hsing, Y.-Le C. and Lin, Y.-R. (2012). Genetic diversity of rice germplasm used in Taiwan breeding programs. Botanical Studies, Vol. 53: 363-376.

[15] Liu A, Burke JM (2006).Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics, 173: 321-330.

[16] Liu T, Shao D, Kovi MR and Xing Y. (2010). Mapping and validation of quantitative trait loci for spikelets per panicle and 1,000-grain weight in rice (*Oryza sativa L.*) Theor. Appl. Genet 120:933–942.

[17] Muthusamy S., Kanagarajan S. and Ponnusamy S. (2008). Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. Electronic Journal of Biotechnology.Vol.11 (3):1-10.

[18] Nuruzzaman M, Alam MF, Ahamed MG, Shohacl AM, Biswas MK, Amin MR, Hassain MM (2002). Studies on parental variability and heterosis in rice. Pak. J. Biol. Sci. 5(10):1006-1009.

[19] Pradhan SK, Boss LK, Meher J. 2006. Studies on gene action and combining ability analysis in Basmati rice. Journal of Centeral European Agriculture, Vol.7: 267-272.

[20] Raju Ch. Damodar, S. Sudheer Kumar, Ch. Surender Raju and A. Srijan (2014).Combining ability Studies in the Selected Parents and Hybrids in Rice (*Oryza sativa L.*), Int. J. Pure App. Biosci. 2 (4): 271-279.

[21] Ray D K, Gerber J S, MacDonald G K, West P C. (2015). Climate variation explains a third of global crop yield variability. Nat Commun, 6: 5989.

[22] Ren F, Lu B-R, Li S, Huang J, Zhu Y (2003).A comparative study of genetic relationships among the AA-genome Oryza species using RAPD and SSR markers. Theor. Appl. Genet. 108: 113-120.

[23] Saleem MY, Mirza JI, Haq, MA, 2010. Combining ability analysis for yield and related traits in Basmati rice (*Oryza sativa* L.). Pak. J. Bot.42: 627 -637.

[24] Sathya R and S Jebaraj. (2015).Evaluation of aerobic hybrid analysis of combining ability in three line hybrids in Rice (*oryza sativa* L.) under aerobic Conditions, African Journal of agricultural Research, Vol. 10(18), pp. 1971-1981.

[25] Seehalak, W.; Tomooka, N.; Waranyuwat, A.; Thipyapong, P.; Laosuwan, P.; KAGA, A. and VAUGHAN, D.A. (2006).Genetic diversity of the Vigna germplasm from Thailand and neighbouring regions revealed by AFLP analysis. Genetic Resources and Crop Evolution. Vol. 53(5): 1043-1059.

[26] Tsumura Y, Ohba K, Strauss SH. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in douglas-®r (Pseu-dotsugamenziesii) and sugi (*Cryptomeria japonica*). Theoretical and Applied Genetics 92: 40-45.

[27] Wright SI, Gaut BS (2005). Molecular population genetics and the search for adaptive evolution in plants. Mol. Biol. Evol. 22: 506-519.

[28] Zhang, Y., and M.S. Kang. 1997. Diallel-SAS: A SAS program for Griffing's diallel analyses. Agron. J. 89:176–182.

[29] Zhu Q, Zheng X, Luo J, Gaut BS, Ge S (2007). Multi locus analysis of nucleotide variation of *Oryza sativa* and its wild relatives: Severe bottle eneck during domestication of rice. Mol. Biol. Evol. 24(3): 875-888.