

GENETICAL AND MOLECULAR STUDIES ON SALINITY AND DROUGHT TOLERANCE IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

The field experiments were conducted during 2013 and 2014 growing seasons at Kafrelsheikh University and Rice Research and Training Center (RRTC), Sakha, Kafrelsheikh, Egypt, facilities at El-Sirw Agriculture Research Station experimental farm, Damietta governorate, Egypt. Eight-parental half diallel cross and eight SSR molecular markers were used to determine combining ability of some common rice genotypes grown under normal, drought and salinity conditions. Analyses of variance were highly significant for days to heading, plant height, total chlorophyll content, proline content, sodium content, potassium content, Na^+/K^+ ratio and panicles plant⁻¹, filled grains panicle⁻¹ and grain yield plant⁻¹ indicated highly significant differences among the studied genotypes for this traits. The estimates of GCA effects indicated that three rice genotypes i.e. Giza178, A22 and WAB56-125 were the best general combiners for total chlorophyll content, proline content, sodium content, potassium content, Na^+/K^+ ratio, panicles plant⁻¹, filled grains panicle⁻¹ and grain yield plant⁻¹ under normal, salinity and drought conditions. The estimates of SCA effects indicated that the most desirable hybrids were Sakha105 x A22, Sakha102 x A22 and Sakha104 x Giza178 for total chlorophyll content, panicles plant⁻¹ and grain yield plant⁻¹; Giza178 x WAB56-125 for filled grains panicle⁻¹; Sakha102 x Sakha104 for days to heading; Sakha104 x Sakha105 for plant height. The genetic distance, measured using SSR markers, differed from 0.00 to 0.79 among the eight genotypes. Microsatellite markers were effective in predicting the mean and the variance of SCA in various cultivar combinations. In addition, RM223 marker elucidated the possibility to use it in MAS for salinity and drought tolerance in the studied rice genotypes according to different alleles.

INTRODUCTION

Rice (*Oryza sativa*, L.) is one of the most important crops in the world's, providing a staple food for nearly half of the global population (FAO, 2004). In Egypt, rice is considered the second important cereal crop, following wheat, as a main food for Egyptian population (Bastawisi *et al.*, 2003). In addition, rice constitutes one of the main agricultural exports. The rice area cultivated was 1.424 million feddans

with an average of 4.00 t fed⁻¹, and a total production of 5.70 million tons over the past five years (RRTC, 2014).

Rice is very sensitive to salinity stress and is currently listed as the most salt sensitive cereal crop with a threshold of 3dSm⁻¹ for most cultivated varieties (USDA, 2013). Rice yield in salt-affected land is significantly reduced with an estimation of 30–50% yield losses annually (Eynard *et al.*, 2005). Drought is the most significant limiting factor for plant agriculture worldwide, which can cause serious losses of yield and productivity in most crop plants in arid and sub-arid regions. The degree of these effects depends on its impact on the plant physiological, biochemical and the ability of plant to adapt to drought stress (Massonnet *et al.*, 2007). The major environmental factor that constrains the productivity and stability of plants is water stress (Araus *et al.*, 2002). Salinity and drought stresses are among the most serious challenges to crop production in the world today, particularly in developing countries (Zhou *et al.*, 2007). Drought and salinity tolerance can be designated as complex traits due to the interplay of multiple pathways that mediate a plant's capacity to withstand these abiotic stresses (Witcombe *et al.*, 2008).

Molecular markers offer specific advantages in assessment of genetic diversity and in trait specific crop improvement. The use genetic of markers in applied breeding programs can range from facilitating appropriate choice of parents for hybrids. SSR markers are more popular in rice because they are highly informative, mostly monolocus, codominant, easily analyzed and cost effective (Gracia *et al.*, 2004). This investigation aimed to study the GCA and SCA and their interaction with different stress conditions, also to identify some markers associated with drought and salinity tolerance. This study aimed to evaluate some rice genotypes under drought and salt stress using SSR marker linked to those traits.

MATERIALS AND METHODS

This investigation was carried out in Genetics Department laboratories, Faculty of Agriculture, Kafrelsheikh University, Egypt. Field experiment were carried out at the Rice Research and Training Center (RRTC) facilities at El-Sirw Agriculture Research Station experimental farm, Damietta governorate, Egypt, during 2013 and 2014 rice growing seasons. Eight rice genotypes namely; Sakha102, Sakha104, Sakha105, Sakha106, Giza178, A22, IRAT170, and WAB56-125 were chosen as a parental lines based on the previous studies according to RRTC data 2011. These genotypes have a wide range of variation for salt and drought tolerance due to their different genetic backgrounds. The pedigree, group type and main characters of these varieties are shown in Table (1). Each parent was grown in five

rows; each row was 5 meter long and contained 25 hills. At flowering the five parents were diallel crossed, i.e., in all possible combinations (excluding reciprocal) to produce F₁ hybrid seeds following the method proposed by Jodon (1938) and modified by Butany (1961).

Table 1: Name, origin, parentage, type, salinity and drought tolerance of the eight rice varieties

Variety	Origin	Parentage	Type	Salinity tolerance	Drought tolerance
Sakha102	Egypt	Giza176/Milyang79	Japonica	Sensitive	Sensitive
Sakha104	Egypt	GZ4096-7-1/GZ4120-2-5-2 (Giza177)	Japonica	Moderate	Moderate
Sakha105	Egypt	GZ5581-46-3/GZ4316-7-1-1	Japonica	Sensitive	Sensitive
Sakha106	Egypt	Giza177/Hexi30	Japonica	Sensitive	Sensitive
Giza178	Egypt	Giza175/Milyang49	Indica/ Japonica	Tolerant	Tolerant
A22	Sri-lank	IR47664	Indica	Tolerant	Tolerant
IRAT170	Côte d'Ivoire	IRAT13/Palawan	Tropical-Japonica	-	Tolerant
WAB56-125	Côte d'Ivoire	IDSA6 / IAC164	Indica	-	Tolerant

The eight parents and their 28 F₁ hybrid were grown in a randomized complete block design with three replications under three conditions (normal, salinity with E_Ce 8.88dsm⁻¹ and drought conditions), where drought stress was imposed by using flush irrigation every 10 days without standing water after irrigation. All the data were recorded on the parents and their crosses in three sites. All recommended agricultural practices were applied according to rice research program, Egypt. At ripening, each plant was harvested individually.

Soil analysis:

The procedure for preparation and measurements of the soil extract was taken according to the method of Black *et al.* (1965). The methods of Chapman and Parker (1961) of soil chemical analysis were followed. The chemical analysis of experimental sites was listed as shown in Table (2).

Table 2: Chemical and physical analyses of the experimental sites during 2014

Character	2014		
	Normal	Salinity	Drought
E.C.	2.37	8.88	2.0
P.H.	8.09	8.42	8.1
Soluble ions:	(meq/L)		
Ca ⁺⁺	2.8	10.9	2
Mg ⁺⁺	2.9	7.1	1
Na ⁺	18.02	70	16.49
K ⁺	0.37	0.39	0.59
Co ₃ ⁼	2.07	5.08	2
Hco ₃ ⁻	1.4	8	1.1
Cl ⁻	15.5	65	12.9
So ₄ ⁼	6	10	4

Studied characters:

Plants of twenty hills were randomly taken from each parent and F₁ cross, from each replicate. Data were collected on ten rice traits, viz; days to heading, plant height, total chlorophyll content, proline content, sodium content, potassium content, Na⁺/K⁺ ratio, panicles plant⁻¹, filled grains panicle⁻¹ and grain yield plant⁻¹. Where, Proline content was estimated according to Sadasivam and Manickam (1996), furthermore, Na⁺ and K⁺ leaf content were determined by flam photometer according to Richards (1954).

Statistical Analysis:

Data of the three treatments were subjected to Randomized Complete Block Design (RCBD) according to Snedecor and Cochran (1967). At first, the data were analyzed by using the ordinary analysis of variance to test the significance of differences among the genotypes studied (eight parents and their crosses). If the genotypic mean squares were found to be significant, there was a need to proceed for further analysis; i.e., Griffing (1956) mode 1, method 2. Estimates of combining ability analysis were estimated according to Griffing, 1956 model 1 method 2.

Molecular studies:-**DNA isolation:-**

Genomic DNA was isolated from 0.5g of three week old leaves of the used rice genotypes using CTAB method described by (Murray and Thompson, 1980).

SSR markers and PCR amplification:-

Eight SSR markers i.e. RM 219, RM 201, RM3805, RM 72, RM 223, RM 315, RM8094 and RM 25 were used. The sequences of

primer pairs are found on the Web database (<http://www.gramene.org>). PCR amplification reactions were done according to Lodha *et al.* (2011). Primers names, repeat motifs and chromosome number are shown in Table (3).

SSR data analysis:-

The amplified bands were scored for each SSR marker based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. Effective alleles per locus (Aep) were calculated according to Weir (1989) ($Aep = 1 / (1 - H_e)$, where H_e , the genetic diversity per locus). Genetic diversity was calculated according to Nei (1973). Polymorphic information content (PIC) values were calculated for each SSR marker by using the formula described by Anderson *et al.* (1993) as follows:

$$(PIC = 1 - \sum p_i^2, \text{ where } p_i \text{ is the frequency of the } i\text{th allele})$$

Phylogenetic tree construction:-

The presence/absence matrix for amplified DNA fragments was analyzed using the PAST program, ver. 1.90 (Hammer *et al.*, 2001). Data matrix was used to calculate genetic similarity based on Jaccard's similarity coefficients, and dendrogram displaying relationships among eight rice genotypes was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Table 3: Name; chromosome number (CN); SSR motifs and the sequences of the SSR markers used in the current study and related traits

No.	Markers	CN	SSR motif	Sequence	Trait	References
1	RM 219	9	(CT)17	F CGTCGGATGATGTAAGCCT	Salinity	Chatterjee and Reddy (2014)
				R CATATCGGCATTGCGCTG		
2	RM 201	9	(CT)17	F CGTTTATTACCTACAGTACC	Drought	Chaitra <i>et al.</i> (2006)
				R CTACCTCCTTTCTAGACCGATA		
3	RM3805	6	(GA)19	F AGAGGAAGAAGCCAAGGAGG	Drought	Ramadan <i>et al.</i> (2015)
				R CATCAACGTACCAACCATGG		
4	RM 72	8	(TAT)5C(ATT)15	F CCGGCGATAAAACAATGAG	Drought	Lin <i>et al.</i> (2007)
				R GCATCGGTCCTAACTAAGGG		
5	RM 223	8	(CT)25	F GAGTGAGCTTGGGCTGAAAC	Drought	Kumar <i>et al.</i> (2005)
				R GAAGGCAAGTCTTGGCACTG		
6	RM 315	1	(AT)4(GT)10	F GAGGTAICTTCTCCGTTTCAC	Salinity	Chatterjee and Reddy (2014)
				R AGTCAGCTCACTGTGCAGTG		
7	RM8094	1	(AT)31	F AAGTTTGTACACATCGTATACA	Salinity	Mohammadi-Nejad <i>et al.</i> (2008)
				R CGCGACCACTACTACTACTA		
8	RM 25	8	(GA)18	F GGAAAGAATGATCTTTTCATGG	Drought	Matin <i>et al.</i> (2012)
				R CTACCATCAAACCAATGTTC		

RESULTS AND DISCUSSION

Analysis of variance

Mean squares of genotypes (parents and their crosses) were found to be highly significant for ten characters studied at the three conditions, indicating overall differences among these populations (Table 4). General and specific combining ability variances were found to be highly significant for all characteristics studied under the three conditions. These results would indicate the importance of both

additive and non-additive genetic variances in determining the performance of these characteristics. GCA/SCA ratio was more than unity for filled grains panicle⁻¹ under the three conditions; grain yield plant⁻¹ under salinity and drought stress; days to heading under normal condition; K⁺ content and Na⁺/K⁺ ratio under drought condition; and panicles plant⁻¹ under salinity, suggesting the importance of additive type of gene action in the inheritance of these traits under these environments. On the other hand, GCA/SCA ratio was less than unity for plant height, Na⁺ content, proline content, total chlorophyll under the three conditions; days to heading under salinity and drought conditions; K⁺ content and Na⁺/K⁺ ratio under normal and salinity conditions; panicles plant⁻¹ under normal and drought; and grain yield plant⁻¹ under normal condition suggesting the importance of non-additive gene action in the inheritance of these traits. It is therefore, could be concluded that selection procedures based on the accumulation of additive effects would be successful in improving these characters. These findings were in agreement with those reported by EL-Mowafi and Abou Shousha (2003), Hammoud *et al.* (2008), Shehata *et al.* (2009) and El-Mouhamady *et al.* (2010).

Mean performance

Data in Table (5) indicated that, the most desirable mean values among parents were Giza178 for panicles plant⁻¹, proline content, sodium content, potassium content, Na/k ratio, filled grains panicle⁻¹ and grain yield plant⁻¹ followed by WAB56-125 and A22 for most traits under three conditions. Moreover, Sakha105 the most desirable mean values among parents for plant height and Sakha102 for days to heading under three conditions. Furthermore, the best crosses for mean values were Sakha102 × Giza178, Sakha102 × A22, Sakha102 × Giza178, Sakha102 × Giza178, Giza178 × WAB56-125, A22 × WAB56-125 and Sakha105 × WAB56-125 for chlorophyll content, proline content, sodium content, potassium content, Na/K ratio, panicles plant⁻¹, filled grains panicles⁻¹ and grain yield plant⁻¹ under three conditions. While, the most desirable mean values under the three conditions were Sakha105 × Sakha106, Sakha106 × IRAT170 for plant height; Sakha102 × Sakha105, Sakha102 × Sakha105 for days to heading. Finally these parents and crosses could be used in breeding programs to enhance these characters. These findings were in conformity with that reported by Weerakoon *et al.* (2008), El-Mouhamady (2009), Shehata *et al.* (2009) and Zayed *et al.* (2014).

Combining ability:

General combining ability effects:

Data in Table (6) showed that, the best combiners under normal, salinity and drought conditions were Sakha105 and Sakha106

for plant height; Sakha102 and Sakha106 for days to heading; Giza178, WAB56-125 and A22 for total chlorophyll content, proline content, sodium content, potassium content, Na⁺/K⁺ ratio and panicles plant⁻¹; Giza178, WAB56-125 and A22 for filled grains panicle⁻¹; Giza178, WAB56-125 and A22 for grain yield plant⁻¹. So, this parents could be used as a good combiner for improve this trait under these conditions. Those parents showed better performance under salt and drought stresses that attributed to its high ion selectivity with high ability to proline releasing resulted in high tolerance and improving rice growth as well as yield and yield components. These results were in agreement with those reported by Shehata *et al.* (2009), El-Mouhamady *et al.* (2010) and Sedeek *et al.* (2012).

Specific combining ability effects:

From the results in Table (7), the most desirable hybrids were, Sakha105 X A22, Sakha102 x A22 and Sakha104 x Giza178 for total chlorophyll content, panicles plant⁻¹ and grain yield plant⁻¹; Giza178 x WAB56-125 for filled grains panicle⁻¹; Sakha102 X Sakha104 for days to heading; Sakha104 X Sakha105 for plant height. These crosses exhibited these results under three studied conditions. Those crosses had the same pattern of their tolerant parents for studied stresses regarding studied traits. Moreover, these crosses could be used in breeding programs to improve this trait under drought and saline soil conditions. These results were reported by Shehata *et al.* (2009), El-Mouhamady *et al.* (2010) and Sedeek *et al.* (2012).

Table 4: Mean square estimates of ordinary analysis and combining ability analysis for all traits under normal, salinity and drought conditions

Sources of variance	df	Days to Heading (days)			Plant height (cm)			Panicles plant ⁻¹			Filled grains panicle ⁻¹			Grain yield plant ⁻¹ (g)		
		N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Replications	2	2.398	7.11	0.15	1.95	8.08	1.42	4.29	0.59	1.24	4.87	0.22	0.46	0.87	0.61	0.99
Genotypes	35	52.64**	41.71**	64.14**	258.4**	328.0**	263.1**	28.23**	59.95**	33.10**	564.10**	612.20**	652.31**	272.1**	142.5**	140.8**
Parents (P)	7	83.61**	48.19**	90.26**	109.5**	145.8**	143.1**	20.99**	82.74**	44.05**	440.82**	614.54**	895.80**	101.9**	156.0**	170.0**
Crosses (C)	27	45.26**	40.66**	58.38**	275.3**	371.8**	251.4**	31.00**	52.94**	31.33**	616.90**	632.97**	612.90**	322.8**	141.6**	137.0**
P. Vs C.	1	34.98**	24.38**	36.84**	843.0**	421.2**	1420**	4.13	89.57**	4.12**	1.62	35.18**	11.94**	94.87**	71.43**	36.49**
Error	70	1.22	3.48	1.14	3.23	2.90	4.76	1.46	0.76	0.75	1.82	1.28	2.25	0.77	0.54	0.99
GCA	7	64.88**	44.57**	65.42**	111.9**	190.5**	146.1**	31.73**	87.36**	35.59**	684.03**	915.88**	948.33**	282.9**	190.4**	167.8**
SCA	28	5.72**	6.23**	10.37**	79.67**	89.04**	73.12**	3.83**	3.14**	4.89**	64.04**	26.12**	34.72**	42.66**	11.77**	16.71**
Error	70	0.41	1.16	0.38	1.08	0.97	1.59	0.49	0.26	0.25	0.61	0.43	0.75	0.26	0.18	0.33
GCA/SCA		1.21	0.86	0.65	0.14	0.22	0.20	0.93	3.02	0.76	1.08	3.56	2.79	0.67	1.64	1.02

Table 4: continue...

Sources of variance	df	Total chlorophyll content (SPAD)			Proline content ($\mu\text{g/g}$)			Na ⁺ /K ⁺ ratio			Na ⁺ content (%)			K ⁺ content (%)		
		N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Replications	2	0.07	0.83	2.41	0.024	0.12	0.72	0.0002	0.0001	0.0003	0.0002	0.0007	0.0013	0.0013	0.0001	0.0002
Genotypes	35	26.46**	54.46**	35.72**	0.756**	30.85**	71.21**	0.0100**	0.2666**	0.0523**	0.0172**	0.2363**	0.0563**	0.1928**	0.0560**	0.1180**
Parents (P)	7	11.13**	25.93**	22.71**	0.814**	36.50**	59.71**	0.0066**	0.2989**	0.0522**	0.0057**	0.2252**	0.0500**	0.1177**	0.0654**	0.1326**
Crosses (C)	27	29.92**	57.31**	36.84**	0.764**	29.43**	74.65**	0.0103**	0.2661**	0.0541**	0.0196**	0.2479**	0.0589**	0.2064**	0.0539**	0.1182**
P. Vs C.	1	40.43**	177.4**	96.51**	0.134	29.73**	58.79**	0.0251**	0.0541**	0.0038**	0.0324**	0.0010	0.0284**	0.3511**	0.0463**	0.0109**
Error	70	1.71	0.70	1.49	0.055	0.33	0.46	0.0003	0.0001	0.0007	0.0006	0.0005	0.0013	0.0033	0.0001	0.0019
GCA	7	10.63**	52.96**	29.45**	0.662**	30.40**	65.51**	0.0100**	0.3105**	0.0633**	0.0116**	0.2321**	0.0551**	0.1964**	0.0637**	0.1460**
SCA	28	8.37**	9.45**	7.52**	0.149**	5.25**	13.29**	0.0017**	0.0335**	0.0060**	0.0043**	0.0404**	0.0097**	0.0312**	0.0074**	0.0127**
Error	70	0.57**	0.23	0.50	0.018	0.11	0.15	0.0001	0.0001	0.0002	0.0002	0.0002	0.0004	0.0011	0.0001	0.0006
GCA/SCA		0.13	0.57	0.41	0.49	0.59	0.50	0.64	0.93	1.10	0.28	0.58	0.59	0.65	0.86	1.21

* and **: Significant at 0.05 and 0.01 levels of probability, respectively, N = Normal environment, S = salinity and D = Drought environment.

Table 5: Mean performance of ten traits in the studied rice genotypes under normal, salinity and drought conditions

Genotypes	Traits	Days to Heading (days)			Plant height (cm)			Panicles plant ⁻¹			Filled grains panicle ⁻¹			Grain yield plant ⁻¹ (g)		
		N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Sakha102		93.00	94.33	92.00	101.0	69.00	80.00	17.33	6.00	9.67	99.85	56.17	65.33	34.67	6.34	12.33
Sakha104		107.0	105.3	103.3	99.00	76.67	83.00	19.33	10.33	13.67	101.8	60.19	70.22	40.77	12.46	17.11
Sakha105		96.67	97.33	93.00	92.00	67.33	70.00	18.33	4.00	8.53	98.14	55.15	60.33	36.62	5.67	11.21
Sakha106		95.33	96.67	91.00	95.67	71.33	74.00	18.67	6.667	12.23	104.8	53.35	62.33	37.72	7.90	14.50
Giza178		104.3	104.7	103.0	94.33	86.00	90.00	23.33	17.33	19.00	128.5	85.98	101.3	49.32	23.33	30.33
A22		106.0	103.3	104.0	98.33	83.67	87.33	20.00	15.33	18.00	120.3	84.43	95.67	47.29	21.43	26.90
IRAT170		103.7	101.3	100.7	112.0	75.33	84.33	15.33	4.333	13.00	115.2	56.59	88.25	34.00	8.37	20.24
WAB56-125		102.0	101.0	100.3	99.33	82.00	86.80	22.67	14.00	17.00	125.5	80.15	97.67	44.33	19.35	28.23
Sakha102 X Sakha104		98.33	98.00	93.67	97.33	87.00	88.33	17.00	8.67	12.33	98.83	65.15	67.96	34.60	9.17	16.17
Sakha102 X Sakha105		97.67	96.67	95.00	95.33	66.00	82.33	14.67	5.67	7.33	89.33	48.67	60.43	25.67	5.08	10.78
Sakha102 X Sakha106		93.00	94.00	92.67	97.00	66.00	80.00	16.00	6.00	10.67	95.26	52.33	63.25	30.80	5.17	12.55
Sakha102 X Giza178		100.7	98.00	96.33	114.3	86.00	100.7	24.33	15.33	18.67	114.7	73.33	88.67	55.33	20.95	24.91
Sakha102 X A22		104.3	102.3	99.33	117.7	97.67	100.0	23.67	14.33	18.33	116.0	77.51	84.26	51.93	21.46	28.16
Sakha102 X IRAT170		102.7	102.3	100.7	116.0	62.33	97.00	18.33	5.00	13.00	100.5	56.29	78.25	39.17	4.96	21.14
Sakha102 X WAB56-125		99.67	99.00	96.00	114.0	88.33	99.00	19.67	13.33	14.67	124.0	77.26	84.19	43.33	20.45	26.21
Sakha104 X Sakha105		101.0	102.3	99.67	93.00	68.67	71.00	17.67	8.33	11.33	97.62	49.99	53.48	26.12	6.82	9.97
Sakha104 X Sakha106		103.0	104.3	104.0	94.33	68.67	72.33	16.00	8.67	9.33	87.85	48.55	53.67	25.71	6.41	8.00
Sakha104 X Giza178		106.7	104.3	105.7	117.3	90.00	93.33	24.67	17.67	18.33	115.8	82.91	85.25	51.73	26.47	28.63
Sakha104 X A22		110.3	110.0	109.3	107.3	95.33	97.67	21.67	18.33	16.00	130.5	81.19	88.67	47.33	21.69	24.17
Sakha104 X IRAT170		108.0	107.0	106.0	107.3	85.67	91.67	16.67	9.67	11.33	116.6	60.23	83.29	38.67	11.24	20.89
Sakha104 X WAB56-125		109.0	108.0	108.3	98.67	90.33	93.00	19.67	15.67	12.67	127.6	78.67	88.30	45.22	22.63	27.21
Sakha105 X Sakha106		98.67	100.0	97.33	85.33	68.67	71.00	18.00	5.67	10.00	87.64	43.35	55.29	27.95	6.63	12.67
Sakha105 X Giza178		100.0	98.33	98.00	106.0	80.67	93.67	21.33	12.67	15.67	111.2	63.56	70.67	44.24	17.92	21.33
Sakha105 X A22		102.0	101.3	100.7	120.3	96.00	102.3	23.67	14.00	18.33	114.5	65.74	77.29	58.33	18.56	30.65
Sakha105 X IRAT170		107.0	102.7	100.7	101.0	79.00	81.67	14.67	5.67	10.00	108.7	55.73	78.67	30.90	6.43	15.77
Sakha105 X WAB56-125		104.7	105.3	103.0	113.0	88.00	96.33	23.33	11.33	15.00	112.0	67.26	74.67	54.92	13.14	22.13
Sakha106 X Giza178		97.67	98.67	98.00	105.3	78.33	96.67	20.33	13.67	16.67	115.3	68.67	72.22	40.59	16.20	17.75
Sakha106 X A22		100.3	103.0	98.67	116.3	98.67	104.3	21.67	15.67	17.33	115.3	68.67	79.33	51.67	21.10	25.36
Sakha106 X IRAT170		99.00	98.67	96.67	99.33	60.00	87.67	15.67	6.67	11.33	106.7	53.15	81.67	40.48	7.13	16.85
Sakha106 X WAB56-125		103.3	102.7	100.7	109.0	80.33	98.00	18.00	12.33	16.33	111.3	64.48	78.67	38.40	14.60	20.68
Giza178 X A22		103.7	98.00	96.00	97.67	81.00	85.00	22.33	17.00	13.33	148.5	94.26	95.22	56.00	18.81	21.74
Sakha106 X IRAT170		100.7	98.33	93.33	114.7	86.00	95.33	19.67	15.33	17.00	111.3	73.67	89.15	41.97	20.31	32.06
Giza178 X WAB56-125		102.3	102.7	97.00	98.00	76.33	89.00	23.00	17.00	18.33	135.9	99.94	110.7	54.11	25.24	32.90
A22 X IRAT170		105.0	105.3	104.7	119.0	86.00	89.67	21.00	13.33	13.67	109.3	64.67	85.67	49.33	15.65	21.76
A22 X WAB56-125		105.7	104.0	101.7	96.00	71.00	89.33	25.33	15.67	15.00	128.5	93.97	110.1	57.60	20.12	26.05
IRAT170 X WAB56-125		102.0	100.7	102.0	108.3	90.67	92.00	17.67	11.67	17.00	106.8	71.22	84.67	37.52	17.37	25.63
LSD 0.05		1.80	3.05	1.74	2.93	2.78	3.56	1.97	1.43	1.42	2.20	1.84	2.45	1.44	1.20	1.62
LSD 0.01		2.40	4.05	2.33	3.90	3.70	4.74	2.62	1.90	1.89	2.93	2.45	3.26	1.91	1.59	2.16

N=normal s=salinity d=drought

Table 5: Continue.....

Genotypes	Traits	Total chlorophyll content (SPAD)			Proline content (µg/g)			Na ⁺ /k ⁺ ratio			Na ⁺ content (%)			K ⁺ content (%)		
		N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Sakha102		45.63	33.60	39.00	1.81	11.17	14.70	0.304	1.641	0.681	0.591	2.024	1.093	1.944	1.234	1.605
Sakha104		48.20	38.63	40.60	2.40	14.67	19.43	0.292	1.252	0.551	0.526	1.700	0.904	1.802	1.359	1.641
Sakha105		44.17	34.43	36.33	1.40	8.90	13.47	0.352	1.867	0.698	0.574	2.381	1.043	1.633	1.276	1.495
Sakha106		46.67	35.40	37.23	2.07	11.33	17.15	0.299	1.457	0.655	0.533	1.878	0.991	1.782	1.290	1.514
Giza178		49.27	41.83	43.33	2.95	19.10	24.70	0.208	0.955	0.357	0.465	1.556	0.730	2.238	1.630	2.046
A22		49.53	40.73	42.33	2.30	17.87	20.03	0.240	1.063	0.398	0.505	1.595	0.758	2.104	1.501	1.907
IRAT170		48.90	36.40	41.63	1.87	13.60	18.40	0.297	1.384	0.568	0.546	1.816	0.952	1.842	1.312	1.675
WAB56-125		48.53	37.53	43.67	2.81	15.57	26.30	0.234	1.055	0.443	0.481	1.645	0.865	2.054	1.559	1.952
Sakha102	X	47.50	35.77	37.67	2.20	10.50	15.50	0.294	1.228	0.671	0.551	1.801	1.007	1.873	1.466	1.501
Sakha104	X	41.67	32.67	37.67	1.77	9.73	10.27	0.330	1.463	0.778	0.595	2.072	1.175	1.808	1.416	1.511
Sakha105	X	40.97	35.50	35.20	2.57	11.73	13.43	0.262	1.299	0.708	0.522	1.824	1.075	1.994	1.405	1.522
Sakha106	X	51.43	46.00	46.33	2.64	16.13	17.53	0.153	1.123	0.366	0.405	1.737	0.697	2.647	1.547	1.905
Giza178	X	48.13	45.00	45.63	2.84	12.83	22.63	0.192	1.167	0.487	0.461	1.754	0.855	2.402	1.503	1.755
Sakha102 X A22		45.60	39.50	41.33	1.67	10.27	19.40	0.258	1.627	0.499	0.522	2.201	0.925	2.026	1.352	1.853
IRAT170	X	46.73	42.33	43.67	2.10	16.73	19.70	0.178	0.971	0.404	0.427	1.419	0.757	2.402	1.461	1.874
Sakha102	X	45.98	38.57	39.33	1.77	14.77	17.83	0.335	1.393	0.709	0.629	1.820	1.132	1.876	1.306	1.597
WAB56-125	X	40.37	34.00	38.67	1.44	13.83	15.13	0.317	1.549	0.707	0.587	2.173	1.123	1.849	1.403	1.589
Sakha104	X	42.87	39.70	38.30	2.87	16.67	29.17	0.177	0.752	0.389	0.365	1.191	0.743	2.065	1.583	1.907
Giza178	X	39.13	37.90	38.90	2.73	15.57	22.43	0.190	1.090	0.520	0.386	1.644	0.958	2.026	1.508	1.842
Sakha104 X A22		45.93	35.63	42.60	2.40	14.77	24.37	0.289	1.711	0.650	0.544	2.055	1.040	1.878	1.201	1.618
IRAT170	X	41.40	37.20	40.63	2.97	17.57	21.27	0.149	1.313	0.478	0.288	1.865	0.861	1.931	1.420	1.803
Sakha104	X	45.20	36.63	38.33	1.54	11.37	12.80	0.321	1.698	0.887	0.499	2.069	1.260	1.610	1.218	1.421
WAB56-125	X	49.00	45.43	46.20	3.10	19.60	25.50	0.168	1.159	0.466	0.377	1.684	0.822	2.241	1.453	1.766
Sakha105	X	49.97	44.43	45.77	2.40	18.53	23.33	0.194	1.106	0.501	0.454	1.568	0.977	2.336	1.418	1.950
Giza178	X	45.90	33.27	44.07	1.67	15.13	18.63	0.299	1.878	0.707	0.537	2.481	1.086	1.795	1.321	1.541
Sakha105 X A22		47.73	42.80	45.47	1.71	15.67	20.80	0.233	1.170	0.598	0.428	1.820	1.004	1.836	1.555	1.680
IRAT170	X	50.93	44.63	46.40	2.97	21.77	27.97	0.256	1.188	0.411	0.536	1.721	0.765	2.093	1.448	1.861
WAB56-125	X	48.43	43.13	46.83	2.22	17.90	26.70	0.274	1.061	0.477	0.524	1.725	0.804	1.915	1.627	1.684
Sakha106	X	46.70	36.73	43.10	1.52	14.57	21.63	0.308	1.914	0.666	0.620	2.381	1.097	2.011	1.244	1.647
IRAT170	X	48.10	42.00	46.03	2.74	16.70	23.70	0.250	1.283	0.549	0.466	1.823	0.984	1.863	1.421	1.791
Sakha106	X	48.10	47.47	47.80	2.07	17.57	18.90	0.182	0.989	0.410	0.427	1.580	0.880	2.349	1.598	2.149
WAB56-125	X	46.03	44.33	43.87	2.44	15.20	25.73	0.286	1.098	0.611	0.513	1.539	0.980	1.796	1.402	1.604
Giza178	X	46.27	44.30	44.30	2.57	16.47	23.53	0.203	0.856	0.447	0.489	1.564	0.882	2.415	1.827	1.975
IRAT170	X	45.33	42.87	44.40	1.94	13.37	18.30	0.257	1.079	0.512	0.533	1.627	1.017	2.074	1.508	1.987
WAB56-125	X	48.43	45.30	45.33	2.53	21.30	30.80	0.173	1.089	0.418	0.447	1.666	0.917	2.581	1.530	2.193
IRAT170	X	48.10	38.20	44.30	2.64	11.80	22.33	0.232	1.588	0.602	0.475	2.075	0.947	2.047	1.306	1.574
WAB56-125	X	2.13	1.37	1.99	0.38	0.94	1.10	0.030	0.019	0.044	0.040	0.035	0.059	0.094	0.016	0.072
LSD 0.05		2.84	1.82	2.65	0.51	1.26	1.47	0.040	0.026	0.059	0.053	0.046	0.078	0.126	0.021	0.096

N=normal s=salinity d=drought

Table 6: Estimates of general combining ability (GCA) effects for all traits for eight parents under normal, salinity and drought conditions

Traits Parents	Days to Heading (days)			Plant height (cm)			Panicles plant ⁻¹			Filled grains panicle ⁻¹			Grain yield plant ⁻¹ (g)		
	N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Sakha102	-3.63**	-3.35**	-3.79**	1.60**	-2.96**	0.89**	-0.93**	-2.28**	-1.39**	-6.96**	-4.52**	-5.80**	-3.09**	-3.17**	-2.62**
Sakha104	3.18**	3.22**	3.77**	-2.43**	1.80**	-2.51**	-0.57**	0.46**	-0.66**	-2.94**	-2.10**	-5.46**	-3.02**	-0.23**	-2.15**
Sakha105	-1.43**	-1.12**	-1.53**	-3.97**	-3.93**	-6.01**	-0.77**	-3.18**	-2.35**	-9.07**	-10.35**	-12.45**	-3.97**	-4.57**	-4.50**
Sakha106	-3.29**	-1.78**	-2.56**	-3.97**	-5.77**	-4.05**	-1.47**	-2.11**	-1.21**	-7.90**	-10.22**	-10.89**	-5.00**	-3.86**	-4.79**
Giza178	0.18	-0.48**	-0.52**	0.43*	2.93**	3.52**	2.47**	4.03**	2.77**	10.17**	12.02**	9.88**	6.15**	6.09**	4.93**
A22	2.48**	1.82**	2.27**	3.33**	7.20**	4.46**	2.17**	3.59**	1.97**	9.54**	10.68**	9.62**	8.57**	4.86**	4.09**
IRAT170	1.31**	0.52**	0.97**	5.20**	-2.07**	0.52*	-2.33**	-2.71**	-0.89**	-1.77**	-6.00**	4.22**	-3.51**	-3.18**	0.38**
WAB56-125	1.21**	1.18**	1.38**	-0.20	2.80**	3.18**	1.43**	2.19**	1.77**	8.92**	10.50**	10.89**	3.87**	4.06**	4.65**
S.E. (gi)	0.19	0.32	0.18	0.31	0.29	0.37	0.21	0.15	0.15	0.23	0.19	0.26	0.15	0.13	0.17
S.E.(gi-gi)	0.28	0.48	0.28	0.46	0.44	0.56	0.31	0.23	0.22	0.35	0.29	0.39	0.23	0.19	0.26

Table 6: continue....

Traits Parents	Total chlorophyll content (SPAD)			Proline content (µg/g)			Na ⁺ /k ⁺ ratio			Na ⁺ content (%)			K ⁺ content (%)		
	N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Sakha102	0.49**	1.35**	1.51**	0.10**	2.48**	3.80**	0.003*	0.053**	0.028**	0.021**	0.049**	0.015**	0.076**	0.028**	0.060**
Sakha104	1.86**	2.14**	2.33**	0.08**	0.21**	-0.13*	0.009**	0.009**	0.023**	0.006**	0.042**	0.014**	0.118**	0.030**	0.060**
Sakha105	0.44**	1.48**	1.11**	0.36**	1.25**	2.98**	0.034**	0.197**	0.105**	0.021**	0.191**	0.102**	0.152**	0.066**	0.128**
Sakha106	0.42**	1.40**	1.15**	0.13**	0.45**	1.02**	0.034**	0.128**	0.072**	0.036**	0.110**	0.056**	0.139**	0.056**	0.119**
Giza178	1.50**	3.81**	1.93**	0.42**	2.65**	3.19**	0.041**	0.256**	0.118**	0.042**	0.224**	0.130**	0.180**	0.122**	0.153**
A22	0.84**	3.01**	1.88**	0.09**	1.77**	1.73**	0.030**	0.192**	0.087**	0.022**	0.161**	0.060**	0.160**	0.079**	0.164**
IRAT170	0.32*	1.41**	0.64**	0.24**	1.28**	0.13*	0.028**	0.204**	0.039**	0.038**	0.162**	0.047**	0.097**	0.094**	0.056**
WAB56-125	0.56**	0.97**	1.65**	0.25**	1.23**	2.89**	0.036**	0.125**	0.061**	0.047**	0.085**	0.044**	0.090**	0.074**	0.106**
S.E. (gi)	0.22	0.14	0.21	0.04	0.10	0.12	0.003	0.002	0.005	0.004	0.004	0.006	0.010	0.002	0.008
S.E.(gi-gi)	0.34	0.22	0.32	0.06	0.15	0.17	0.005	0.003	0.007	0.006	0.006	0.009	0.015	0.003	0.011

* and **: Significant at 0.05 and 0.01 levels of probability, respectively, N = Normal environment, S= salinity and D = Drought environment

Table 7: Estimates of specific combining ability (SCA) effects for agronomic traits of 28 hybrids under normal, salinity and drought conditions

Hybrids	Days to Heading (days)			Plant height (cm)			Panicles plant ⁻¹			Filled grains panicle ⁻¹			Grain yield plant ⁻¹ (g)		
	N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Sakha102 X Sakha104	-3.28**	-3.26**	-5.83**	-6.02**	8.06**	1.24	-1.24	-0.97	0.14	-3.26**	4.21**	-0.30	-1.63*	-2.06**	-0.26
Sakha102 X Sakha105	0.65	-0.26	0.81	-6.49**	-7.21**	-1.26	-3.37**	-0.34	-3.17**	-6.63**	-4.03**	-0.83	-9.61**	-1.80**	-3.29**
Sakha102 X Sakha106	-2.15**	-2.26*	-0.49	-4.82**	-5.38**	-5.56**	-1.34	-1.07	-0.98	-1.87*	-0.49	0.43	-3.45**	-2.42**	-1.24
Sakha102 X Giza178	2.05*	0.44	1.14	8.12**	5.92**	7.54**	3.06**	2.13**	3.04**	-0.53	-1.73*	5.07**	9.93**	3.41**	1.41
Sakha102 X A22	3.42**	2.48*	1.34	8.55**	13.32**	5.94**	2.69**	1.56*	3.50**	1.42	3.79**	0.92	4.11**	5.14**	5.49**
Sakha102 X IRAT170	2.92**	3.78**	3.97**	5.02**	-12.7**	6.87**	1.86*	-1.47*	1.04	-2.78**	-0.76	0.32	3.42**	-3.31**	2.19**
Sakha102 X WAB56-125	0.02	-0.22	-1.09	8.42**	8.39**	6.21**	-0.57	1.96**	0.04	10.08**	3.72**	-0.42	0.21	4.93**	2.98**
Sakha104 X Sakha105	-2.82**	-1.16	-2.09**	-4.79**	-9.31**	-9.19**	-0.74	-0.40	0.10	-2.36**	-5.13**	-8.13**	-9.24**	-3.00**	-4.59**
Sakha104 X Sakha106	1.05	1.51	3.27**	-3.45**	-7.48**	-9.83**	-1.71*	-1.14	-3.04**	-13.31**	-6.69**	-9.50**	-8.61**	-4.13**	-6.26**
Sakha104 X Giza178	1.25	0.21	2.91**	15.15**	5.16**	3.61**	3.03**	1.73*	1.97**	-3.40**	5.43**	1.31	6.25**	5.98**	4.66**
Sakha104 X A22	2.62**	3.58**	3.77**	2.25*	6.22**	7.01**	0.33	2.83*	0.44	11.95**	5.05**	4.99**	-0.56	2.43**	1.03
Sakha104 X IRAT170	1.45	1.88	1.74*	0.38	5.82**	4.94**	-0.17	0.46	-1.36	9.35**	0.76	5.02**	2.85**	0.02	1.46
Sakha104 X WAB56-125	2.55**	2.21*	3.67**	-2.89**	5.62**	3.61**	-0.94	1.56*	0.30	9.63**	2.71**	3.35**	2.03**	4.17**	3.51**
Sakha105 X Sakha106	1.32	1.51	1.91*	-10.9**	-1.74	-7.66**	0.49	-0.50	-0.68	-7.38**	-3.64**	-0.89	-5.42**	0.44	0.76
Sakha105 X Giza178	-0.82	-1.46	0.54	5.35**	1.56	7.44**	-0.11	0.36	1.00	-1.90*	-5.68**	-6.28**	-0.28	1.77**	-0.29
Sakha105 X A22	-1.12	-0.76	0.41	16.78**	12.62**	15.17**	2.53**	2.13**	4.46**	2.06*	-2.15**	0.60	11.39**	3.64**	9.87**
Sakha105 X IRAT170	5.05**	1.88	1.71*	-4.42**	4.89**	-1.56	-1.97*	0.10	-1.00	7.52**	4.52**	7.38**	-3.97**	-0.45	-1.30
Sakha105 X WAB56-125	2.82**	3.88**	3.64**	12.98**	9.02**	10.45**	2.93**	0.86	1.33	0.11	-0.46	-3.29**	12.68**	-0.97	0.78
Sakha106 X Giza178	-1.28	-0.46	1.57*	4.68**	1.06	8.47**	-0.41	0.30	0.86	0.99	-0.70	-6.29**	-2.91**	-0.66	-3.58**
Sakha106 X A22	-0.92	1.58	-0.56	12.78**	17.12**	15.21**	1.23	2.73**	2.32**	1.66	0.65	1.09	5.76**	5.47**	4.86**
Sakha106 X IRAT170	-1.08	-1.46	-1.26*	-6.09**	-12.3**	2.47**	-0.27	0.03	-0.81	4.40**	1.80*	8.82**	6.65**	-0.45	0.07
Sakha106 X WAB56-125	3.35**	1.88	2.34**	8.98**	3.19**	10.15**	-1.71*	0.80	1.52*	-1.68	-3.37**	-2.85**	-2.81**	-0.23	-0.38
Giza178 X A22	-1.05	-4.72**	-5.26**	-10.3**	-9.24**	-11.7**	-2.04*	-2.07**	-5.66**	16.81**	3.99**	-3.80**	-1.07	-6.77**	-8.47**
Giza178 X IRAT170	-2.88**	-3.09**	-6.63**	4.85**	5.02**	2.57*	-0.21	2.56**	0.87	-9.05**	0.08	-4.47**	-3.02**	2.77**	5.56**
Giza178 X WAB56-125	-1.12	0.58	-3.36**	-6.42**	-9.51**	-6.42**	-0.64	-0.67	-0.46	4.81**	9.86**	10.37**	1.75*	0.46	2.13**
A22 X IRAT170	-0.85	1.61	1.91*	6.28**	0.76	-4.03**	1.43	1.00	-1.66*	-10.50**	-7.58**	-7.69**	1.93**	-0.65	-3.91**
A22 X WAB56-125	-0.08	-0.39	-1.49	-11.3**	-19.1**	-7.02**	1.99*	-1.57*	-3.00**	-1.95*	5.23**	10.08**	2.82**	-3.43**	-3.89**
IRAT170 X WAB56-125	-2.58**	-2.42*	0.14	-0.85	9.82**	-0.42	-1.17	0.73	1.87**	-12.36**	-0.85	-9.96**	-5.19**	1.86**	-0.60
LSD at 0.05	1.56	2.02	1.53	1.99	1.93	2.19	1.63	1.39	1.38	1.72	1.58	1.81	1.39	1.27	1.48
LSD at 0.01	2.10	2.73	2.07	2.68	2.61	2.95	2.20	1.87	1.86	2.32	2.12	2.45	1.88	1.71	1.99

Table 7: continue.....

Hybrids	Traits	Total chlorophyll content (SPAD)			Proline content (µg/g)			Na ⁺ /K ⁺ ratio			Na ⁺ content (%)			K ⁺ content (%)		
		N	S	D	N	S	D	N	S	D	N	S	D	N	S	D
Sakha102 X Sakha104		3.39**	-0.46	-0.79	-0.042	-1.82**	-1.22*	0.033	-0.108	0.065	0.040	-0.025	0.030	-0.116	0.091	-0.127
Sakha102 X Sakha105		-3.87**	-4.22**	-2.01*	-0.033	-1.55**	-3.61**	0.044	-0.080	0.090	0.058	0.013	0.111	-0.148	0.078	-0.049
Sakha102 X Sakha106		-4.59**	-1.47*	-4.43**	0.531	-0.34	-2.40**	-0.025	-0.174*	0.053	-0.030	-0.154	0.056	0.026	0.055	-0.047
Sakha102 X Giza178		3.96**	3.83**	3.63**	0.055	0.95	-2.50**	-0.059	0.033	-0.099	-0.069	0.094	-0.136	0.360*	0.020	0.065
Sakha102 X A22		1.32	3.62**	2.98**	0.577	-1.47*	4.05**	-0.030	0.015	-0.008	-0.034	0.048	-0.047	0.134	0.018	-0.097
Sakha102 X IRAT170		-0.70	2.55**	-0.08	-0.258	-0.98	2.42**	-0.022	0.079	-0.123	-0.032	0.171	-0.084	0.016	0.042	0.221
Sakha102 X WAB56-125		0.19	2.99**	1.24	-0.309	2.98**	-0.04	-0.039	-0.249**	-0.118	-0.041	-0.364**	-0.161	0.205	-0.018	0.080
Sakha104 X Sakha105		1.82*	2.47**	0.48	-0.211	1.21*	0.29	0.043	-0.087	0.026	0.119	-0.148	0.069	0.114	-0.031	0.036
Sakha104 X Sakha106		-3.82**	-2.17**	-0.14	-0.781*	-0.51	-4.37**	0.025	0.138	0.057	0.061	0.286*	0.104	0.075	0.055	0.019
Sakha104 X Giza178		-3.23**	-1.68*	-3.59**	0.110	-0.79	5.46**	-0.041	-0.275**	-0.071	-0.083	-0.362**	-0.090	-0.028	0.057	0.066
Sakha104 X A22		-6.31**	-2.69**	-2.94**	0.289	-1.01	0.18	-0.038	-0.001	0.029	-0.082	0.029	0.056	-0.048	0.025	-0.011
Sakha104 X IRAT170		1.01	-0.53	2.01	0.297	1.24*	3.71**	0.003	0.225**	0.033	0.016	0.116	0.031	0.062	-0.108	-0.015
Sakha104 X WAB56-125		-3.77**	-1.35	-0.98	0.379	1.54**	-2.14**	-0.073	0.155	-0.039	-0.154	0.173	-0.056	-0.073	-0.057	0.008
Sakha105 X Sakha106		-0.41	-0.20	-1.69**	-0.239	-1.94**	-3.85**	0.004	0.082	0.155	-0.053	-0.051	0.155	-0.131	-0.093	-0.080
Sakha105 X Giza178		1.48*	3.39**	3.09**	0.785*	3.19**	4.64**	-0.075	-0.075	-0.076	-0.098	-0.101	-0.097	0.181	-0.036	-0.007
Sakha105 X A22		3.10**	3.19**	2.71**	0.408	3.00**	3.93**	-0.059	-0.191*	-0.071	-0.041	-0.280*	-0.012	0.295	-0.028	0.165
Sakha105 X IRAT170		-0.44	-3.56**	2.25**	0.006	2.65**	0.83	-0.012	0.186*	0.009	-0.017	0.309**	-0.010	0.012	0.048	-0.024
Sakha105 X WAB56-125		1.15	3.59**	2.64**	-0.444	0.68	0.24	-0.014	-0.195*	-0.001	-0.041	-0.105	-0.001	-0.134	0.114	-0.046
Sakha106 X Giza178		3.39**	2.51**	3.34**	0.415	4.56**	5.15**	0.013	0.024	-0.098	0.046	0.016	-0.109	0.020	-0.051	0.079
Sakha106 X A22		1.54	1.81	3.83**	-0.010	1.57**	5.34**	0.020	-0.167*	-0.062	0.014	-0.043	-0.139	-0.139	0.170*	-0.110
Sakha106 X IRAT170		0.33	-0.17	1.33	-0.381	1.29*	1.87**	-0.003	0.291**	0.001	0.051	0.289*	0.046	0.215	-0.040	0.074
Sakha106 X WAB56-125		1.49	2.71**	3.25**	0.351	0.92	1.18	0.003	-0.012	-0.016	-0.018	-0.021	0.025	-0.120	-0.031	0.056
Giza178 X A22		-0.70	0.93	1.71*	-0.705	-1.87**	-6.67**	0.003	0.144	0.060	-0.005	0.147	0.123	-0.023	-0.036	0.084
Giza178 X IRAT170		-2.25*	2.22**	-0.99	-0.006	-1.18*	1.76**	0.049	-0.142	0.135	0.021	-0.218*	0.115	-0.318	-0.059	-0.241
Giza178 X WAB56-125		-2.26*	-0.20	-1.57	-0.358	-2.42**	-3.19**	0.030	-0.056	0.071	0.083	0.054	0.109	0.114	0.198**	-0.032
A22 X IRAT170		-2.29**	1.55*	-0.40	-0.184	-2.14**	-4.21**	0.010	-0.224**	0.005	0.021	-0.192	0.083	-0.021	0.090	0.131
A22 X WAB56-125		0.56	1.60*	-0.48	-0.076	3.29**	5.53**	-0.010	0.114	0.012	0.021	0.094	0.074	0.299	-0.056	0.174
IRAT170 X WAB56-125		0.75	-1.08	-0.27	0.363	-3.16**	-1.34*	-0.009	0.218**	0.070	-0.011	0.179	-0.003	0.023	-0.107	-0.225
LSD at 0.05		1.69	1.36	1.64	0.717	1.13	1.22	0.200	0.161	0.243	0.231	0.217	0.281	0.356	0.146	0.311
LSD at 0.01		2.29	1.83	2.21	0.968	1.52	1.64	0.270	0.218	0.328	0.312	0.292	0.379	0.481	0.197	0.420

* and **. Significant at 0.05 and 0.01 levels of probability, respectively, N = Normal environment, S = salinity and D = Drought environment.

Genetic diversity analysis of the tested rice varieties using SSR markers

The eight parents used in the present study were subjected to DNA polymorphism screening and assessment using SSR markers which offer a great potential for generating large numbers of markers evenly distributed throughout the genome and have efficiently been used to give reliable and reproducible genetic markers. Eight SSR primer pairs related to salinity and drought tolerance with known map positions distributed in the rice genome were used to screen a set of eight selected Indica, Japonica and Tropical-Japonica rice genotypes with different levels and mechanisms of drought and salinity tolerance. The presence, absence matrix for SSR amplified fragments for the studied genotypes are found in Table (8).

Number of alleles and allelic diversity

The eight SSR markers spread on four chromosomes (1, 6, 8 and 9) generated polymorphic alleles. Data in Table (8) showed that, a total number of 29 alleles were detected at the loci of the eight markers across the eight rice genotypes. The number of alleles per locus generated by each marker varied from 2 (RM201) to 5 (RM223 and RM25) alleles with an average of 3.63 alleles per locus. Only one SSR

marker generated two alleles, three markers generated three alleles, two markers generated four alleles and two generated five alleles. The eight primers gave a total of 29 DNA fragments from all tested varieties with an average of 3.63 bands per primer. The varieties Sakha104, Sakha106, Giza178 and A22 displayed the highest number of DNA fragments (13 fragments), while variety IRAT170 revealed the lowest number of fragments (8). Data in Table (9) showed that, the effective number of alleles per locus ranged from 1.60 alleles to 4.92 alleles with an average of 3.26 alleles. The highest and effective numbers of alleles per locus was observed for RM72 (4.92), RM3805 (4.74) and RM223 (4.44). Results in Table (9) showed also that the lowest numbers of alleles per locus were obtained by RM201 (1.60) and RM315 (2.13) similar results were obtained by Sajib *et al.*, 2012 (3.33) and Vanniarajan *et al.*, 2012 (2.5). On the other hand, high number of alleles per locus was obtained by El-Malky *et al.* 2007 (8.57); Ni *et al.* 2002 (6.8) and Ram *et al.* 2007 (4.86). On average, 45% of the eight rice genotypes shared common major allele at any given locus ranging from 31% (RM219, RM72 and RM223) and 75% (RM201).

Table 8: The presence (1), absence (0) matrix for SSR amplified fragments for the studied genotypes

Marker	No. of alleles.	Sakha 102	Sakha 104	Sakha 105	Sakha 106	Giza 178	A22	IRAT 170	WAB 56-125	M.W. (bp)
RM 219	1	1	1	1	1	0	0	0	0	600
	2	0	1	0	0	1	0	0	0	500
	3	1	0	1	1	0	1	0	0	250
	4	0	0	0	0	0	0	1	1	200
RM 201	1	1	1	1	1	1	1	0	0	150
	2	0	0	0	0	0	0	1	1	100
RM 3805	1	1	1	0	1	0	0	0	0	125
	2	0	0	0	0	1	1	0	1	75
	3	1	1	0	1	1	1	0	1	50
RM 72	1	1	1	1	1	0	0	0	0	200
	2	0	0	0	0	1	0	0	0	125
	3	0	0	0	0	0	1	0	0	100
	4	0	1	1	1	1	1	0	0	50
RM 223	1	1	1	0	1	0	0	0	0	400
	2	0	0	0	0	1	1	0	1	370
	3	0	0	0	0	1	1	0	1	350
	4	0	0	0	0	1	1	1	1	200
	5	1	1	1	1	1	0	0	0	150
RM 315	1	1	1	1	1	0	0	1	0	150
	2	0	0	0	0	1	1	0	0	140
	3	0	0	0	0	0	0	0	1	125
RM 8094	1	1	1	1	1	1	1	0	0	500
	2	1	1	1	1	0	1	0	0	250
	3	0	0	0	0	1	0	1	0	200
RM 25	1	0	0	0	0	0	0	1	0	230
	2	0	0	0	0	0	0	1	0	210
	3	0	0	0	0	0	0	1	0	190
	4	0	0	0	1	1	1	0	1	150
	5	1	1	0	0	0	0	0	0	130
Total band		12	13	9	13	13	13	8	9	

A total of 29 amplified fragments were polymorphic (Table 9). The monomorphic fragments are constant and cannot be used to study the diversity while polymorphic fragments revealed differences and could be used to examine and establish systematic relationships among the genotypes (Hadrys *et al.* 1992). Results presented in Table

(9) showed that the total number of polymorphic fragments was 29 bands, out of them six were unique, and the highest unique bands number was detected by primer RM25 with three unique bands. On the other hand, two unique fragments were scored by RM72 and one unique band was scored by RM315. Unique DNA fragments with different sizes were detected in a particular genotype but not in the others using different primers. The presence of a unique fragment for a given genotype is referred as positive marker, while the absence of common fragments served as negative marker. Such bands could be used as DNA markers for genotype identification and discrimination. In this respect, three DNA unique fragments were detected in the varieties, IRAT170 and one fragment in each of the genotypes Giza178, A22 and WAB56-125.

Table 9: List of SSR markers used including name, polymorphic bands (PM), unique bands (UN), number of amplified alleles (AN), effective number of alleles (ENA), common alleles (CA) and polymorphic information content (PIC)

No.	Marker	PIC	ENA	AN	CA	UN.	PM
1	RM 219	0.74	3.88	4	0.31	0	100%
2	RM 201	0.38	1.60	2	0.75	0	100%
3	RM 3805	0.79	4.74	3	0.38	0	100%
4	RM 72	0.80	4.92	4	0.31	2	100%
5	RM 223	0.78	4.44	5	0.31	0	100%
6	RM 315	0.53	2.13	3	0.63	1	100%
7	RM 8094	0.73	3.66	3	0.38	0	100%
8	RM 25	0.68	3.15	5	0.50	3	100%

PIC value:-

PIC value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency; thus, it provides an estimate of the discriminating power of the marker (Nagy *et al.*, 2012). As it is shown in Table (9), the PIC values for the SSR used in this study varied from 0.38 to 0.80 with an average of 0.68. This result is consistent with that at Sajib *et al.* (2012) who reported greatly variations in PIC values for all tested SSR loci (from 0.14 to 0.71 with an average of 0.48). Higher averages of PIC values (0.57) were reported by Zeng *et al.* (2004) and (0.707) by Ram *et al.*, (2007). According to Anderson *et al.* (1993), there were seven highly informative markers ($PIC > 0.50$), one informative marker ($0.50 < PIC < 0.25$) and no slightly informative markers ($PIC < 0.25$). The highest PIC values were observed for RM72 (0.80), RM3805 (0.79) and RM223 (0.78).

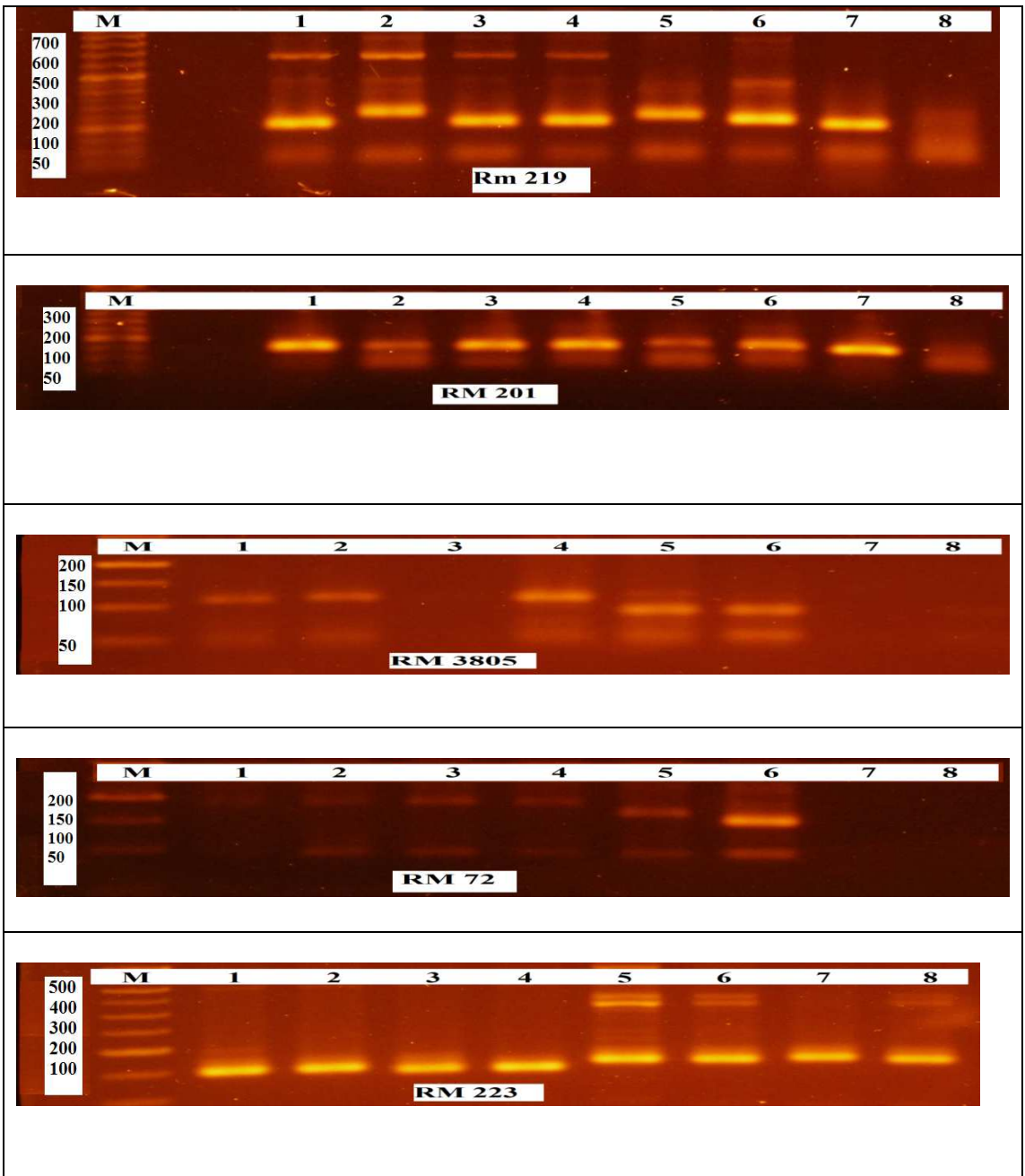
Genetic similarity and phylogenetic tree based on the SSR markers.

Data in Table (10) showed that, the similarity matrices resulting from the SSR markers. In the present investigation, eight SSR markers were used to study the genetic differences and relationships among the eight rice varieties (Fig. 1). Polymorphism was evident in all tested SSR markers, polymorphism due to SSR markers analysis could be caused by differences in nucleotide sequences at the priming sites (such as point mutations), or by structural rearrangements within the amplified sequence, (e.g., insertions, deletions, inversions) (Welsh and McClelland, 1990).

The genetic similarity among the eight rice varieties was ranging from 0% to 79%. The highest similarity 79% and shortest genetic distance were scored between Sakha102 with each of Sakha104 and Sakha106; these three varieties are Japonica rice. Moreover, the obtained results confirmed the sensitivity and moderately tolerant of those varieties to salt and drought stresses. The lowest genetic similarity (0%) and longest genetic distance were found between WAB56-125 and Sakha105. These results were substantiated by the fact that these two genotypes have different origin, the genotypes WAB56-125 is Indica rice while, Sakha105 is Japonica rice, which may be due to the absence of common parents between them. Also, the large distance between Sakha105 and WAB56-125 fixed their difference in the ability of drought and salt tolerance. These results were in agreement with those of Chakravarthi and Naravaneni (2006) who reported that low similarity coefficient between Japonica type and Indica type genotypes were found, and Kanawapee *et al.* (2011) who reported relatively high level of similarity between closely related genotypes.

Table (10): Genetic similarity and distance values of SSR markers among the eight rice varieties

	Sakha 102	Sakha 104	Sakha 105	Sakha 106	Giza 178	A22	IRAT 170
Sakha102							
Sakha104	0.79						
Sakha105	0.62	0.57					
Sakha106	0.79	0.73	0.69				
Giza178	0.14	0.24	0.16	0.24			
A22	0.25	0.24	0.29	0.37	0.63		
IRAT170	0.05	0.05	0.06	0.05	0.11	0.05	
WAB56-125	0.05	0.05	0.00	0.10	0.38	0.38	0.21



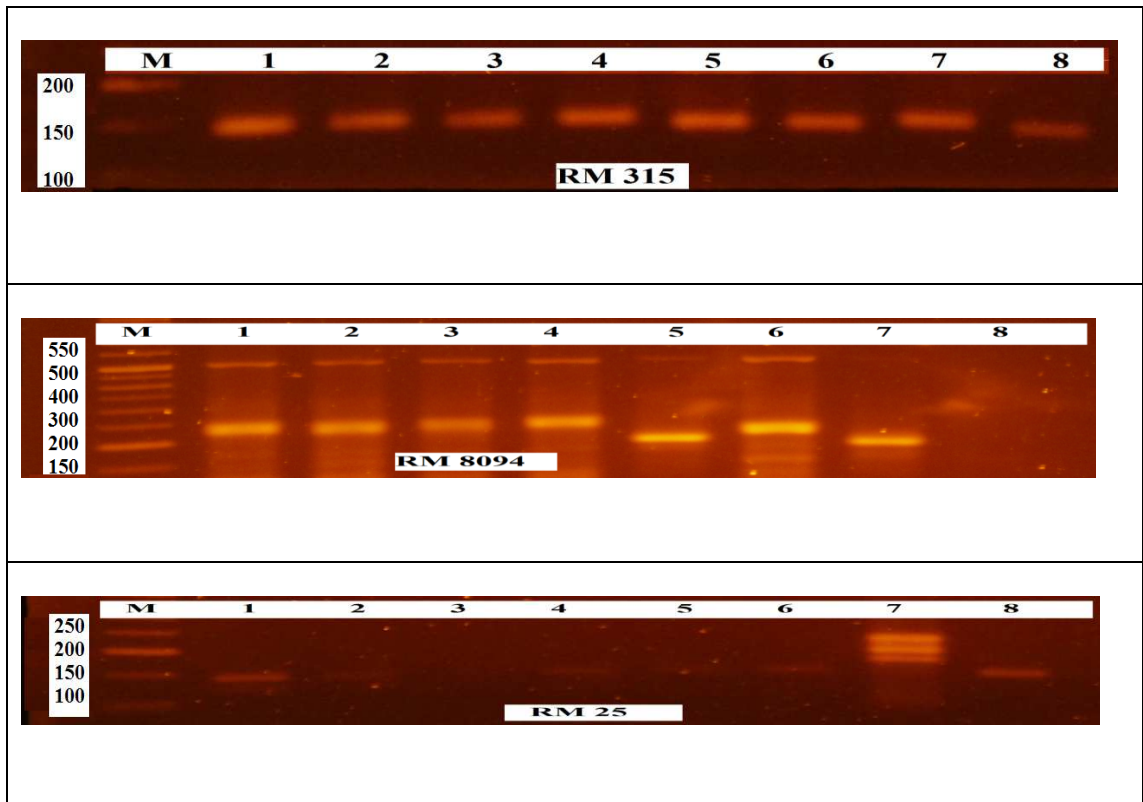


Fig. 1: Agarose gel electrophoresis of PCR amplified fragments for eight SSR markers. M is 50 bp DNA ladder; 1, Sakha102; 2, Sakha104; 3, Sakha105; 4, Sakha106; 5, Giza178 6, A22; 7, IRAT170; and 8, WAB56-125

Cluster analysis:-

The genetic relationships among rice genotypes are presented in a dendrogram based on informative microsatellite alleles (Fig. 2). All genotypes clearly grouped into two major clusters in the dendrogram at 9% similarity based on Jaccard's similarity index. The first cluster represents the tropical Japonica rice IRAT170. While, the second cluster represents two sup clusters, the first sub cluster include the Indica and Indica/Japonica rice Giza178, A22 and WAB56-125 at 38% similarity based on Jaccard's similarity index and the second cluster include Japonica rice Sakha102, Sakha104, Sakha105 and Sakha106 at 62% similarity based on Jaccard's similarity index. Below the sub cluster Indica and Indica-Japonica in the dendrogram, Giza178 and A22 were in one group at 62% similarity and WAB56-125 was in another group at 38% similarity with other indica rice in the same sub

cluster. Below the second sub cluster in the dendrogram, genotypes were grouped into three groups, the first group contains Sakha105, the second group contains Sakha106, and the third group contains Sakha102 and Sakha104 at about 62, 74 and 79% similarity. As seen in cluster result, IRAT170 came in first cluster since it was found to be only drought tolerance and salt sensitive. It was observed that, the four Japonica rice varieties; Sakha102, Sakha104, Sakha105 and Sakha106 occupied on the second sup cluster while all of them drought and salt sensitivity, except Sakha104 was moderately tolerance for salinity and drought stresses. Continuously, A22, Giza178 and WAB56-125 were in the first sup cluster and were characterized as drought and salt tolerance varieties. El-Malky *et al.* (2007) reported the ability of SSR makers to divide the genotypes into two groups, one included the Indica genotypes and the other included the Japonica genotypes. Also, Zeng *et al.* (2004) found that, all genotypes clearly grouped into two major branches in the dendrogram with less than 10% similarity based on Jaccard similarity index, one branch represented the subspecies Japonica rice and the other branch represented the subspecies Indica or the hybrids between Japonica rice and Indica rice.

Identified MAS marker:-

Among eight polymorphic SSR markers, RM223 was able to divide the studied genotypes into two groups depending on their drought tolerance (**Figure 1**). The first group showed the first allele with molecular size of 150bp included the drought susceptible genotypes i.e. Sakha102, Sakha104, Sakha105 and Sakha106. Where the second allele with molecular size of 200bp appeared in the second group included the drought tolerant genotypes i.e. Giza178, A22, IRAT170 and WAB56-125. Furthermore, this marker indicated that the three parent i.e. Giza178, A22 and WAB56-125 exhibited two allele differed with other parent this molecular result indicated that this three parent tolerant to salinity stress and this result supported agronomic and yield characters under this study, so this marker can used in breeding program under salinity and drought stress. These results agree with Lang *et al.* (2008) they used RM223 for salinity tolerance. Furthermore, Kumar *et al.* (2005) used this primer for drought tolerance in rice.

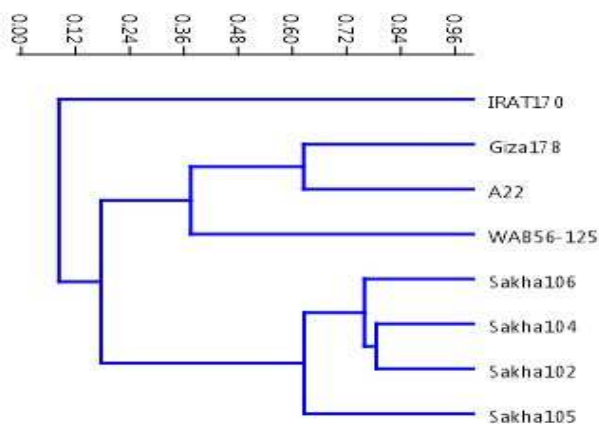


Figure 2: Dendrogram derived from UPGMA cluster analysis of eight rice genotypes based on Jaccard's similarity coefficient using eight SSR markers

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الملخص العربي

دراسات وراثية وجزيئية على تحمل الملوحة والجفاف في الأرز

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تضمنت الدراسة ثمانية اصناف ارز وتم التهجين بينها بطريقه التهجين النصف دورى half-diallel وكذلك الدلائل الوراثيه SSR-markers لتقييم تلك التراكيب الوراثيه وتقدير القدره على الانتلاف تحت الظروف الطبيعيه وظروف الجفاف والملوحة. وأشار تحليل التباين الى وجود فروقات عالية المعنوية بين السلالات في جميع الصفات المدروسة. وأشارت تقديرات القدره العامه على الانتلاف أن الثلاثة اصناف الأرز التاليه: جيزه 178، أ 22 و اب 125-56 كانت أفضل الاصناف لمعظم الصفات تحت الثلاثة ظروف البيئيه. وأشارت تقديرات القدره الخاصه على الانتلاف أن أكثر الهجن المرغوب فيها كانت، سخا 105 × أ 22 ، سخا 102 × أ 22 و سخا 104 × جيزه 178 لمحتوى الكلوروفيل الكلى، عدد السنابل والمحصول؛ جيزه 178 × اب 125-56 لعدد الحبوب الممتلئه فى السنبله؛ سخا 102 × سخا 104 لصفه التزهير و سخا 104 × سخا 105 لطول النبات. وقدرت القرابه الوراثيه باستخدام الثمانيه دلائل الجزيئيه SSR بين اصناف الأرز الثمانيه وتراوحت بين 0% إلى 79%. وكانت الواسمات الوراثية SSR فعالة في التنبؤ بمتوسط التباين فى القدره الخاصه على الانتلاف بين مختلف التراكيب الوراثيه بين الأصناف. وبالإضافة إلى ذلك، اظهرت النتائج امكانيه استخدام الدليل RM223 في الانتخاب بمساعده الدلائل (MAS) لتحمل الملوحة والجفاف.