

Field Performance and Molecular Evaluation of New Promising Lines (PLS) for Water Deficit Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

Developing new tolerant varieties for water shortage condition is the best way to overcome water limitation problem. Using the selection of the best rice lines under stress condition could be useful strategy during breeding for tolerant genotypes. The present study aimed evaluate new promising lines along with their parental varieties Sakha 105 and IRAT 170 to identify superior lines under water shortage conditions. Field experiments were conducted at Rice Research and Training Center, Sakha, Kafr Elsheikh, Egypt during 2015 and 2016 rice growing seasons. This study was established in order to identify adapted and promising lines under both normal and water shortage conditions. The obtained results revealed that all promising lines showed good performance under both normal and stress conditions, the promising lines PL3, PL4 and PL6 showed the highest yield potential under normal conditions, with values 1246.7, 1071.7 and 990g/m² respectively, which were higher than the high-yielding parental variety Sakha 105. While the promising lines PL5, PL6 and PL10 showed the highest yield under water shortage conditions with values (738.3, 709 and 736.7 g/m²) higher than the tolerant parental variety IRAT 170. The promising lines PL2, PL5 and PL10 showed the lowest yield reduction caused by water stress according to drought susceptibility index (DSI) values (0.232, 0.255 and 0.181). These promising lines will be further evaluated for grain quality on multi-locations yield trials and could be used as a donor to improve cultivars ability for tolerance of water shortage condition. Significant amounts of genetic variations were observed for all studied traits. The phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) in all studied traits. High broad sense heritability and genetic advance as percent of mean was observed for most studied traits indicating the importance of genetic variance for the studied traits. The SSR markers RM259, RM241, RM263 and RM201 showed a polymorphic pattern among the susceptible and tolerant genotypes with different alleles which detected at molecular weight ranged between 80bp to 190 bp. These markers could be used for the screening in further studies. The molecular analysis confirmed the existence of significant amounts of genetic variations, and hence the usefulness of studied materials for improving water deficit tolerance breeding program.

Keywords: Rice, New lines, Water deficit, SSR.

INTRODUCTION

Rice is considered as drought susceptible crop, especially at the reproductive stage (Agarwal *et al.*, 2016) due to the semi aquatic nature of rice growth conditions. Recent drought conditions caused by global climate change seriously threatened commercial rice production in irrigated as well as rain-fed conditions (Naylor *et al.*, 2007). Thus, the development of drought-tolerant varieties is an important global strategy to maintain sustainable rice production. Modern rice varieties are sensitive to drought stress at seedling, vegetative, and reproductive stages and even mild drought stress can result in a significant yield reduction (O'Toole 1982; Torres and Henry 2016). At seedling stage, drought affects crop establishment and seedling survival rates. At vegetative stage, it reduces leaf formation and tillering, which subsequently reduces panicle's development, thus causing a yield loss. Furthermore, at reproductive stage, drought causes a reduction in the number of grains per panicle, increases spikelet sterility, and reduces grain weight (Pantuwan *et al.*, 2002). Water resources in Egypt are limit and not sufficient to cover all live activities including agricultural irrigation, particularly with high population growth rate. Some rice cultivated areas, especially at canal's terminal suffer from shortage of irrigation water during different growth stages, which are considered to be one of the most serious constraints to rice production in Egypt (Abd Allah *et al.*, 2010a). Drought research at the International Rice Research Institute (IRRI) over the past decade has concentrated on direct selection for grain yield under drought conditions. This approach has led to the successful development and release of 17 high-yielding drought-tolerant rice varieties in South and Southeast Asia, and Africa (Kumar *et al.*, 2014). Climate change is predicted to increase the frequency and severity of water scarcity,

causing serious constraints to rice production worldwide (Wassmann *et al.*, 2009). Understanding the genetic basis of drought tolerance in rice is fundamental to enable breeders and molecular biologists to develop new varieties with more drought tolerance characters (Nguyen and Buu, 2008). Molecular dissecting of water deficit tolerance and identification of linked markers enhanced selection efficiency for this complex trait. Among the ideal marker systems used, microsatellites, also known as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). They are more amenable to high throughput methods with higher abilities for polymorphism detection, among even closely related individuals. Rice genome sequence has been found to contain sum of 40,000 SSRs (Goff *et al.*, 2002). The objective of the current study was to evaluate a set of eleven promising lines resulted from selection of a cross between drought sensitive, high yielding local variety Sakha 105 and drought tolerant check IRAT 170, under both normal and water deficit conditions as well as molecular confirmation of the existence of previously reported linked SSR markers.

MATERIALS AND METHODS

Plant Materials and Designation:

The present study was carried out at the Experimental Farm of Sakha Agricultural Research Station, Sakha, Kafr El-Sheikh, Egypt. Eleven promising lines derived under water stress condition from two different parents, Sakha 105 Egyptian japonica cultivar characterized as high yield, semi-dwarf, early and susceptible to water shortage stress used as a female parent, IRAT 170 from Ivory Coast, indica type and drought tolerant variety used as male and donor parent.

During 2015 and 2016 growing seasons all genotypes (two parental varieties and eleven selected

promising lines) were evaluated under normal and stress conditions in separated experiments using randomized complete block design (RCBD) in three replicates. The first experiment was normally irrigated with continuous flooding while, the second experiment was carried out in aerobic conditions, dry seeds were sown in dry soil, and irrigated every 12 days without any standing water (flush irrigation) as stress treatment. Each genotype of parents and promising lines were planted in five rows per replicate. The row was five meters long with 20 × 20 cm spacing. All the recommended agronomic and plant protection practices were uniformly followed according to RRTC recommendations throughout the crop growth period for raising ideal crop stand.

Data collection

Data were recorded on the average of each plot (5m²) per replicate. The studied traits were; days to heading (day), plant height (cm), number of tillers/plant, number of panicles/plant, panicle length (cm), panicle weight (g) Spikelet fertility (%), root thickness (mm), number of metaxylem vessels and grain yield/plant (g). Root traits were measured by the microscope with micrometer slide after fixing and staining for root cross sections according to the procedure of Terashima *et al.*, (1987). Drought susceptibility index (DSI) for each genotype were calculated according to the formula given by Ali-Dib *et al.*, (1990). $DSI = (XN - XS)/XN$ Where: XN is the observation under normal condition and XS is the observation under stress condition.

Data Analysis:

The analysis of variance for each experiment (normal and stress conditions) was done according to Steel and Torrie (1980). The genetic parameters were computed according to formula suggested by Burton (1952) and Hanson *et al.*, (1956). The cluster analysis tree construction was expressed by using the Paleontological Statistics (PAST) software package using the mean performance of the studied genotypes (Hammer *et al.*, 2001).

SSR Markers analysis

The fresh leaves of the 13 rice genotypes were sampled for DNA extraction according to modified CTAB (Cetyl Try Methyl Ammonium Bromide) method (Rogers and Bendich, 1988). A total of 31 SSR markers associated

with drought tolerance from previous studies were used in the polymorphism survey. PCR was performed as follow: The 10-μL PCR reaction mixture contained 1 x buffer, 0.2 μM of each primer, 50 μM of DNTPs, 0.5 unit of *Taq* polymerase (Tiagen Company, Beijing, China), and 10 ng of genomic DNA as a template. The thermal cycler was programmed for a first denaturation step of 4 min at 94°C, followed by 30 cycles, each of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s and final extension step of 5 min at 72°C. The PCR products were separated on 8.0% non-denaturing polyacrylamide gel and detected using the silver staining method. Generated DNA bands were analyzed and scored 1 for the presence- or 0 for the absence of allele. Four SSR markers (RM 259, RM263, RM241 and RM201) were highly polymorphic among the studied genotypes which used for genotyping of tested genotypes (Elgamal 2013, Lang *et al.*, 2013 and Aziza Aboulila 2015) and these markers located on chromosomes 1, 2, 4 and 9, respectively.

RESULTS AND DISCUSSION

Analysis of variance

The mean square estimates for genotypes were significant and highly significant for all studied traits under both normal and stress conditions indicating the existence of genotypic variances among all studied genotypes (Table 1). On the other hand, there were no significant mean squares due to years for all studied traits under normal condition while, were significant just for number of tillers/plant and number of panicles/plant under stress condition. The interaction mean squares between years and genotypes were no significant for all studied traits except for plant height under stress condition. A little effect of years under Egyptian conditions due to stability conditions within years and lines stability after F₆ generation. Abdelmaksoud *et al.*, 2013; Elgamal 2013 and Elgamal *et al.* 2015 reported that the effect of years was not highly significant on rice growing under Egypt conditions. These results agreed with previous studies by Abdallah *et al.* 2010a, Elgamal 2013 and Elgamal *et al.* 2018 in their studies on genetic diversity of some rice cultivars.

Table 1. Mean squares and analysis of variance model for all studied traits under all experimental treatments.

S.O.V.	d.f		Plant height	Heading date	Number of tillers /plant	Number of panicles /plant	Panicle Length	Panicle Weight	Fertility %	Grain yield/m ²	Root thickness	Number of metaxylem vessels
Years	1	N	5.13	0.1154	2.513	2.167	0.948	0.0009	6.93	96.96	0.0004	0.4615
		S	168.08	14.821	44.628*	24.821*	1.206	0.0949	4.89	26	0.0026	0.6282
Residual _(a)	4	N	14.79	1.1026	5.744	8.513	3.632	0.7592	7.104	76.3	0.0384	0.0385
		S	27.48	6.333	2.859	0.897	1.159	0.258	46.92	42	0.0031	0.1667
Genotypes	12	N	355.58**	37.809**	78.972**	69.288**	17.86**	2.4349*	40.013**	109519**	0.1337**	2.7671**
		S	567.54**	204.68**	69.850**	4.806*	18.476**	2.1428**	304.74**	23160**	0.2120**	4**
Years x Genotypes	12	N	4.49	0.2543	0.929	0.861	5.748	0.667	9.819	97.6	0.0263	0.1004
		S	30.21*	1.654	4.767	3.737	2.065	0.3813	16.85	106.5	0.0088	0.2393
Residual _(b)	48	N	14.53	0.4776	7.563	5.152	4.741	0.9849	9.513	465.7	0.0185	0.0940
		S	11.78	2.694	3.873	2.175	4.264	0.3727	25.33	188.2	0.0034	0.1389

N: normal conditions, S: stress condition

Mean Performance

To evaluate the studied genotypes (eleven promising lines and their parents), we determined the performance of these genotypes under normal and stress

conditions replicated in two years by ten traits measurements. Under normal condition the parent Sakha 105 was superior and better than IRAT 170 for most studied yield and yield components traits during the two

evaluation seasons, which recorded the highest values of number of panicles, spikelets fertility and grain yield in addition to the lowest values of heading dates and plant height which preferred to lodging resistance and supporting the harvest index. On the other hand, the parent IRAT 170 showed the performance better than Sakha 105 under stress condition during two years for all studied traits Except for Number of tillers and number of panicles per plant.

Tables from 2 to 6 present the mean performances of the studied genotypes for ten studied traits under normal and water shortage conditions during 2015 and 2016 growing seasons. Regarding plant height, the promising lines PL9, PL4 and PL7 were the shortest among all

studied promising lines under both normal and stress conditions during two years with values ranged from 91.00 cm under stress condition, season 2016 to 108.33 cm under normal condition, season 2015 (Table 2). Also, the lowest values of heading dates were recorded for promising lines PL4, PL8 and PL11, as their values ranged between 78.00 days under stress conditions and 92.33 days under normal conditions at 2015 season (Table2). The water shortage stress affected plant height and heading date due to lake metabolisms and following drought escape mechanism. Abdallah *et al.*, 2010b; Elgamal 2013; Elgamal *et al.*, 2015; Kumar *et al.* 2016 and Elgamal *et al.* 2018.

Table 2. Mean performances of studied genotypes for plant height and heading date under both normal and stress conditions during 2015 and 2016 seasons.

Genotypes	Plant height (cm)				Heading date (days)			
	2015		2016		2015		2016	
	N	S	N	S	N	S	N	S
Sakha 105	101.33	65.67	102.00	64.50	93.33	94.33	93.67	95.00
IRAT 170	126.67	110.67	126.00	106.67	98.67	96.67	99.00	96.00
PL1	120.00	95.00	121.67	98.33	96.67	88.00	96.33	87.67
PL2	111.00	96.33	107.67	97.00	95.00	89.33	95.00	88.67
PL3	115.67	91.33	117.33	92.67	98.33	89.00	97.67	89.00
PL4	105.33	93.33	105.67	92.33	93.33	85.33	92.67	86.33
PL5	122.00	102.00	123.33	95.33	93.67	90.00	93.33	90.00
PL6	108.33	93.33	108.33	94.00	95.33	89.33	95.67	88.67
PL7	103.33	93.00	101.67	91.00	94.33	89.00	94.33	88.00
PL8	110.00	94.33	108.33	93.00	92.67	85.00	92.33	86.67
PL9	108.33	94.00	106.00	93.00	97.67	91.67	98.33	91.67
PL10	113.33	101.67	111.67	99.00	95.00	93.00	95.00	93.00
PL11	110.00	101.33	108.33	96.67	92.33	78.00	92.33	79.67
LSD 5%	5.697	5.519	7.179	6.035	0.8133	2.605	1.432	2.919
LSD 1%	7.544	7.479	9.729	8.179	1.102	3.53	1.941	3.955

N: normal conditions, S: stress condition

In the same trend, water shortage affecting on number of tillers and panicles when occurred during vegetative stage as reported by Elgamal 2013, Hadifa 2012 and Kumar *et al.*, 2016. The highest numbers of tillers and panicles per plant observed on promising lines PL6, PL2 and PL3 under both normal and drought conditions during 2015 and 2016 seasons and the recorded values ranged from 16.33 to 22.67 for number of tillers/plant under normal condition and from 11.33 to 13.67 under stress conditions. For number of panicles/ plant, the values ranged between 18.67 and 15.00 under normal conditions and ranged from 11.00 to 13.00 under water shortage conditions (Table 3).

The promising lines PL1, PL3 and PL5 exhibited large panicles among the studied lines under normal and stress conditions during the two evaluation seasons, with values ranged from 21.27 to 24.77 cm. While the promising Lines PL1, PL5 and PL10 were the heaviest panicles among studied genotypes under both growing seasons and treatments which scored the highest values of panicle weight. The highest values ranged from 4.89 to 5.60 g at normal and ranged from 3.66 to 4.90 under stress (Table 4).

Spikelets fertility were affected by water shortage stress especially at heading stage, fertility percentage for all studied genotypes during two growing seasons under stress conditions were lower than under normal condition. The

highest values of Spikelets fertility were recorded at the promising lines PL3, PL5 and PL8 and ranged from 86.72 under stress to 97.64 under normal conditions % (Table 5). Fertility percentage affected on grain yield by positive effect and sterility determine the grain yield (Pantuwan *et al.*, 2002 and Kumar *et al.*, 2014)

Grain yield considers the main target for all breeders and farmers even under normal or stress conditions, in totally it is due to many components like as some morphological and physiological traits but finally, the direct measurements of mass grain weight per unit is the easiest way to have the optimum results. The results were presented in table 5 show that all studied lines gave grain yield higher than their parents under both normal and stress conditions during 2015 and 2016 rice crop season. Among new lines, the promising lines PL3, PL4 and PL6 gave the highest amount of grain yield /m² under normal conditions with values 1246.7, 1071.7 and 1055 g, respectively. Other two different promising lines PL5, PL10 along PL6 gave the highest yield under stress conditions during the two years with values ranging from 696.7 to 738.3 g (Table5). In general, water shortage stress causing grain yield reduction when occurs at any growth stages as Hsiao 1973; Pantuwan *et al.*, 2002; Abdallah *et al.*, 2010a and Elgamal *et al.*, 2018 reported and emphasized by the present results.

Table 3. Mean performances of studied genotypes for number of tillers and number of panicles/plant under both normal and stress conditions during 2015 and 2016 seasons.

Genotypes	Number of tillers/plant				Number of panicles/plant			
	2015		2016		2015		2016	
	N	S	N	S	N	S	N	S
Sakha105	20.33	13.67	21.67	13.33	19.67	13.33	20.00	12.67
IRAT170	15.33	12.33	14.67	11.67	14.67	12.33	14.33	11.00
PL1	16.00	10.33	16.33	9.33	14.67	10.00	16.33	9.00
PL2	16.67	12.33	17.33	13.67	15.00	11.00	16.67	11.00
PL3	16.33	11.33	16.67	13.33	15.33	11.33	15.00	12.67
PL4	14.00	11.00	13.00	12.33	11.67	11.00	11.33	11.00
PL5	11.67	11.00	16.00	11.00	10.67	10.00	14.33	10.33
PL6	22.67	12.33	21.67	13.67	18.00	12.00	18.67	13.00
PL7	15.67	10.67	15.33	11.33	13.67	9.33	13.33	11.00
PL8	13.00	11.00	15.33	13.00	11.33	10.67	13.00	11.00
PL9	15.33	11.33	14.67	13.00	13.00	10.33	13.67	11.67
PL10	12.67	10.33	13.67	11.67	11.00	10.00	13.33	10.67
PL11	13.33	11.00	12.67	11.67	11.00	10.67	12.00	10.67
LSD 5%	3.239	2.167	5.698	4.159	3.393	1.275	4.213	3.275
LSD 1%	4.389	2.937	7.721	5.637	4.598	1.728	5.709	4.439

N: normal conditions, S: stress condition

Table 4. Mean performances of studied genotypes for panicle length(cm) and panicle weight(g) under both normal and stress conditions during 2015 and 2016 seasons.

Genotypes	panicle length (cm)				panicle weight (g)			
	2015		2016		2015		2016	
	N	S	N	S	N	S	N	S
Sakha105	24.67	16.33	24.50	16.10	3.53	2.27	3.57	2.33
IRAT170	27.00	19.33	27.17	18.87	4.67	3.53	4.70	3.31
PL1	24.77	22.17	23.97	22.60	5.27	3.66	5.60	3.79
PL2	22.60	21.00	22.40	21.20	4.77	2.65	4.71	3.11
PL3	24.60	21.27	24.50	22.00	4.24	3.36	4.25	2.91
PL4	22.83	21.03	23.13	21.47	5.09	3.64	3.46	2.98
PL5	24.53	21.60	23.20	21.77	5.20	4.31	5.50	4.90
PL6	23.33	20.27	22.00	18.47	4.57	3.61	4.39	3.71
PL7	22.40	19.50	20.83	19.70	3.98	3.60	4.26	3.67
PL8	19.50	19.00	22.50	18.83	3.57	2.52	3.46	2.83
PL9	22.33	19.43	23.17	20.50	4.19	2.58	4.31	2.58
PL10	23.47	19.60	20.73	16.93	5.13	3.67	4.89	3.72
PL11	23.13	19.90	22.70	18.27	4.63	2.46	4.25	3.60
LSD 5%	3.905	4.176	3.417	2.604	1.789	1.0386	1.547	1.0187
LSD 1%	5.291	5.659	4.656	3.529	2.424	1.4075	2.097	1.3805

N: normal conditions, S: stress condition

Table 5. Mean performances of studied genotypes for spikelets fertility% and grain yield/m² (g) under both normal and stress conditions during 2015 and 2016 seasons.

Genotypes	Spikelets fertility%				Grain yield/m ² (g)			
	2015		2016		2015		2016	
	N	S	N	S	N	S	N	S
Sakha105	94.79	75.83	95.52	76.67	985.00	536.70	995.00	523.30
IRAT170	90.45	82.55	90.78	81.87	621.00	518.30	640.30	523.30
PL1	64.76	80.19	95.16	88.77	877.30	618.30	878.30	631.70
PL2	95.14	85.80	95.48	87.55	880.00	680.00	891.70	680.00
PL3	96.46	91.16	97.71	91.07	1246.70	680.00	1211.70	683.30
PL4	91.69	86.43	95.87	88.49	1071.70	650.00	1085.00	648.30
PL5	97.02	89.29	97.64	90.48	990.00	738.30	988.30	735.00
PL6	87.04	83.24	93.27	84.34	1055.00	709.00	1061.70	696.70
PL7	93.00	84.58	94.95	84.14	913.30	631.70	880.00	638.30
PL8	95.80	86.72	94.96	89.54	888.30	668.30	893.30	656.70
PL9	92.00	71.81	92.61	67.64	961.70	683.30	963.30	685.00
PL10	90.06	81.77	90.12	85.84	895.00	736.70	898.30	731.70
PL11	92.29	82.71	93.19	80.21	943.30	606.70	965.00	610.00
LSD 5%	5.843	8.169	4.46	8.781	40.01	25.64	32.32	20.28
LSD 1%	7.918	11.07	6.044	11.9	45.22	34.75	43.8	27.48

N: normal conditions, S: stress condition

Studying the root characters are very important for scientists whose works in breeding and developing new tolerant genotypes for water shortage conditions, some of these traits like as root depth, root volume, root thickness and metaxylem vessels consider as indicators for the tolerance (Elgamal 2013; Abd Allah *et al.*, 2010b; Wu and Cheng 2014 and Elgamal *et al.*, 2018). In the present investigation, two of these traits (root thickness and metaxylem vessels numbers) were used to evaluate the

genotypes for drought tolerance under normal and stress condition during 2015 and 2016 growing seasons (Figure 1). The lines PL5, PL7 and PL10 scored the highest values of root thickness and metaxylem vessels numbers under both normal and stress conditions during two years, the scored values were higher than both parents and ranged from 1.68 to 2 mm for root thickness and ranged from 6.67 to 9 for metaxylem vessels number (Table 6).

Table 6. Mean performances of studied genotypes for the studied root traits under both normal and stress conditions during 2015 and 2016 seasons.

Genotypes	Root thickness (mm)				Number of metaxylem vessels			
	2015		2016		2015		2016	
	N	S	N	S	N	S	N	S
Sakha105	1.18	1.18	1.18	1.18	4.00	4.00	4.33	3.67
IRAT170	1.70	1.72	1.70	1.72	6.00	6.33	6.00	6.33
PL1	1.62	1.68	1.67	1.70	6.00	6.00	6.67	6.00
PL2	1.60	1.68	1.62	1.70	6.00	6.33	6.00	6.00
PL3	1.73	1.77	1.68	1.78	6.67	7.00	7.00	7.00
PL4	1.73	1.68	1.65	1.70	6.00	6.33	6.33	5.00
PL5	1.73	1.97	1.79	1.97	8.00	8.00	8.00	8.00
PL6	1.63	1.70	1.65	1.70	6.00	6.67	6.00	6.33
PL7	1.73	1.87	1.68	1.78	6.67	7.00	7.00	7.00
PL8	1.65	1.73	1.67	1.72	6.00	6.33	6.33	6.33
PL9	1.70	1.80	1.70	1.67	6.00	6.33	6.33	6.00
PL10	1.83	2.00	1.70	1.90	8.66	9.00	9.00	9.00
PL11	1.23	1.80	1.65	1.77	6.00	6.00	6.00	6.33
LSD 5%	0.3045	0.0988	0.1114	0.098	0.3736	0.6701	0.628	0.5829
LSD 1%	0.4126	0.1339	0.628	0.5829	0.5063	0.908	0.8511	0.79

N: normal conditions, S: stress condition

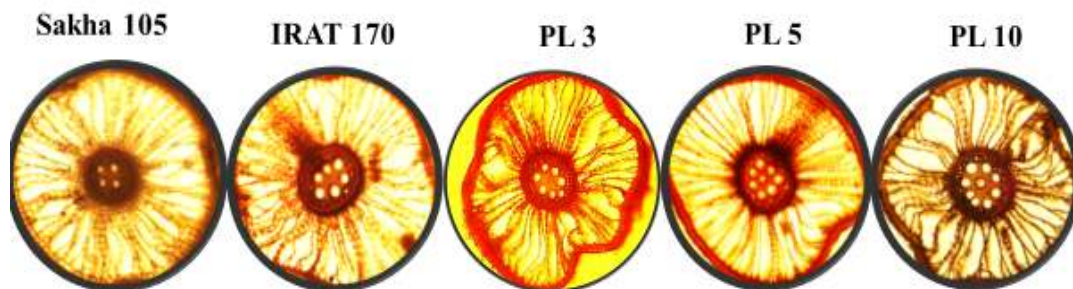


Figure1. variations of studied genotypes based on metaxylem root vessels numbers

Drought susceptibility index (DSI): Drought susceptibility index is an important indicator for drought susceptibility, which presents the reduction of estimated trait values due to water shortage stress. The drought susceptibility index (DSI) was presented in Table 7. The highest values of DSI were recorded at the susceptible parent Sakha 105 for most studied traits, which are undesirable except for heading date and plant height. Most of the studied new lines showed DSI values less than both parents, but there are no lines showed the best for all studied traits. PL4 recorded the lowest DSI values at no. of tillers, no. of panicles and panicle length. Regarding grain yield the promising lines PL2, PL5 and PL10 showed the lowest yield reduction caused by water shortage stress with DSI values 0.232, 0.255 and 0.181, respectively. In case of root traits, there are some negative values due to the drought effect on root traits and the roots responding to drought tolerance mechanisms such as drought avoidance.

Estimation of genetic parameters for the studied traits in parental genotypes and its promising lines under normal and water shortage conditions

Estimation of genetic parameters is very important for successful breeding programs depend on the amount of genetic variability among genotypes, which selected further manipulation to achieve the breeding target. A survey of genetic variability with the help of suitable parameters such as genotypic (σ^2_g) and phenotypic (σ^2_p) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h²b.s) in broad sense and genetic advance (GA) is necessary to start an efficient breeding program. Different values of estimated parameters were observed between the parental genotypes and their progenies (Table 8). The genotypic (σ^2_g) and phenotypic (σ^2_p) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h²b.s) in broad sense and genetic advance (GA) of parental genotypes and its 11 derived lines were estimated for each normal and stress

conditions based on the collected data during two seasons. Generally, phenotypic variance was higher than the genotypic variance in all the studied traits under both normal and stress conditions and this indicated the influences of environmental factor on these studied traits. Similar results were reported by Anis *et al.*, 2017 and Ghidan *et al.*, 2018. High genotypic and phenotypic variances were observed in grain yield followed by plant height. The highest estimated value of genotypic (GCV) and phenotypic coefficients of variation (PCV) were recorded for No. of panicle (22.19 and 27.00) and No. of tillers (21.39 and 26.34) under the normal conditions. Furthermore, high estimates of heritability in broad sense (h^2_b) were exhibited for plant height, heading date, fertility percentage (F%), grain yield and root studied traits with values ranged from 77.54% for F% under stress condition to 97.88% for grain yield under normal condition. These findings indicated that most of studied traits are mainly controlled by genetic factors. From low to

moderate values of broad sense heritability were observed of tillers and panicles traits with estimated values ranged from 29.46 % for No. of panicles under stress conditions to 49.23% for panicle length under normal conditions. In addition, the results revealed that the genetic advance% was high for number of panicles (37.57) followed by number of tillers (35.80) and grain yield (28.56), while, the lowest value was detected for F% (3.32) under normal condition. The genetic advance is an efficient evidence of the progress that is predicted as a result of the related choice population. Heritability in conjunction with genetic advance would give a more reliable index of better selection. High heritability compared with high genetic advance were recorded for most studied traits, suggesting the effectiveness of selection in early generation to improve the yield potential under stress conditions. These results agreed with early reports by Elgamal 2013 and El-Hity *et al.*, 2015.

Table7. Drought susceptibility index for all studied traits based on the two years mean results.

Genotypes	Plant height	Heading date	No. of tillers /plant	No. of panicles /plant	Panicle length	Panicle weight	Spikelets Fertility (%)	Grain yield/m ²	Root thickness (mm)	No. of metaxylem vessels
Sakha 105	0.360	-0.012	0.357	0.345	0.340	0.352	0.199	0.465	0.000	0.079
IRAT 170	0.140	0.025	0.200	0.196	0.295	0.270	0.093	0.319	-0.012	-0.055
PL1	0.200	0.090	0.392	0.387	0.081	0.315	0.057	0.288	-0.027	0.053
PL2	0.116	0.063	0.235	0.305	0.062	0.392	0.091	0.232	-0.050	-0.028
PL3	0.210	0.092	0.253	0.209	0.119	0.261	0.061	0.453	-0.026	-0.024
PL4	0.120	0.077	0.136	0.015	0.075	0.235	0.067	0.398	0.000	0.081
PL5	0.196	0.037	0.205	0.187	0.091	0.139	0.076	0.255	-0.119	0.000
PL6	0.135	0.068	0.414	0.318	0.145	0.183	0.071	0.345	-0.037	-0.083
PL7	0.102	0.062	0.290	0.247	0.093	0.118	0.102	0.292	-0.070	-0.024
PL8	0.142	0.072	0.153	0.109	0.099	0.239	0.076	0.256	-0.039	-0.027
PL9	0.128	0.065	0.189	0.175	0.122	0.393	0.245	0.289	-0.021	0.000
PL10	0.108	0.021	0.165	0.068	0.174	0.255	0.070	0.181	-0.171	-0.019
PL11	0.093	0.145	0.128	0.029	0.167	0.318	0.122	0.362	-0.240	-0.028

Table 8. Grand mean, variance components, estimates of phenotypic (PCV) and genotypic (GCV) coefficient of variation, heritability in broad sense (h^2_b) and genetic advance for ten characters in rice based on pooled data under normal and stress.

Traits	Treatment	Mean	Variance		Coefficient of variance		H^2_b	Genetic advance(GA)	GA% of mean
			Genotypic	Phenotypic	GCV %	PCV %			
Plant height(cm)	N	111.7	55.31	67.16	6.66	7.34	82.36	13.90	12.45
	S	94.2	93.02	97.74	10.24	10.50	95.17	19.38	20.58
Heading date(days)	N	95.3	6.22	6.47	2.62	2.67	96.02	5.03	5.28
	S	87.9	33.54	35.27	6.59	6.75	95.08	11.63	13.23
No. of tillers/plant	N	15.7	11.23	17.02	21.39	26.34	65.98	5.61	35.80
	S	12.0	0.69	2.11	6.94	12.11	32.80	0.98	8.19
No. of panicles/plant	N	14.2	9.95	14.74	22.19	27.00	67.56	5.34	37.57
	S	11.3	0.45	1.51	5.90	10.84	29.64	0.75	6.62
Panicle length(cm)	N	23.6	2.22	4.50	6.32	9.00	49.23	2.15	9.13
	S	19.9	2.32	4.59	7.64	10.74	50.55	2.23	11.19
Panicle weight(g)	N	4.5	0.24	0.74	10.81	18.97	32.44	0.57	12.68
	S	3.3	0.29	0.49	16.61	21.47	59.84	0.86	26.47
Fertility %	N	94.4	4.87	10.26	2.34	3.39	57.45	3.13	3.32
	S	85.5	46.32	59.73	7.96	9.04	77.54	12.35	14.44
Grain yield/m ² (g)	N	950.7	18122.39	18515.02	14.01	14.16	97.88	274.36	28.56
	S	661.6	3802.43	3975.16	9.32	9.53	95.65	124.24	18.78
Root thickness(mm)	N	1.62	0.02	0.03	8.50	10.71	62.90	0.22	13.88
	S	1.72	0.03	0.04	10.74	11.10	93.66	0.37	21.41
Number of metaxylem vessels	N	6.42	0.45	0.49	10.98	11.55	90.46	1.31	21.52
	S	6.43	0.63	0.73	12.90	13.89	86.24	1.52	24.68

N: normal conditions, S: stress condition

Correlation coefficient

The results in Table 9 showed the estimates of correlation coefficients among all studied traits, the data setup as the mean of two years under each experiment; Stress conditions (above) and normal conditions (below). The results revealed that, highly significant positive correlation between number of panicles/plant and number of tillers /plant under both experiments conditions. Highly significant positive correlations were recorded also between root thickness and each of plant height, grain yield and number of metaxylem vessels in addition to between grain yield and number of metaxylem vessels under drought condition only. Some cases of significant positive

correlation were detected under stress conditions, between root traits and plant height, panicle weight and number of panicles/plant. Under normal condition experiment, there were significant positive correlations between plant height and each of panicle length, panicle weight and between heading date and panicle length, and between number of metaxylem root vessels and panicle weight and root thickness. From the above-mentioned results, it could be concluded that, root traits are very important to improve the yield potential under stress condition and could be used as indicator at early stage. These results are in agreement with Philippe *et al.*, 2010, Abdulmajid 2011 and Elgamel 2013.

Table 9. Correlation coefficients among the studied traits under stress (above) and normal (below).

		Stress									
Traits		PH	HD	NTP	NPP	PL	PW	F%	GY	RT	NMV
Normal	PH	1.00	-0.145	-0.497	-0.478	0.472	0.527	0.305	0.495	0.810**	0.651*
	HD	0.491	1.00	0.282	0.317	-0.300	0.007	-0.291	-0.051	-0.251	0.019
	NTP	-0.322	0.050	1.00	0.827**	-0.459	-0.616*	-0.233	-0.221	-0.601*	-0.492
	NPP	-0.186	0.156	0.953**	1.00	0.614*	-0.471	-0.231	-0.297	-0.571*	-0.377
	PL	0.693*	0.585*	0.141	0.333	1.00	0.486	0.508	0.461	0.500	0.282
	PW	0.691*	0.277	-0.276	-0.195	0.275	1.00	0.512	0.530	0.677*	0.683*
	F%	-0.295	-0.332	-0.061	-0.123	-0.187	-0.497	1.00	0.376	0.421	0.408
	GY	-0.083	0.121	0.246	0.158	0.145	-0.191	0.394	1.00	0.788**	0.826**
	RT	0.461	0.285	-0.624*	-0.719**	-0.146	0.470	-0.053	0.053	1.00	0.911**
	NMV	0.383	0.010	-0.637*	-0.666*	-0.258	0.580*	-0.018	-0.047	0.692*	1.00
		Normal									

PH: plant height, HD: heading date, NTP: number of tiller/plant, NPP: number of panicles/plant, PL: panicle length, PW:panicle weight, F%: fertility percentage, GY: grain yield/m², RT: root thickness and NMV: number of metaxylem vessels.

SSR analysis

The objective of this part of investigation to differentiate the eleven new promising lines for water shortage tolerance comparing with their parents using SSR markers related to drought tolerance. Out of 31 SSR markers used in this study, four markers showed highly polymorphic patterns among parental genotypes. The banding patterns for the amplified DNA fragments for these markers are shown in Figures 2, 3, 4 and 5. Based on the main band, two different molecular weight (MW) of fragments were differentiated by each marker for the two parental genotypes. The studied promising lines always divided in two groups following the same molecular weight of both parents. Regarding to RM259 which amplified two alleles, the allele at 180 bp to detect the tolerant parent (IRAT170) followed by all new promising lines except PL9 which followed the susceptible parent (Sakha 105) at 120 bp molecular weight (Figure 2). The RM241 marker divided the studied genotypes into two levels of fragments, the highest level for susceptible parent along four promising lines (PL2, PL3, PL9 and PL10) at 190 bp, while the lowest level of band for the tolerant parent and the other seven lines at the same level at 180bp (Figure 3). Regarding to the marker RM 263, there were two promising lines (PL7 and pL9) had the same banding pattern with the susceptible parent at 90 bp molecular weight while the other lines followed the tolerant parent at 80 bp (Figure 4). Regarding to the marker RM 201, all studied promising lines had the same banding pattern with the tolerant parent at 120 bp molecular weight except two lines (PL6 and PL7) patterned with the susceptible parent at 110 bp (Figure 5). To conclude the SSR analysis result,

four markers related to drought tolerance showed high polymorphic pattern among the parental genotypes. Based the selected SSR markers, four promising lines showed the tolerant pattern with all polymorphic markers, while the other lines gave the tolerant pattern with some markers. It confirmed the morphological results which no superior lines for all studied traits. Due to complexity of drought tolerance, which controlled by a lot of genes there were some lines showed the tolerance with some markers and showed the opposite with others. The reason was the random distribution of drought tolerance genes into all promising lines during selections by observations under stress conditions.

Cluster analysis

To determine the strong degree of the relation of the genotypes and present in a simple way, we use the cluster analysis depending on the data out of all studied morphological traits during two years under normal, water shortage conditions and molecular analysis (Figures 6, 7 and 8). The cluster analysis based on normal condition data showed that the phylogenetic divided the 13 studied genotypes into two main groups; the first group just including the promising line PL3, while the second group including 12 genotypes. The second group divided into nearly three sub-groups; one of them including five lines along the tolerant parent IRAT170 and second sub-group including three lines along the susceptible parent Sakha 105, while the third sub-group including two lines PL4 and PL6, in addition to, some sub-groups belong to each main group. Regarding the data obtained from stress condition experiment, the cluster analysis showed different phylogenetic, present the genotypes into two main groups,

the first group just including the susceptible parent Sakha 105 with long distance from the second group which including the tolerant parent and all new lines. The large

group was divided in to sub-groups but the majority of the new lines were close to tolerant parent IRAT 170

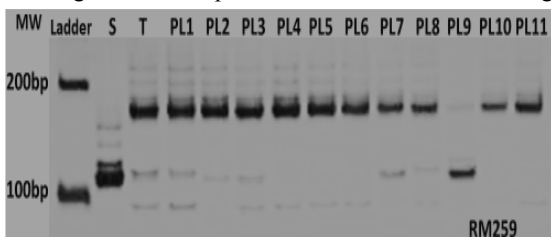


Figure 2. Banding patterns of 13 genotypes amplified with the SSR primer RM259

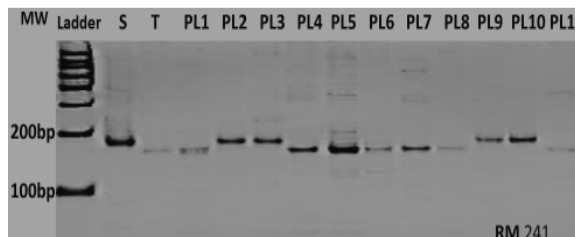


Figure 3. Banding patterns of 13 genotypes amplified with the SSR primer RM241

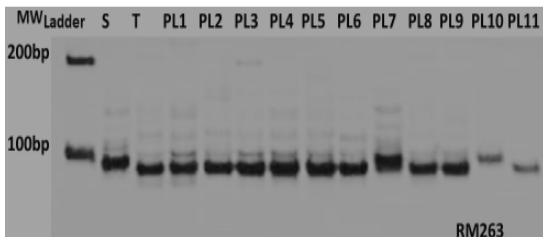


Figure 4. Banding patterns of 13 genotypes amplified with the SSR primer RM263

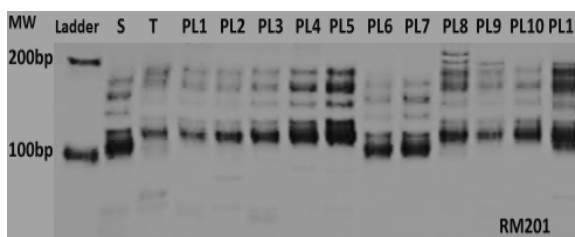


Figure 5. Banding patterns of 13 genotypes amplified with the SSR primer RM201

S and T are the parental genotypes; S: Sakha 105 as the susceptible and T: IRAT 170 as tolerant. PL1-PL11 are the new promising lines.

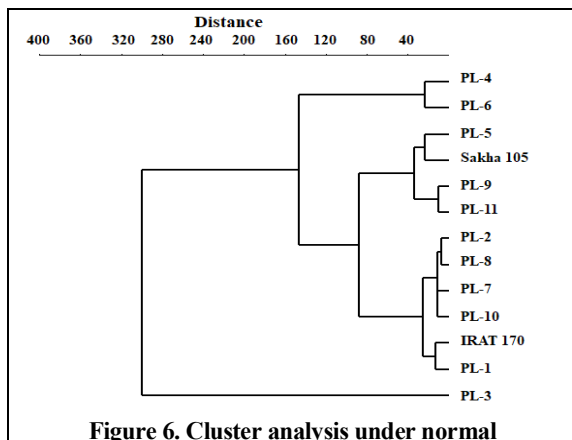


Figure 6. Cluster analysis under normal

Based on the data out of all SSR polymorphic markers (Figure 8), the cluster analysis showed that, the phylogenetic divided the studied genotypes into two main groups, in addition to some sub-groups belong to the second main group. The first group just including the susceptible parental genotype Sakha105 and the second group included the tolerant parental genotype along all promising lines in different subgroups. The promising lines PL1, PL4, PL5, PL8 and PL11 were too close to IRAT 170 in one sub-group with zero genetic distance. There are similar results obtained from cluster analysis based on molecular analysis and morphological data under stress conditions, which supports the importance of the selected primer for genetic differentiation in case of drought tolerance.

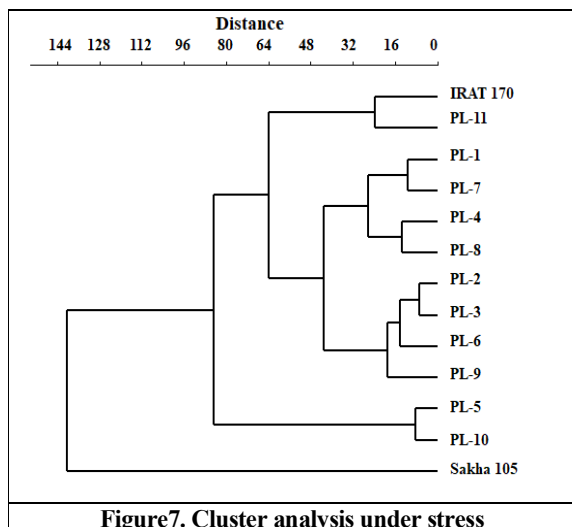


Figure7. Cluster analysis under stress

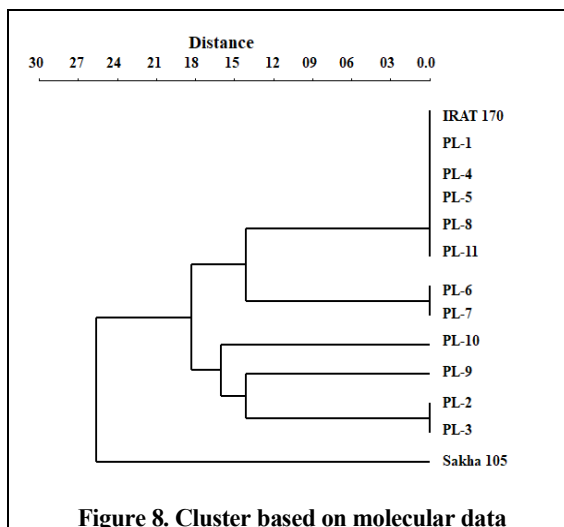


Figure 8. Cluster based on molecular data

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التقييم الحقلّي و الجزيني لسلاسل جديدة مبشره لتحمل ظروف نقص المياه في الارز وليد حسن الجمل مركز البحوث و التدريب في الأرز- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية- مصر

إنتاج أصناف متحملة لظروف نقص المياه تكون الطريقة الأحسن لمواجهة مشكلة محدودية المياه، كما أن الإختيار تحت الظروف المعاكسه يحتمل ان يكون الاستراتيجية الانفع خلال التربية ل مواد وراثية جديدة متحملة. تهدف الدراسة الحالية الي انتاج وتقييم سلاسل جديدة مبشرة الي جانب الاصناف الابوية لهم الصنف سخا 105 و الصنف IRAT170 لتحديد أي هذه السلاسل تبدو عالية الانتاجية تحت ظروف ندرة المياه و أجريت التجارب الحقلية بمزرعة محطة البحوث الزراعية بسخا كفر الشيخ مصر وتم التقييم في المواسم الزراعيه 2015 و 2016. صممت هذه الدراسة من أجل الوقوف علي أهم السلاسل المبشرة و المتأقلمة تحت ظروف نقص المياه وكذلك طريقه ملائمة لإنتاج مثل هذه السلاسل. أفادت النتائج المتحصل عليها بأن كل السلاسل المبشرة أظهرت أداء متميز تحت كل من الظروف الطبيعية والظروف الغير ملائمة و أظهرت السلاسل المبشرة PL3 و PL4 و PL6 أعلى قدرة محصولية تحت الظروف الطبيعية بقيم (1246,7 و 1071,7 و 990 جم / م²) تفوق محصول الاب الأعلى محصولا سخا 105 بينما أظهرت السلاسل المبشرة PL5 و PL6 و PL10 أعلى محصولا تحت ظروف ندرة المياه بقيم (783,3 و 709 و 736,7 جم / م²) أعلى من من الاب المتحمل IRAT170. أظهرت السلاسل PL2 و PL5 و PL10 أقل نسبة انخفاض محصولي ناتج عن ظروف نقص المياه طبقا لقيم دليل حساسية الجفاف بقيم (0,232 و 0,255 و 0,181) و هذه السلاسل المبشره سوف تقيم لصفات الجوده و في مواقع متعددة للمقارنه المحصوليه و يمكن استخدامها لتحسين الاصناف المنزرعه لتحمل ظروف نقص المياه. سجلت درجات تباين عاليه لكل الصفات و كان التباين البيئي أعلى من التباين الوراثي لكل الصفات المدروسة. كانت قيم المكافي الوراثي بالمعني الواسع عاليه لمعظم الصفات كما لوحظت قيم عاليه للتحسين الوراثي نسبة الي المتوسط لمعظم الصفات المدروسه دلالة علي أهمية دور التباين الوراثي لتوريث هذه الصفات. أظهرت المعلمات الجزئيه RM259 و RM241 و RM263 و RM201 تباين بين المواد وراثيه الحساسه و المتحملة لنقص المياه حيث كشفت عن أليلات مختلفه عند أوزان جزئيه تتراوح بين 80 و 190 زوج من القواعد و يمكن استخدام هذه المعلمات الجزئيه لتقييم المواد وراثيه في الدراسات المقبله. أكد التحليل الجزئي وجود محتوى كبير من التباين الوراثي ، وبالتالي الاستفادة من المواد المدروسة في تطوير برنامج التربية لتحمل نقص المياه.