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Utilization of ISSR marker and tolerance indices for selecting adapted wheat genotypes under water stress

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The study aimed at identifying and assessing drought tolerance and genetic diversity of twelve wheat genotypes in two diverse irrigation treatments during the two successive seasons of 2016/2017 and 2017/2018. Drought caused decreases in flag leaf area, relative water content and grain yield. Catalaise and perodixdase activities and proline content increased due to stress. Grains yield was significantly different between water stress and normal conditions. Differences varied among wheat genotypes. The highest grain yield was by Gemmiza 11 followed by Giza 168. The genotype Yakora Rojo gave lowest yield but was highly reinstate to water stress. The 5 ISSR primers produced 25 out of 48 amplicons and were polymorphic. The primer 844A gave 14 amplicons, while, the primer HB 8 exhibited 6 amplicons. Sids 13 followed by Yakora Rojo exhibited highest total number of amplicons 36 and 35 amplicons, respectively and may be high of resistance for drought. Cultivars Sids 12, Giza 168 and line 127 followed by Giza 171 gave highest grain yield and stress tolerance index (STI), while Line 145 and Yakora Rojo gave the lowest STI and grain yield. Gemmiza 11 recorded highest stress tolerance (TOL), while, Sids 12 and Giza 168 showed highest mean productivity (MP) and harmonic mean (HM) indicating more stress tolerance. Yakora Rojo exhibited highest yield stability Index (YSI) but gave low yield, though not very low, due to its drought resistance; and can be recommended as a parent in breeding programmers to transfer drought resistance.

Keywords: Bread wheat, Cluster analysis, Drought stress, Genotypes, ISSR marker, Stress tolerance indices.

INTRODUCTION

Wheat is the vital crop in the world. Egypt imports about 45% of its wheat requirements. Demand for wheat increased considerably with restriction of its available production resources. This requires more efforts to increase wheat production. Thus, improving varieties with wider adaptability and stable performance through crop improvement programs is needed. During recent years increasing wheat production under abiotic stress conditions has become very important. The main stress is drought, which has negative effects on plant and production (Al Saadoown et al., 2018). Selection of genotypes under environmental stress condition is one of the main tasks of plant breeders for exploiting the genetic variations to obtain the most tolerant cultivars (Clarke et al., 1984 and1992 and Al Saadoown et al., 2017). Various quantitative criteria have been proposed for selection of genotypes based on their yield, and tolerance to stress coditions. Based on such criteria, genotypes are compared under stress and non-stress conditions (Taghian and Abo-Elwafa 2003).

Stress resistance is defined by Hall (1993) as the relative yield of genotype compared with other

genotypes subjected to the same stress. Stress susceptibility of a genotype is often measured as a function of the reduction in characteristic performance under stress (Bidinger et al., 1978).

Oxidative damages and decreased CO_2 availability occur as a result of drought, therefore inhibiting photosynthesis and respiration through the reactive oxgen species (ROS) damage and electron transport proteins. Also, drought may lead to accumulation of ROS and disturbance of antioxidant defense, oxidative stress to proteins, membrane lipids and other cellular components (Tang et al., 2002 and Shan et al., 2012).

The DNA molecular markers which are based on PCR (such as inter simple sequence repeats "ISSRs") are excellent tools for plant breeders to select the genetic materials that are tolerant to stresses, regardless of any interaction with the environment. Presence of genetic diversity and genetic relationships among genotypes is a prerequisite and of a paramount importance for successful wheat breeding programs. Developing wheat varieties with desirable traits requires knowledge about the existing genetic variability (Singhal and Upadhyay 1977, Maniee et al., 2009, Kahrizi et al., 2010 and El-Hosary and Nour El Deen 2015). Some selection indices have been proposed based on a mathematical relation between stress and adequate conditions. Utilization of the selection indices is to evaluate the response of plant genotype to abiotic stress and provide a measure of injury based on loss of characteristic performance under stress in comparison with normal conditions (Mitra 2001). These indices are either based on stress resistance or on susceptibility of the genotype (Fernandez 1992).

The current study was undertaken to study some physiological traits and grain yield of twelve bread wheat genotypes across two diverse environments during two years to screen tolerance criteria and select the most adaptable genotypes under drought stress based on ISSR marker and some tolerance indices.

MATERIALS AND METHODS

Plant materials

This study was conducted on six recently released Egyptian bread wheat (*Triticum aestivum*) cultivars (Gemmeza 11, Sids 12, Giza 171, Sids 13, Sakha 93 and Giza 168) obtained from the Agricultural Research Center, as well as six introduced lines from CIMMYT . The code, pedigree and/ or selection history of those genotypes are presented in Table 1.

Table 1. Origin, pedigree and selection history of the five bread wheat cultivars and lines used in the present study

No.	Genotype	Origin	Pedigree and/or selection history
G1	Gemmeza 11	Egypt	Bow “s”/ Kvz “s”//7C/Seri 82 /3/ Giza 168 / Sakha 61 GM 7892-2GM-1GM-2GM-1GM-0GM
G2	Sids 12	Egypt	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4 /CHAT"S"/6/MAYA/VUL//CMH74A.630/4 *SX SD7096-4SD-1SD-1SD-0SD
G3	Giza 171	Egypt	Sakha 93/Gemmeiza 9
G4	Sids 13	Egypt	Kauz “s” // Tsi / Snb”s” ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APA-050AP-0AP-0SD
G5	Shaka 93	Egypt	S 92/TR 810328 S8871-1S-2S-1S-0S
G6	Giza 168	Egypt	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B
G7	Line 116	CIMMYT	MILAN \ S7116 \\ Hall //(Ne700011)
G8	Line 124	CIMMYT	MILAN \ S87124 \\ BABAX
G9	Line 127	CIMMYT	MILAN \ S7147 \\ OAPYMex
G10	Line 145	CIMMYT	MILAN \ S7145 \\ OAPYMex
G11	Line 150	CIMMYT	CMH.S87.150\ ELVIRA
G12	Yakora Rojo	CIMMYT	Ciano 67/Sonora 6411 KlienRendidor /3/1L815626Y-2M-1Y-0M-302M

CIMMYT: Centro International de Mejoramiento de Maize Y Trigo (Mexico) = International maize and wheat improvement center

Table 2. ISSR primers codes and its sequences used in PCR analysis.

No.	Name	Sequence (5`→ 3`)
1	844A	CT CT CT CT CT CT CT CTAC
2	844B	CT CT CT CT CT CT CT CTGC
3	17898B	CA CA CA CA CA CAGT
4	17899B	CA CA CA CA CA CAGG
5	HB 8	GA GA GA GA GA GAGG

Table 3. Chemical analysis of soil during 2016/2017 and 2017/2018 growing seasons.

Season	OM %	$CaCO_3$ g kg^{-1}	pH	EC (dSm^{-1})	Soluble anions (mmolcL-[1])			Soluble cations (mmolcL-[1])			
					Cl-	HCO_3-	SO_4--	Na^+	K^+	Ca^{2+}	Mg^{2+}
Soil analysis (0 – 30cm)											
2016/2017	2.1	2.8	8.0	0.5	9.8	1.1	7.1	8.7	0.4	5.7	3.2
2017/2018	1.9	3.2	8.1	0.8	6.56	2.2	10.7	8.6	1.2	8.2	1.4

Notes: pH of 1:2.5 with water suspension for soil.

DNA extraction and ISSR-PCR amplification

Genomic DNA from each genotype was isolated according to the CTAB method (Doyle and Doyle 1990). Five primers (Table 2) were used to detect polymorphism among the aforementioned genotypes. These primers were selected from a set of Operon kits (Operon Technologies Inc., Alameda California, USA).

Reactions were carried according to Weising et al., (2005) using a total volume of 25 µL containing 30 ng of genomic DNA (as a template) along with 30 pmoles of primer, 2mM of dNTP's mix (dATP, dCTP, dTTP and dGTP, ABgene, Surrey, UK), 10 X PCR buffer, 25 mM MgCl2, and 2 units Taq DNA polymerase (Promega, USA). PCR cycling was programmed at 94ºC for 4 min, (one cycle); followed by 94ºC for 45 sec, 38ºC for 1 min and 72ºC for 1 min (35 cycle) then by 72ºC for 10(one cycle) ,then 4ºC(infinitive). The PCR products against 1Kb DNA Ladder (Promega USA) as a size marker were separated by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 µg mL^{-1}). The productive bands were visualized with ultraviolet light and documented on Gel documentation UVITEC, UK. Distance coefficients between a pair of genotypes were calculated using Nei and Li's formula (1979) after fragments were scored as binary data, where (1) and (0) refers to presence or absence of band, respectively. Cluster analysis was performed using UPGMA clustering algorithm from the SAHN option of NTSYS-PC version 2.1 (Rohlf, 2000) to produce a dendrogram.

Field trial design and treatments

The field trials were carried out at Moshtohor Experiment Research Station (30° 21´ 07´´ N and 31° 13´ 34´´ E), Al- Kalubia, Egypt (Texture Clay) in the successive seasons of 2016/2017 and 2017/2018. The chemical analysis of soil samples (10-30 cm soil surface) were done according to Jackson (1962) and Piper (1947), Table 3 shows results of analysis.

Meteorological data from November to May in the seasons of 2016/2017 and 2017/2018 were obtained from the Agro-meteorological Station at Moshtohor, Benha Univ., the maximum temperatures were 19.8, 19.7, 17.7, 20.4, 25.8, 29.1 and 34.5°C, and the minimum temperatures were 9.5, 9.2, 6.1, 7.8, 11.4, 14.4, and 19.0 °C, relative humidity were 52.2, 51.3, 55.9, 47.2, 37.3, 38.9 and 32.1% and the mean precipitation were 0.2, 0.5, 1.6, 0.8, 0.4, 0.3 and 0.00 mm respectively in season 2016/2017. As for the second season the maximum temperatures were 18.9, 18.5, 16.3, 19.6, 24.5, 28.3 and 32.6°C, and the minimum temperatures were 8.9, 8.4, 7.0, 6.7, 10.9, 15.3, and 19.5 °C, relative humidity were 46.8, 50.1, 52.8, 45.2, 34.3, 35.9 and 29.8% and the mean precipitation were 0.1, 0.4, 2.1, 1.5, 0.8, 0.5 and 0.03 mm, respectively.

In each season a split-plot design in three replicates was laid out. The planting date was on 24th and 26th Nov. in the first and second season, respectively. The main plots were allotted to two irrigation (water stress) treatments of (1) water stress giving two irrigations one after sowing and one tillering then no irrigation till harvest and (2) non- stress giving 5 irrigations at sowing, tillering,

stem elongation, booting and reproductive stages. When irrigation treatments were applied, all precautions were taken to separate the two treatments to prevent infiltration. The sub-plot was allocated to the twelve wheat genotypes. An average of random samples of ten plants of each genotype was collected 2 weeks after heading to measure the following physiological measurements:

1- Relative water content (RWC) calculated according the following equation:

$$RWC = \frac{FW-DW}{TW-Dw}$$

FW refers to fresh weight of leaves, TW refers to turgid weight after leaves were rehydrated in distilled water for 24 h and DW refers weight of a dry leaves.

2- Flag leaf area (cm^2) was determined using Handheld Laser Leaf Area meter (CI-203 by Bio-Science, Inc).

3- Catalase activity was assayed according the method described by Sadasivam and Manickam (1996), 100 µL of enzyme extract of each genotype then, mixed with 100 µL of 100 mM H_2O_2 and the total volume was made up to 1 mL by 250 mM phosphate buffer pH 6.8. The decrease in optical density at 240 nm against blank was recorded every minute.

4- Peroxidase activity expressed as the change in absorbance according to Allam and Hollis (1972) using spectrophotometer at 425nm/ 15 minute/ g fresh weight in the reaction mixture (0.5 mL of 0.1 M potassium phosphate buffer solution at pH 7.0; 0.3 ml enzyme ectract; 0.3 ml 0.05 M pyrogallol and 0.1 mL 1.0 %H_2O_2)

5- Proline content (mg g^{-1}) was determined according the method of Bates, (1973) using spectrophotometer at 520 nm.

6-Grain yield m^{-2}after physiological maturity (within 155 days after planting) 1 meter from each plot was harvest then grains were separated and weighted

Data analysis

Each year was separately variance analyzed according to Gomez and Gomez, (1984) then the combined across year was made after test the homogeneity of errors. Analysis of variance of each year and the combined analysis were obtained. Then, Duncan's multiple range test (Duncan, 1955) was used to verify the significance of mean performances for all traits recorded in both years.

Calculation of the tolerance indices

Some selection indices were calculated to evaluate the response to stress and provide a measure of injury based on loss of yield under stress in comparison with normal conditions calculations were as follows:

-Tolerance index (TOL) and mean productivity (MP) according to Rosielle and Hamblin (1981):

$TOL = (Y_p - Y_s)$ and $MP = (Y_s + Y_p) / 2$

-Harmonic mean (HM) and Stress susceptibility index (SSI) according to (Kristin et al., 1997) and (Fisher and Maurer 1978), respectively as follows:

$HM = 2(Y_p * Y_s) / (Y_p + Y_s)$

$SSI = 1 - (Y_s / Y_p) / SI$, while $SI = 1 - (\hat{Y}_s / \hat{Y}_p)$

Where, SI is the stress intensity and $\hat{Y}s$ and $\hat{Y}p$ are the means of all genotypes under stress and normal conditions, respectively.

- stress tolerance index (STI) according to Fernandez (1992) and Kristin *et al* (1997):

$STI = (Y_p * Y_s) / (\hat{y}_p)^2$

-Yield Index (YI) according to Gavuzzi *et al* (1997) and Lin *et al* (1986):

$YI = Y_s / \hat{Y}_s$

-Yield Stability Index (YSI) according to Bouslama and Schapaugh (1984):

$YSI = Y_s / Y_p$

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) of split plot design in ea iscussiondch across the successive years of 2016/2017 and 2017/2018 (Table 4) showed that mean squares due to the treatments of irrigation (I), genotypes (G) and their interaction (IG) were significant for all studied traits. These results indicate that water stress had a marked effect on all studied characters. There were differences among genotypes due to genetic-background and behavior under water stress. Therefore, there is a possibility of selecting genotypes for improved performance under a water stress conditions as stated by Jones (2007) and Khakwani et al., (2011). For combined analysis, mean squares for seasons (Y), irrigation treatments (I), genotypes (G), first order of interaction (YI, YG and IG) and second order of interaction (YIG) were significant ($P \leq 0.05$ or $P \leq 0.05$) for most cases of the studied traits, indicating significance differences among the twelve genotypes under each environment. Also, the results reflect the fact that genotypes under study behave differently from one environment to another, and are agree with those of Changhai et al., (2010), who note that the four wheat genotypes under their study were varied significantly for some physiological studies under

different water regimes.

Effect of seasons

The results show increases in relative water content (RWC), flag leaf area (FLA), and grain yield m^{-2} (GY) and decreases in activity of catalase and peroxidase enzymes at the first season. This can be due to the different environmental conditions, like increase in the rainfall rate and the moderate temperature in the second season.

Effect of water stress

Relative water content, flag leaf area, proline and grain yield m^{-2} exhibited reduction in performance due to exposure to water stress in both seasons of study (Table 5). The decrease in water supply decreased flag leaf area for all genotypes in both seasons. With this respect, numerous studies have shown a strong negative relationship between water stress and leaf area reduction. The decrease in leaf area is a common drought avoidance mechanism (Clarke et al 1984). It reflects a reduction in leaf growth of the main shoot and the primary and secondary tillers (Davidson and chevalier (1987). Akram (2011) reported that leaf area is a reflection of transpiration and assimilation. Under drought condition, maintenance of leaf turgor may be achieved by osmotic adjustment in response to the accumulation of proline, in cytoplasm increasing water uptake from drying soil. The process of accumulation of such solutes under drought is an osmotic adjustment which strongly depends on the extent of water stress. Wheat has low levels of these compatible solutes and the accumulation and mobilization of proline was observed to enhance its tolerance to water stress (Batool et al., 2012). Water stress must have increased the activity of catalase and peroxidase enzymes and proline in leaves of wheat genotypes during the studied seasons. These results are in harmony with those by several researchers (Shao et al., 2007 and Abdul Jaleel et al., 2008) they reported that, genotypes have developed a wide range of adaptive/resistance mechanisms to maintain productivity and ensure survival under drought stress condition. Antioxidant defense system is one of the stress defense mechanisms. Therefore, cells of resistant genotypes have developed an antioxidants system, as protective enzymes or antioxidant enzymes like catalase (CAT) and peroxidase (POD) to reduce the toxicity of ROS**.**

Effect of genotypes

To describe the differences among genotypes, data of the selected traits were averaged for the twelve genotypes differing in their stress tolerance, as well as in grain yield m^{-2} under normal and water regime in both seasons of study (Table 5). For relative water content and flag leaf area and grain yield m^{-2} the cultivates gemeza 11, and sids 12 and giza 168 exhibited high values for this trait under normal irrigation in both seasons. For the traits of Catalase, peroxidase activities and proline content, superiority occurred for of the two genotypes shandaweel 1 and Yakora compared with the other genotypes under stress condition. Antioxidant enzymes are higher in stress tolerant genotypes than in sensitive under diverse water stresses (Wang et al., 2009). High peroxidaes activity is linked with protection from oxidative damage (Hashminasab et al., 2012).

Genotype identification by unique DNA markers

Unique markers are bands that specifically identify an accession from the other by their presence or absence (Table 6). The bands that shown in one accession but not in the others are termed positive unique markers, in contrast with the negative unique markers, which are absent in a specific genotype. These bands could be used for genotype identification (Sajida et al., 2010).

As shown in tables 6, the ISSR assay identified of the nine out of 12 wheat genotypes by one or more unique positive (+) and / or negative (-) markers. The nine genotypes were characterized by positive and two negative unique markers G1(1+ and 1-), G2 (2-), G3(1-), G4 (2+), G5(1+), G6 (1+), G8(1-), G9(2+ and 1-) and G11 (2+), while these genotypes were resistant to drought stress except G1 (gemizza 11), G5 (Sakha 93) and G8 (Line 124). The susceptibility of these genotypes may be the reason of appearance or absence of one unique marker. However, these genotypes showed high yield in normal environment.

The G4 (Sids 13) followed by G12 (Yakora Rojo) exhibited the highest total number of amplicons 36 and 35 amplicons, respectively. Results indicate that these genotypes may be high resistance for drought stress.

Table 4. Mean squares from ordinary analysis of variance for the studied traits in both and across seasons of 2016/2017 and 2017/2018.

S.O.V		Relative water content	Flag leaf area (cm^2)	Catalase activity min-1 mg-1 protein	Peroxidase min-1 mg-1 protein	Proleine mg/g F.W.	Grain yield m^{-2}
First season 2016/2017							
Irrigation (I)	1	5786.925**	278.904**	0.642**	0.22*	1826.799**	1783531**
Error a	2	45.198	0.117	0.014	0.022	0.0107	1526.778
Genotype (G)	11	350.846**	161.767**	0.012**	0.026**	12.889**	160790.1**
I X G	11	169.343**	77.309**	0.011**	0.004*	11.724**	26060.59**
Error b	44	23.753	1.597	0.0015	0.002	0.306	843.323
Second season 2017/2018							
Irrigation (I)	1	11665.54**	2717.418**	0.763**	1.597**	909.767**	1778677**
Error a	2	38.614	4.686	0.028	0.036	8.372	4461.113
Genotype (G)	11	379.003**	145.769**	0.045**	0.029**	27.685**	174195.6**
I X G	11	226.776**	59.918**	0.016**	0.049**	34.074**	26627.14**
Error b	44	24.36	1.06	0.0029	0.003	0.489	988.568
Combined analysis across seasons							
Year (Y)	1	677.735*	73.59**	0.21**	0.005	25.927*	61935.04**
R (L)	4	42.544	4.037	0.018	0.047	4.527	5487.168
Irrigation (I)	1	16942.54**	5505.09**	1.403**	1.501**	2657.454**	3562206**
YxI	1	509.932*	0.23	0.003	0.316**	79.112*	1.653
Error a	4	41.268	0.73	0.023	0.011	3.953	500.722
genotype (G)	11	702.32**	303.23**	0.041**	0.051**	38.076**	332964**
YxG	11	27.529	4.31**	0.016**	0.004	2.499**	2021.006*
IxG	11	377.899**	134.22**	0.024**	0.026**	41.17**	52559.31**
YxIXG	11	18.221	3.01*	0.003	0.026**	4.628**	128.417
Error b	88	24.057	1.33	0.002	0.002	0.398	915.946

*and**: Significant at $P \leq 0.05$ and $P \leq 0.01$ probability level, respectively.

ISSR markers analysis

For ISSR primers revealed discernible amplification profiles, therefore were employed to investigate the genetic polymorphism among the 12 wheat genotypes (Table 6 and Fig 1). The number of amplified fragments from the gnomic DNA of the 12 wheat genotypes generated by the different ISSR primers is presented in table 7. Each of the 5 primers produced multiple band profiles with 12 wheat genotypes. The highest number of amplicons (14 amplicons) was generated by the primer 844A, while, the primer HB 8 exhibited 6 amplicons.

The 5 ISSR primers produced 48 amplicons, out of which 25 were polymorphic and the average percentage polymorphism was 52.08% (Table, 7). The number of amplicons per primer ranged from 6 (HB8) to 14 (844A) with an average of 9.6 fragments /primer across the different genotypes. However, the number of polymorphic amplicons varied from 3(HB8 and 17899B) to 7 (844A) with an average number of polymorphic amplicons of 5 fragments / primer.

Different ISSR markers were used for genetic characterization in wheat and related species by Ijaz and Khan, (2009) and Islam et al., (2012).

Table 5. Mean performance of the genotypes for all studied traits in normal (N) and drought stress (S) treatments during the two season of 2016/2017 and 2017/2018.

	Relative water content						Flag leaf area (cm^2)					
	2016/2017			2017/2018			2016/2017			2017/2018		
	S	N	Mean	S	N	Mean	S	N	Mean	S	N	Mean
Gemmiza11	58.53de	90.06a	74.3A	57.53jk	98.73a	78.13A	57.57n	76.8a	67.19B	58.9h	77.47a	68.19BC
Sids12	7.67de	90.06a	69.66AB	57.53jk	98.73a	78.13A	57.57n	71.53c	664.65c	59.09hl	72.87b	65.98D
Giza 171	68.18bc	72.35b	70.27AB	69.18fh	85.68cd	77.43A	69.38f	65.29i	62.34D	60.72h	67.96e	64.34E
Sids 13	46.83fg	65.49bd	56.16C	49.29lm	77.49df	63.39DE	61.38k	67.37h	64.37C	65.38g	68.92de	67.15CD
Shaka 93	49.86ef	62cd	55.93C	51.42kl	75.89eg	63.66DE	53.67o	68.68g	61.17D	57i	71.57bc	64.29E
Giza 168	52.1ef	83.33a	67.72B	53.66jl	94.56ab	74.11AB	64.44j	75.95b	70.19A	65.77fg	76.62a	71.19A
Line 116	66.27bd	73.21b	69.75AB	67.29gi	86.1bd	76.7A	41.86q	69.09fg	55.48E	4.64l	70.2cd	57.92F
Line 124	41.17g	72.98b	57.08C	40.83m	83.21de	62.02E	53.36o	69.97e	61.66D	54.03j	72.63b	63.33E
Line 127	61.44cd	81.81a	72.13AB	61.44hj	82.8de	72.13AC	64.12j	76.33b	70.23A	64.12g	67.33ef	70.23A
Line 145	41.38g	61.02cd	51.2C	41.38m	62.35hj	51.86F	48.29p	60.54l	54.42E	48.29k	60.31h	54.3G
Line 150	61.37cd	73b	67.18B	61.37hj	74.33eg	67.85CE	67.37h	71.08d	69.22A	67.37ef	70.62cd	69B
Yakora Rojo	66.83bd	68.9bc	67.86B	66.83gi	70.23fh	68.53BD	58.29m	64.54j	61.42D	59.63h	67.87e	63.75E
Mean	55.97B	73.9A		56.55B	82A		57.29B	69.64A		58.83B	71.12A	
	Catalase activity Unit min^{-1} mg^{-1} protein						**Peroxidase Unit min^{-1} mg^{-1} protein**					
	2016/2017			2017/2018			2016/2017			2017/2018		
	S	N	Mean	S	N	Mean	S	N	Mean	S	N	Mean
Gemmeza 11	0.51e	0.41i	0.46BC	0.42bc	0.3de	0.36B	0.44ch	0.29kl	0.36EG	0.56bc	0.16j	0.36D
Sids 12	0.53d	0.32l	0.42CD	0.5b	0.15h	0.33BC	0.36fl	0.29kl	0.32G	0.54bc	0.13j	0.34D
Giza 171	0.6c	0.32l	0.46BC	0.5b	0.18gh	0.34BC	0.61a	0.47 ce	0.54A	0.71a	0.32fh	0.52A
Sids 13	0.52de	0.37j	0.45BC	0.32de	0.24fg	0.28C	0.46cf	0.37ek	0.42CD	0.61b	0.27gh	0.44BC
Shaka 93	0.49f	0.43h	0.46BC	0.36cd	0.3de	0.33BC	0.48bd	0.4dj	0.44C	0.51cd	0.19ij	0.35D
Giza 168	0.52de	0.37j	0.5B	0.5b	0.28df	0.39B	0.43di	0.39dk	0.41CE	0.46de	0.25hi	0.35D
Line 116	0.61c	0.36j	0.48B	0.47b	0.21fh	0.34BC	0.46cf	0.35gl	0.4CE	0.57bc	0.15j	0.36D
Line 124	0.52de	0.42hi	0.47BC	0.43bc	0.24eg	0.33BC	0.39dk	0.33il	0.36EG	0.61b	0.15j	0.38CD
Line 127	0.47g	0.3m	0.38D	0.47b	0.3de	0.38B	0.45cg	0.3jl	0.38DF	0.57bc	0.15j	0.36D
Line 145	0.46g	0.34k	0.4D	0.46b	0.34d	0.4B	0.39dk	0.26l	0.33FG	0.39ef	0.26hi	0.33D
Line 150	0.63b	0.33kl	0.48B	0.71a	0.34d	0.52A	0.54ac	0.46cf	0.5AB	0.44de	0.62b	0.53A
Yakora Rojo	0.69a	0.42hi	0.56A	0.69a	0.48b	0.58A	0.57ab	0.34hl	0.45BC	0.56bc	0.34fg	0.45B
Mean	0.55A	0.37Bhi		0.49A	0.28B		0.46A	0.35B		0.55A	0.25B	

Table 5. Continues

	Proleine mg/g F.W.						Grain yield m^{-2}					
	2016/2017			2017/2018			2016/2017			2017/2018		
	S	N	Mean	S	N	Mean	S	N	Mean	S	N	Mean
Gemmeza 11	16.95de	7.87ij	12.41DF	14.12de	9.64h	11.88C	474.0i	971.3a	722.7AB	510.9f	1008.6a	759.8B
Sids 12	15.2f	7.58j	11.39G	12.09g	8.8hj	10.45E	644.0f	846.6c	745.3A	697.4d	907.8b	802.5A
Giza 171	19.99b	6.59k	13.29BC	16.44c	7.39k	11.92C	627.3fg	816.0cd	721.7AB	692.7d	887.1b	789.9AB
Sids 13	16.88de	6.76k	11.82EG	13.51ef	7.59jk	10.55E	380.0j	814.0cd	597.0C	431.8gh	869.2b	650.5D
Shaka 93	15.08f	9.18h	12.13DF	11.41g	9.98h	10.7E	276.0kl	736.0e	506.0D	342.2i	806.3c	574.3E
Giza 168	18.4c	6.93k	12.67CD	14.98d	7.67jk	111.33A	566.0h	943.3ab	754.7A	621.3e	999.4a	810.4A
Line 116	17.21d	9.15h	13.18BC	13.5ef	9.8h	11.65CD	262.0kl	652.0f	457.0E	322.7ij	718.5d	520.6F
Line 124	16.58e	8.42i	12.5DE	12.52fg	9.22hi	10.87DE	230.0lm	622.0fg	426.0E	284.3j	685.0d	484.7F
Line 127	18.23c	9.01h	13.62B	14.77d	9.01hi	11.89C	588.0gh	896.6b	742.3A	629.3e	896.7b	763.0B
Line 145	16.43e	7.03k	11.73FG	16.43c	7.03k	11.73C	198.0m	478.0i	438.0E	198.0k	478.0fg	338.0G
Line 150	12.02g	9.34h	15.68A	23.53b	9.34h	16.43B	619.3fg	788.6d	704.0B	619.3e	788.7c	704.0C
Yakora Rojo	23.88a	8.09ij	15.98A	25.57a	8.09ik	16.83B	312.0k	390.0j	351.0F	327.7ij	404.7h	366.2G
Mean	18.07A	8.0B		15.74A	8.63B		431.4B	746.2A		473.1B	787.5A	

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (P < 0.05)

Table 6. ISSR-PCR polymorphism in the twelve bread wheat genotypes using the five primers.

Amplicon	Mol. S (bp.)	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	MM*
ISSR1														
AF01	800	0	0	0	0	0	0	0	0	1	0	0	0	M+
AF02	750	1	1	1	0	0	0	1	0	0	0	0	0	
AF03	695	1	0	0	0	0	0	0	0	0	0	0	0	M+
AF04	684	0	0	0	0	0	0	0	0	0	0	1	0	M+
AF05	628	1	0	1	1	1	1	1	1	1	1	1	1	M-
AF06	612	1	1	1	1	1	1	1	1	1	1	1	1	
AF07	582	1	0	1	1	1	1	1	1	1	1	1	1	M-
AF08	542	1	1	1	1	1	1	1	1	1	1	1	1	
AF09	490	1	1	1	1	1	1	1	1	1	1	1	1	
AF10	380	1	1	1	1	1	1	1	1	1	1	1	1	
AF11	320	1	1	1	1	1	1	1	1	1	1	1	1	
AF12	300	1	1	1	1	1	1	1	1	1	1	1	1	
AF13	280	0	0	0	0	0	0	0	0	1	1	1	1	
AF14	200	1	1	1	1	1	1	1	1	1	1	1	1	

Table 6. Continues

Amplicon	Mol. S (bp.)	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	MM*
ISSR2														
AF15	605	0	0	0	0	0	0	0	0	1	0	0	0	M+
AF16	532	1	1	1	1	1	1	1	1	1	1	1	1	
AF17	491	0	0	0	0	0	1	0	0	0	1	0	0	
AF18	410	0	0	0	1	0	0	0	0	0	0	0	0	M+
AF19	400	1	1	1	1	1	1	1	1	0	1	1	1	M-
AF20	360	1	1	1	1	1	1	1	1	1	1	1	1	
AF21	300	0	1	0	1	1	0	1	0	0	0	0	0	
AF22	270	1	1	1	1	1	1	1	1	1	1	1	1	
AF23	200	1	1	0	1	1	1	1	1	1	1	1	1	M-
ISSR 3														
AF24	1000	1	1	1	1	1	1	1	1	1	1	1	1	
AF25	800	0	0	0	1	0	0	0	0	0	0	0	0	M+
AF26	700	0	0	0	0	0	0	0	0	0	0	1	0	M+
AF27	600	1	1	0	1	1	0	1	1	1	0	1	1	
AF28	520	1	1	1	1	1	1	1	1	1	1	1	1	
AF29	420	1	0	1	0	0	0	0	1	0	0	0	0	
AF30	300	1	1	1	1	1	1	1	1	1	1	1	1	
AF31	280	0	0	0	0	1	1	0	1	1	0	1	0	
AF32	200	1	1	1	1	1	1	1	1	1	1	1	1	
AF33	180	1	1	1	1	1	1	1	0	1	1	1	1	M-
ISSR4														
AF34	720	0	0	0	0	1	0	0	0	0	0	0	0	M+
AF35	600	1	1	1	1	1	1	1	1	1	1	1	1	
AF36	580	1	1	1	1	1	1	1	1	1	1	1	1	
AF37	550	1	1	1	1	1	1	1	1	1	1	1	1	
AF38	460	1	1	1	1	1	1	1	1	1	1	1	1	
AF39	440	1	1	1	1	1	1	1	1	1	1	1	1	
AF40	315	0	0	0	0	0	1	0	0	0	0	0	0	M+
AF41	210	0	1	1	1	1	1	1	1	1	1	1	1	M-
AF42	190	1	1	1	1	1	1	1	1	1	1	1	1	
ISSR5														
AF43	590	0	1	1	1	0	0	0	0	0	0	0	0	
AF44	580	1	1	1	1	1	1	1	1	1	1	1	1	
AF45	505	1	1	1	1	1	1	1	1	1	1	1	1	
AF46	450	1	1	1	1	1	0	0	0	0	0	0	1	
AF47	420	0	1	1	1	1	1	1	0	0	0	0	1	
AF48	350	1	1	1	1	1	1	1	1	1	1	1	1	
Total		33	33	33	36	35	33	33	31	33	31	34	35	

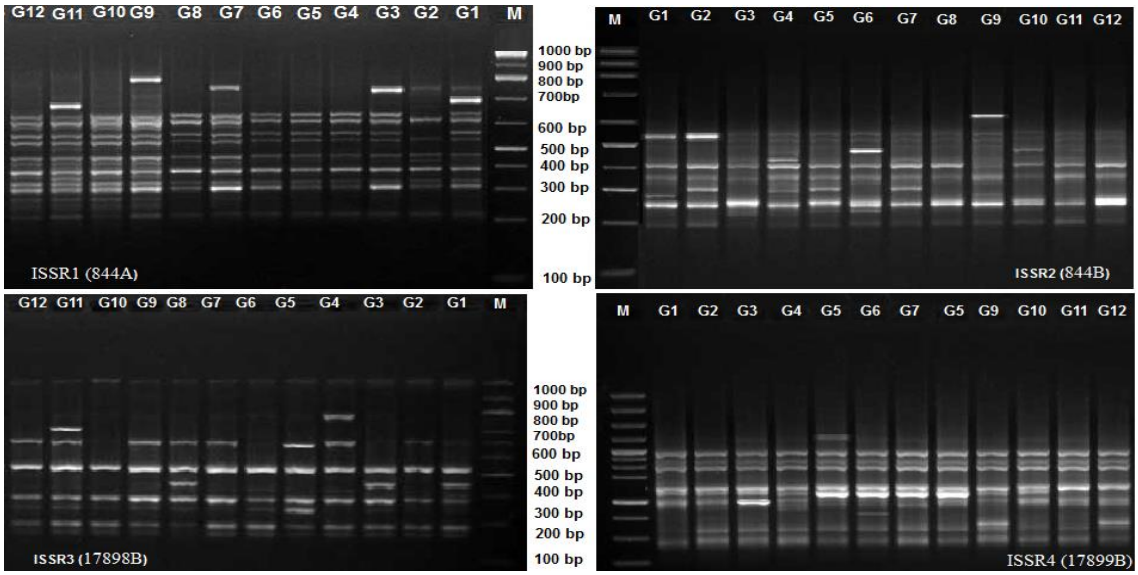

Figure. 1. ISSR fingerprints of the twelve wheat genotypes tested using ISSR-PCR primers 844A, 844B, 17899B and 17899B.

Table (7): Number of monomorphic and polymorphic amplicons and percentage of polymorphism, as revealed by ISSR primers for 12 wheat genotypes

Primer Name	Total number of amplicons	No. of monomorphic Amplicons	No. of polymorphic Amplicons	polymorphism (%)
844A	14	7	7	50
844B	9	3	6	66.6
17898B	10	4	6	60
17899B	9	6	3	33.3
HB 8	6	3	3	50
Total	48	26	25	
Average	9.6	5.2	5	52.08

Table 8. similarity matrix among the twelve bread wheat genotypes based on five ISSR –PCR primers amplification analysis.

	Gemmeza 11	Sids 12	Giza 171	Sids 13	Shaka 93	Giza 168	Line 116	Line 124	Line 127	Line 145	Line 150	Yakora Rojo
Gemmeza 11	1.000											
Sids 12	0.789	1.000										
Giza 171	0.784	0.833	1.000									
Sids 13	0.750	0.892	0.789	1.000								
Shaka 93	0.769	0.865	0.763	0.864	1.000							
Giza 168	0.718	0.763	0.757	0.769	0.838	1.000						
Line 116	0.811	0.914	0.806	0.865	0.889	0.833	1.000					
Line 124	0.806	0.757	0.750	0.763	0.833	0.829	0.829	1.000				
Line 127	0.763	0.718	0.667	0.725	0.789	0.784	0.784	0.829	1.000			
Line 145	0.757	0.757	0.750	0.763	0.784	0.882	0.829	0.824	0.829	1.000		
Line 150	0.744	0.744	0.692	0.750	0.816	0.811	0.811	0.857	0.861	0.857	1.000	
Yakora Rojo	0.711	0.757	0.703	0.763	0.784	0.778	0.778	0.824	0.829	0.824	0.857	1.000

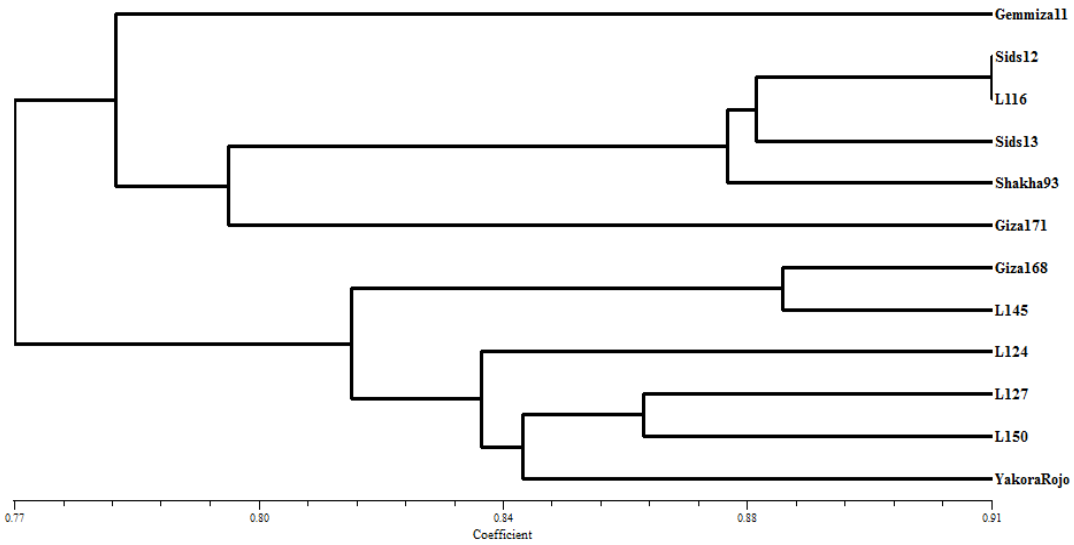

Figure. 2. Dendrogram of the twelve genotypes generated based on UPGM clustering method and Jacquard s coefficient using data of five ISSR markers.

Table 9. Tolerance indices of grain yield m^{-2} measured for 12 bread wheat genotypes cultivated under adequate and stress environments.

	Grain yield/ m^{-2}		Tolerance indices						
Genotype	**S**	**N**	**TOL**	**MP**	**HM**	**SSI**	**STI**	**YI**	**YSI**
First season 2016/2017									
Gemmeza 11	474	971.3	497.30	722.65	637.09	1.21	0.83	1.10	0.49
Sids 12	644	846.6	202.60	745.30	731.53	0.57	0.98	1.49	0.76
Giza 171	627.3	816	188.70	721.65	709.31	0.55	0.92	1.45	0.77
Sids 13	380	814	434.00	597.00	518.12	1.26	0.56	0.88	0.47
Shaka 93	276	736	460.00	506.00	401.45	1.48	0.36	0.64	0.38
Giza 168	566	943.3	377.30	754.65	707.49	0.95	0.96	1.31	0.60
Line 116	262	652	390.00	457.00	373.79	1.42	0.31	0.61	0.40
Line 124	230	622	392.00	426.00	335.82	1.49	0.26	0.53	0.37
Line 127	588	896.6	308.60	742.30	710.23	0.82	0.95	1.36	0.66
Line 145	198	478	280.00	338.00	280.01	1.39	0.17	0.46	0.41
Line 150	619.3	788.6	169.30	703.95	693.77	0.51	0.88	1.44	0.79
Yakora Rojo	312	390	78.00	351.00	346.67	0.47	0.22	0.72	0.80
Mean	431.4	746.2							
Second season 2017/2018									
Gemmeza 11	510.9	1008.6	497.70	759.75	678.24	1.24	0.83	1.08	0.51
Sids 12	697.4	907.8	210.40	802.60	788.81	0.58	1.02	1.47	0.77
Giza 171	692.7	887.1	194.40	789.90	777.94	0.55	0.99	1.46	0.78
Sids 13	431.8	869.2	437.40	650.50	576.97	1.26	0.61	0.91	0.50
Shaka 93	342.2	806.3	464.10	574.25	480.48	1.44	0.44	0.72	0.42
Giza 168	621.3	999.4	378.10	810.35	766.25	0.95	1.00	1.31	0.62
Line 116	322.7	718.5	395.80	520.60	445.37	1.38	0.37	0.68	0.45
Line 124	284.3	685	400.70	484.65	401.83	1.47	0.31	0.60	0.42
Line 127	629.3	896.7	267.40	763.00	739.57	0.75	0.91	1.33	0.70
Line 145	198	478	280.00	338.00	280.01	1.47	0.15	0.42	0.41
Line 150	619.3	788.7	169.40	704.00	693.81	0.54	0.79	1.31	0.79
Yakora Rojo	327.7	404.7	77.00	366.20	362.15	0.48	0.21	0.69	0.81
Mean	473.1	787.5							

TOL: Tolerance index, MP: Mean productivity, HM: Harmonic mean, SSI: Stress susceptibility index, STI: Stress tolerance index, YI: Yield index, YSI: Yield stability index.

Genetic relationship among the 12 wheat genotypes

The data in Table 8 show that the genetic similarity which ranged from 71.1% between gemmiza 11 and yakora rojo to 91.4% between sids 12 and line 116 is attributed to the fact that yakora Rijo is resistant to drought, although it gave low yield. On the other hand Gemmiza 11 is gave high yield under normal irrigation environment and was susceptible to drought.

Cluster analysis

A dendrogram of the 12 wheat genotypes was constructed using UPGMA (Fig. 2) to obtain the relationships among the 12 wheat genotypes using the scoring data of the 5 ISSRs primers. The twelve wheat genotypes were divided into two main groups. The first group was divided in two sub-clusters. The first one involved gemmiza 11 and the second involved Sids 12, L116 Sids 13, Sakha 93 and Geiza 171. However, Gemmiza 11 in one sub-group from the remaining 5 genotypes in anther sub-group. Also, Sids 12 and L116 were closely related, and the other with small different distance ratios. Most of the first major cluster genotypes have high grain yields. The second group was divided into Yakora Rojo in one sub-class, and the other genotypes Giza 168 and L145 join in sub-clustar, Also, L127 and L150 was collected together in one sub cluster. However L124 was as one sub-cluster

Comparing genotypes based on the tolerance indices basis

To investigate drought stress resistance indices for screening of wheat genotypes under normal and drought stress condition during the successive seasons of 2016/2017 and 2017/2018, grains yield m^{-2} were measured for calculating different sensitivity and tolerance indices (Table 9). A suitable index must correlate to any measured parameter under both tested conditions (Farshadfar et al., 2013). Grains yield m^{-2} across genotypes exhibited significant differences between stress (drought) and normal (normal irrigation) conditions. The differences varied among wheat genotypes (Table 4). The highest grain yield was given by Gemmiza 11 and Giza 168 under both normal and stress conditions followed by Sids 12 and Giza 171 under normal environment and Line 127 under the drought stressed environment in both seasons of study (Table 5). The lowest grain yield m^{-2} under normal as well as drought condition was shown by Line 145 and Yakora Rojo. Variations among the genotypes are in agreement with results of Fayaz and Arzani (2011), who reported that grain yield varied considerably from adequate to stress conditions and that genotypes had a high yield under adequate environment.

Since drought is a serious problem reducing crop productivity, improvement of tolerance in crops such as wheat is a major objective for most crop breeding programs. Based on the stress tolerance index (STI) and grain yield, Sids 12, Giza 168 and line 127 followed by Giza 171 were drought tolerant with the highest STI and grain yield, while Line 145 and Yakora Rojo displayed the lowest STI and grain yield under these conditions. The genotype with high STI showed high difference in yield under the two different conditions. In general, similar ranks for the genotypes were observed by HM parameters as well as YI, which suggests that these three parameters are equal for screening tolerant genotypes (Mevlut and Sait 2011).

Gemmiza 11 recorded the highest stress tolerance (TOL). Cultivars Sids 12 and Giza 168 showed highest MP, HM as well as STI as compared with other genotypes suggesting more stress tolerance mechanism. Yakora Rojo exhibited the highest YSI but gave low yield, though not a big decrease, due to its drought resistance mechanism. Thus, this genotype can be recommended as a parent in breeding programmers to transfer drought resistance.

CONCLUSION

Twelve genotypes were grown under two irrigation treatments during the two seasons of 2016/2017 and 2017/2018 to evaluate their response to drought stress. Also, ISSR marker experiment were done in order to select the most adaptable genotypes under drought stress Gemmiza 11 exhibited the highest values for grain yield was by followed by Giza 168. The genotype Yakora Rojo gave lowest yield but was reinstate to water stress. The amplicons ranged from 14 for primer 844A to 6 for primer HB 8. The high tolerant genotypes Sids 13 followed by Yakora Rojo gave highest total number of amplicons 36 and 35 amplicons, respectively.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors performed the field trail treatments as well as ISSR experiments, data collection, and data analysis. The first author El Hosary A.A.A. wrote the manuscript. All authors read and approved the final version.

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