The Impact of Drought Stress on some Morpho-Physiological Traits and RAPD Markers in Wheat Genotypes

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Abstract: Water limitation is a well-known problem for wheat plants. Lack of water affects their biomass and yield. This is the most conspicuous in case of crops causing severe uncertainty of agricultural productivity. Progress in breeding to improve drought tolerance has been limited by its high sensitivity to environmental factors, low heritability, and the complexity and size of wheat genome. In this study eight genotypes of bread wheat were used for screening them under three water regimes; control 100% Field Capacity (FC), 75% FC and 50% FC. Five drought resistance indices including Mean Productivity (MP), Tolerance Index (TOL), Drought Susceptibility index (DSI), Geometric Mean Productivity (MP) and Yield Stability Index (YSI) were calculated for each genotype based on grain yield under stress (50% FC) and normal (100% FC) conditions. Physiological parameters, chlorophyll content (SPAD values), proline accumulation and expression levels of drought related genes were analyzed in wheat plants at heading stage, comparing eight genotypes with different drought tolerance capacity. The imposed drought stress induced a decreasing of plant growth and chlorophyll content, a strong increase in proline and expression of drought related genes. The correlation coefficients showed that YSI, MP, DSI and GMP had the most desirable selection criteria for high yielding and drought tolerant genotypes. The development of molecular markers for physiological traits has made significant headway in recent years with the advancement of new technologies. Consequently, in our study the use of molecular markers; RAPD technique with 9 primers was detected 91 polymorphism alleles for the genotypes with 79.12% polymorphism. The most Polymorphic Information Content (PIC) value and polymorphism percentage was detected by OPA-07primer that showed the high score from bands 13 with polymorphism 69.23%. While, OPO-19 revealed low level from bands was 6 with percentage 83.33%. Also, OPA-02, OPA-04 and OPO-13 revealed 9 fragments with 77.78% polymorphism. While, primers OPB-07, OPB-10 and OPO-14 showed 11 bands with 81.82% polymorphism. The last primer revealed 12 bands with 75% polymorphism. Therefore, these recently developed techniques could be enable faster identification and characterization of drought-related gene(s).

Keywords: Drought susceptibility index, DNA, Proline and chlorophyll content (SPAD) values.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the most important cereal crop not only in Egypt but also all over the world, that play an important role in people's nutrition. The annual consumption of wheat grains in Egypt is about 14 million tons, while the annual local production in 2014 is about 8.6 million tons (Wheat Res. Dept., 2014). Therefore, increasing wheat production is an important goal to reduce the gap between production and consumption. Drought is the most significant factor that restricting plant growth and crop productivity in the majority of agricultural fields of the world. Wheat is essential nourishment for more than 1/3 of the world population and crop yield will be considerably influenced by global climate changing and limitation of water resources in the environment (AL-Ghamdi, 2009). One of the possible ways to ensure future food needs of an increasing world population involves the better water use through the development of crop varieties which need less water and are more tolerant to drought. For successful breeding of bread wheat cultivars tolerant to drought through conventional approach, basic information about the breeding material must be available to the breeders. Firstly, there must be significant variability in genotypic responses to water stress and secondly, this variation must be genetically controlled. Thus, an understanding of the knowledge of these two components about the breeding material under consideration is necessary (Mitra, 2001). Plants are known to adjust morphologically, physiologically, and biochemically to water stress (Vinocur and Altman 2005). Monitoring growth and development of plants grown under controlled conditions like hydroponics provides much effective and less costly ways to investigate genetic variability in morpho-physiological and biochemical traits (Tuberosa *et al.,* 2002).

Although development of higher-yielding crops under water-limited environments is the most viable solution to stabilizing and increasing wheat production under current climatic conditions, it is challenged by the nature of drought response as a trait and the complex genomic constitution of wheat (Bayoumi *et al.,* 2002 and Farooq, 2009). However, recently, the utilization of drought tolerant wild species and the rapid advances in molecular biological, functional genomics, and transgenics technologies have facilitated drought-related studies, resulting significant progress in the identification of related genes and generations and dissection of some of its molecular aspects. Recent advances in molecular biological, functional, and comparative tools open up new opportunities for the molecular improvement of modern wheat. Recently developed techniques enable faster identification and characterization of drought-related gene(s) and gene

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region (s). Introduction of drought-related components of wheat can be performed either with breeding through marker-assisted selection or transgenic methods. Recent increase in sequence availability due to recently developed high-throughput sequencing strategies has provided several high quality genetic markers for breeding. The association mapping (AM) which is used to make an association between marker alleles and phenotypic traits is now extensively being used as an alternative approach to overcome shortcomings of pedigree-based quantitative trait loci (QTL) mapping. As the improvement in wheat yield under drought is still a complicated task to achieve, therefore the main purpose of this study was to screen wheat genotypes with better grain yield and to identify reliable selection indices for drought tolerant wheat genotypes. Wheat genotypes were carried out for important morpho-physiological and biochemical traits. Further, to obtained new markers, this can be useful in traditional and molecular breeding programs; to use as marker assisted selection (MAS).

MATERIALS AND METHODS

The Present research was conducted at the experimental farm, Fac. of Agric., Suez Canal Univ., Ismailia, Egypt in two winter seasons 2013/2014 and 2014/2015. In this study eight genotypes of bread wheat (*Triticum aestivum* L.) were used for screening them under different water deficit treatments. These genotypes included three Egyptian cultivars (Maser 2, Gemmiza 10 and Sids 13) characterized moderate to high sensitivity for drought tolerant respectively. Three strains (Strain 1, Strain 2, Strain 3) and two hybrids (Strain 1 x Sakha 93 and Strain 1 x Giza 167) which were obtained from (Abd El-Raheem,1990). The genotypes were sown under two drought treatments and control conditions following randomized complete block design (RCBD) with three replications. Recommended irrigations were given to control treatment and soil moisture was maintained to field capacity (100%) until harvest. Drought stress treatments were applied by preventing irrigation to maintain field capacity of 75% and 50%. All suggested agricultural practices were followed as and when required.

Measurement traits

Data were recorded for growth traits; shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW). Moreover, at harvest grain yield/m^2 for various water stress treatments were determined. Physiological and biochemical attributes included:

Total chlorophyll content (SPAD)

Chlorophyll meter readings as a SPAD values (502 plus-Minolta, Japan) were repeatedly taken at fully expanded flag leaves throughout the experiments three times and averaged was calculated.

Proline content in leaves

The proline was extracted by methanol and then measured following the ninhydrin method described by (Bates *et al.*, 1973) using L-proline as a standard.

Tolerance indices

Relative decrease (RD%) was calculated as the ratio of unstressed - stressed to unstressed plants (control).

Stress tolerance and susceptibility indices including mean productivity (MP), geometric mean productivity (GMP), tolerance (TOL), drought susceptibility index (DSI) and yield stability index (YSI) for water deficit environment were calculated based on grain yield under sever water stress (50% FC) and unstressed (100% FC). Stress tolerance attributes were calculated by the following formulae:

Mean productivity (MP) and Tolerance (TOL) was calculated according to Gupta *et al.* (2001).

1. Geometric Mean Productivity (GMP) = $\sqrt{Yp * Ys}$
2. Mean productivity = $Y_p + Y_s/2$
3. Tolerance index (TOL) = $Y_p - Y_s$
4. Yield stability index (Y SI) = Y_s/Y_P
5. Drought susceptibility index = $(1- Y_s/Y_p)/DII$

According to Chaudhuri and Kanemasu (1982) where,

Ys = mean yields of a given genotype in WS (50% FC) condition;

Yp = mean yields of a given genotype in NS (100% FC) condition;

DII = Drought intensity index.

The drought intensity index (DII) for each water regime was calculated as

DII =1 - Xs/X_p

Genotyping DNA

Genomic DNA was extracted following the phenol–chloroform method of Pallotta *et al.* (2000) with some minor modifications. All DNA samples were diluted to a concentration (20 ng/µl) and kept at 40°C, while the stock was being kept at −20°C. PCR reactions were carried out according to Roder *et al.* (1998).

PCR- amplification of RAPD

Amplification reaction was carried out in 25µl reaction mixture contained 2µl of genomic DNA, 3µl of the primer, 2.5µl of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase and 200 mm of each dNTPs. The following PCR program was used in a DNA Thermo cycler (PTC-100 PCR version 9.0-USA). Initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30s, 42°C for 90 sec. for annealing temperature, 72°C for 90 Sec. and final extension at 72°C for 2 min. Products by RAPD-PCR were separated on 1.5% agarose gels in 1X TAE buffer and detected by staining with ethidium bromide according to Sambrook *et al.* (1989). DNA ladder 100 bp was used and PCR products were visualized by UV-trans illuminator and photographed by gel documentation system, the amplified bands were scored as (1) for presence and (0) for the absence of all studied wheat varieties according to gel analyzer protocol.

RAPD analysis:

A set of nine random 10-mer primers Table (1) was used in the detection of polymorphism among the eight broad wheat accessions. These primers were

synthesized at RAPD-PCR was carried out according to the procedure given by Williams *et al.* (1990) with minor modifications.

Table (1): Code and sequences of nine RAPD primers.

Primer code	Sequence (5`→3`)
OPA-02	CAGGCCCTTC
OPA-04	AATCGGGCTG
OPA-07	GAAACGGGTG
OPB-07	GGTGACGCAG
OPB-10	CTGCTGGGAC
OPO-10	TCAGAGCGCC
OPO-13	GTCAGAGTCC
OPO-14	AGCATGGCTC
OPO-19	CAATCGCCGT

Statistical analysis

A randomized complete block design with three replicates was used for each water regime. In both seasons analysis of variance and least significant difference (LSD) were used separately to evaluate the response of each character within treatments according to Steel *et al.* (1997). To confirm the relative importance of various characters, heritability in broad sense and coefficient of variation (CV) were calculated for each trait. The calculation of these summary statistics requires knowledge of the error variance for the trait. Moreover, correlation analysis among grain yield and the other characters were calculated according to Hallauer and Miranda (1988).

RESULTS AND DISCUSSION

To utilize any new genotypes effectively in breeding for drought tolerance, it is necessary to characterize and evaluate these genotypes for desirable traits. So, plant morpho-physiology and biochemical approach may help us to identify traits or set of traits that improving yield under stress conditions.

Growth

Drought stress reduced the plant growth as characterized by fresh and dry mass, irrespective of the genotypes were evident from the decline in dry weights of shoots with water stress (Fig. 1). The reduction in dry weight under water stress may be attributed to inhibition of hydrolysis of reserve synthesizing food and its translocation to the growing shoots (Munns and James, 2003). While, root dry weight was increased with drought stress. The coefficient of determination (R^2) between water stresses and shoot dry weight (R^2 = 0.513); root dry weight (R^2 = 0.606) was significant. Our results clearly showed that various wheat genotypes differently responded to soil water stress in terms of shoot and root dry weight, implying that these genotypes could have a large impact on wheat production for water stress. Similar observation was reported by Bayoumi *et al*. (2008) who found that shoot length was always decreased by exposure to all the stress levels tested, whereas , there was a an increase in root length associated with water stress. The development of the root system in response to water deficit suggests that the expression of certain genes controlling root formation is stimulated by drought conditions (Rauf *et al.,* 2007). In addition to, dominant alleles controlled the length of roots and that this feature could be easily incorporated in breeding for drought resistance (Zulu and Modi, 2010).

Grain yield

Analysis of variance was used for the identification of significant genotypic differences. The results of ANOVA showed that in water stress treatments, there was considerable variability among the studied genotypes with regard to grain yield (Table 2). The results showed that genotypic differences were highly significant in water stress conditions and the magnitudes of variances were for treatment 50% FC.

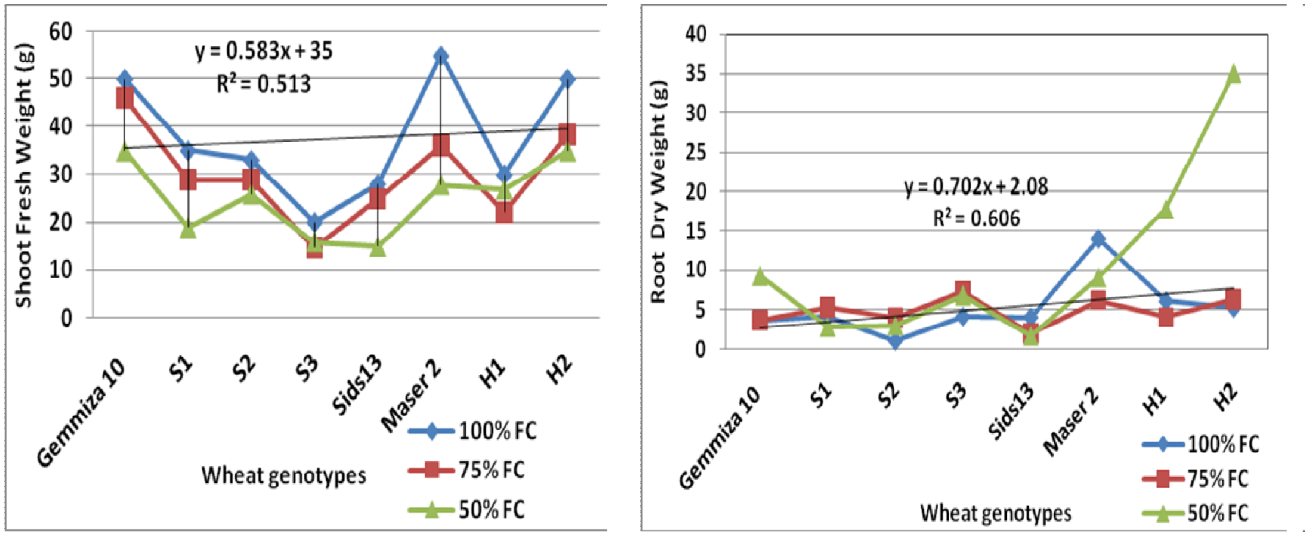

Fig. (1): Effect of water stress on shoot and root dry weight of wheat genotypes

Table (2): Analysis of variance for grain yield (g /m^2) in severe stress (50% FC), moderate stress (75% FC) and non-stress (100% FC) conditions.

SOV	df	Mean of Squares		
		50% FC	75% FC	100% FC
Replications	2	533.6	446.8	254.4
Genotypes	7	1982.3**	1278.5**	1011.2**
Error	14	234.2	187.3	84.4

Grain yield (Table 3) varied from a high yield of 920.0 g m-^{2} (L3) to a low yield of 710 g m-^{2} (Maser 2) under 100% FC, from 680 g m-^{2} (H1) to 290 g m-^{2} (L3) in 75% FC and from 652 g m-^{2} (H1) to 180 g m-^{2} (Sids 13) under 50% FC). Grain yield decreased about 35% to 49% when plants exposed to 75 and 50% FC, respectively. The availability of current assimilates for extending seed filling will often be severely reduced. In such circumstances, a genotype that can mobilize reserves of carbohydrates in the stem will be able to maintain better seed filling.

The values of geometric mean productivity (GMP) ranged from 476.2 to 726.7 g m-^{2} and the genotypes H1 and H2 were the most productive (> 690 g m^{-2}). Yield Stability index (YSI) ranged from 0.24-0.72 the higher Values indicate high stress tolerance. Besides the yield stability, mean productivity (MP) and geometric mean productivity (GMP) showed similar ranking pattern as in drought susceptibility index (DSI). Drought susceptibility index has been used to characterize relative drought tolerance of wheat genotypes. Low drought susceptibility index (S < 1) is synonymous with higher stress tolerance. The DSI ranged from 0.3 for (H1) to 1.33 for Gemmeza 10. Generally, the previous indices indicated that the genotypes H1 and H2 followed by L1 were the most tolerant genotypes.

Table (3): Mean grain yield for various water regimes, mean productivity (MP), geometric mean of productivity (GMP), tolerance index (TOL), yield stability index (YSI) and drought susceptibility index (DSI).

Genotypes	Grain Yield g/m^2			MP	GMP	TOL	YSI	DSI
	100% FC	75% FC	50% FC					
Gemmiza 10	810	670	280	586.7	476.2	530	0.35	1.33
S1	720	640	475	611.7	584.8	245	0.66	0.52
S2	890	490	460	613.3	639.8	430	0.52	0.74
S3	920	290	285	498.3	512.1	635	0.31	1.06
Sids13	755	330	180	421.7	368.6	575	0.24	1.17
Maser 2	710	435	340	495.0	491.3	370	0.48	0.80
H1	810	660	652	707.3	726.7	158	0.80	0.30
H2	820	680	590	696.7	695.6	230	0.72	0.43
LSD	5.4	9.3	10.7	4.3	2.6	12.7	0.19	0.04
RD %		35.0	49.0					

Proline content

In view of fact that the accumulation of proline is tightly controlled by genes and cDNA encoding osmolyte biosynthesis and only achieved when the rate of synthesis prevails over that degradation, probably because too much proline is toxic to cell plant (Yokota *et al.,* 2006). In present work, the sharp increased in proline content might theoretically, attribute to the genes for synthesis and degradation of proline which are up-regulated strongly under drought stress. It might be an adaptation the purpose of which is to overcome the stress condition and it could supplies energy for growth and survival and thereby helps the plant to tolerate stress (Sankar *et al.,* 2007).

Proline is considered to be the most important organic compatible osmolyte and it is also a protecting of biological macromolecules in the protoplasm. The change in proline content in flag leaf was monitored (Fig. 2) as accumulation of proline is considered to be associated with water stress. The results showed that water stress caused marked highly significantly increases in proline contents with various water regimes. Proline content increased by 3 fold in the most genotypes under water stresses (50% FC) compared to control (100% FC). The regression coefficient R^2=0.648 for proline content in wheat genotypes was highly significant under water stress.

In addition to its role as an osmolyte and a reservoir of carbon and nitrogen, proline has been shown to protect plants against free radical induced damage and slow utilization of proline for protein synthesis and stimulation of glutamate conversion to proline during stress may be the possible reason for proline accumulation. Proline is one of the most important osmoprotectant in plants. Under water stress most plant species exhibit a remarkable increase in their proline content (Errabll *et al.,* 2006; Ehab *et al.,* 2011).

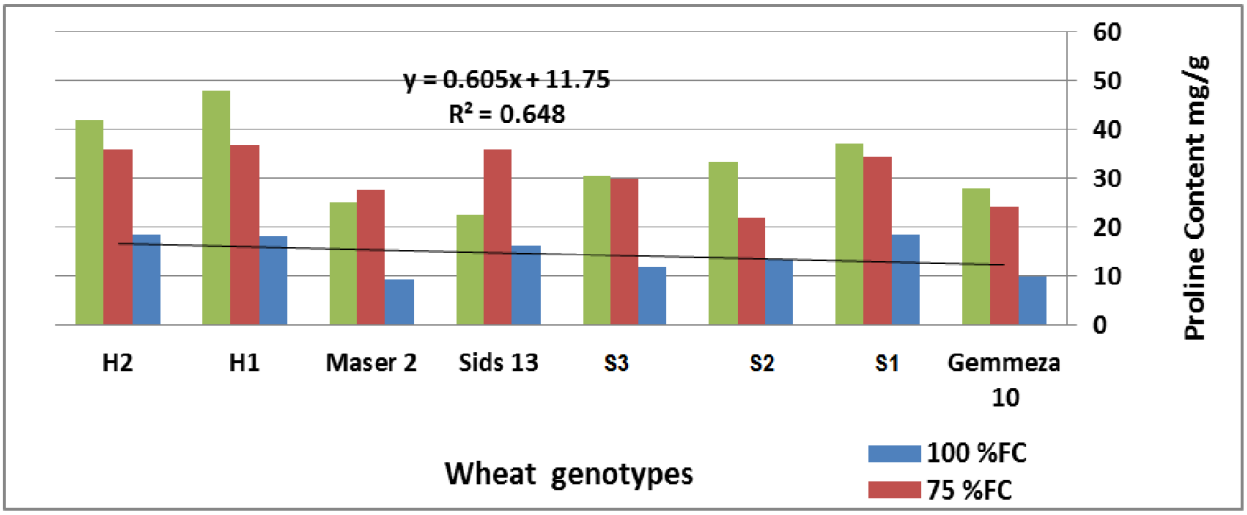

Fig. (2):.Proline content for wheat genotypes under various water stress

Chlorophyll content

Drought -induced restriction in water supply can cause stoma closure; which will in turn lead to decreased absorption of CO2 and eventually result in reduction of photosynthesis. However, chlorophyll content is associated directly with light harvesting potential and is normally considered as one of the important components in photosynthetic capacity. Data presented in (Figure 3) showed that water stress decreased chlorophyll content for the tested genotypes. The percent reduction of chlorophyll content was greater in Sids 13, Gemmiza 10 and Maser 2 than H1, H2, S1, S2 and S3 under the water stress. Several investigators reported that chlorophyll and total carotenoid contents of leaves decrease, in general, under water stress. The ability of plants to tolerate drought is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast function and maintain ion homeostasis (Parida and Das, 2005). The marked decrease of chlorophyll content was observed in all varieties. Leaf chlorophyll content is considered to be a good indicator of photosynthetic capacity. Lower chlorophyll content would limit photosynthetic potential and lead to a decrease in biomass and production (Naumann *et al.,* 2008). Decreasing chlorophyll content index of wheat leaves with water stress (Figure 3) could be related to increasing the activity of chlorophyll degrading enzyme: chlorophyllase (Jamil *et al.,* 2007). Moreover, the destruction of the chloroplast structure and the instability of pigment protein complexes (Singh and Dubey, 1995).

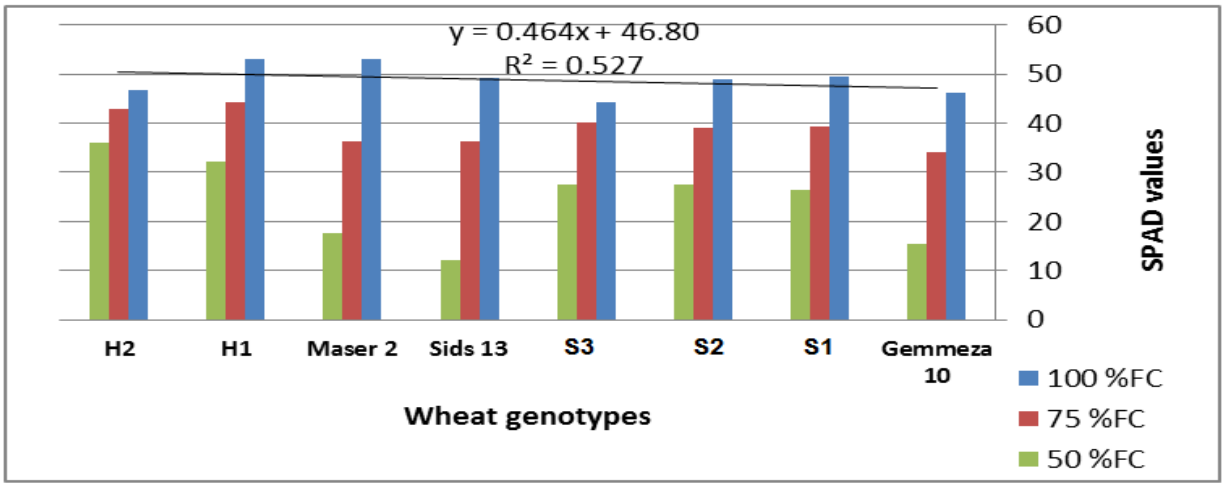

Fig. (3): Chlorophyll content for wheat genotypes under various water stresses.

Mean genotype heritability and coefficient of variability (CV %) for each trait

To investigate the reliability of the analysis of the studied traits, the CV and mean genotype heritability has been calculated for each trait (Table 4). The traits PC, CHL, GMP and TOL have the highest heritability values (0.73–0.86); other traits that have a heritability around 0.60 such as YS, MP, RDY, could be provide useful information for breeding selection whilst, GYP and DSI were 0.46 and 0.41. Moreover, Grain yield for both stresses were around (0.30) and considered unacceptably low for use as breeding tools. The traits GY_{SS}, GY_{IS} and GY_P have very high CVs (Table 4).

This study confirmed the known trend to lower heritability of yield in more stressful selection environments, but it also evidenced for yield in these environments a trend to higher correlation with yield over target environments. This latter result may be a contributing reason to the fact that unfavorable environments tend to be more useful than favorable ones for the selection of wheat genotypes aimed at identifying material that performs well across environments with different stress levels in the region (Ceccarelli *et al.,* 1991). The choice between favorable and unfavorable test sites, sometimes addressed especially for target regions where a relatively low year-to-year climatic variation and/or the adoption of site-specific agronomic practices (e.g., irrigation) make the difference between locations rather constant in the time (e.g., Braun *et al.,* 1992; Cooper *et al.,* 1995), can be considered of lesser importance in the current target region, in which the same location may act as a favorable or unfavorable environment for selection depending on the year.

Table (4): Mean genotype heritability and coefficient of variation (CV %) for each trait

Trait	Heritability	CV (%)
Shoot dry weight (FDY)	0.42	14.8
Root dry weight (RDY)	0.59	13.6
Grain yield for 100 % FC (GY_P)	0.46	25.2
Grain yield for 75 % FC (GY_{IS})	0.31	27.3
Grain yield for 50 % FC (GY_{SS})	0.28	29.77
Mean productivity (MP)	0.60	14.18
Geometric Mean productivity (GMP)	0.77	9.97
Tolerance Index (TOL)	0.73	11.32
Yield Stability (YS)	0.61	12.9
Drought susceptibility index (DSI)	0.41	11.8
Chlorophyll content (CHL)	0.81	6.15
Proline content (PC)	0.86	7.98

Association between grain yield and other tolerance indices

The indices GMP, MP and YS were very similar to the selection based on Y_{is} and Y_s. This was confirmed by positive and highly correlations between Y_s and GMP (r = 0.97), MP (r = 0.93), and YS (r = 0.98) and the correlation between Y_{is} and GMP (r = 0.83), MP (r = 0.86) and YS (r = 0.73) (Table 5). MP is the mean production under both stress and non-stress conditions, and it was highly correlated with yield under both conditions. Thus, MP can be used to identify genotypes in the tolerant group. While, there was a high negative correlation between drought susceptibility index (DSI) and the other tolerance indices except grain yield for 100% FC (Yp). Therefore, these indices were able to identify superior genotypes for both droughts stressed and non stressed treatments.

DSI, YS, GMP and MP were strongly correlated with yield under both stress conditions, suggesting that these parameters are suitable for screening drought tolerant and high yielding genotypes in both drought stressed and non stressed conditions. Similar results were reported by Farshadfar and Sutka (2003) on wheat (*Triticum aestivum*), Golabadi *et al.* (2006) on durum wheat (*Triticum durum*), Sio Se-Mardeh *et al.* (2006) and Mollasadeghi *et al.* (2011) on wheat (*Triticum aestivum*), all of whom found these parameters to be suitable for discriminating the best genotypes under drought stress and irrigated conditions.

Table (5): Simple correlation of yield in 100% FC (Y_p), 75% FC (Y_{is}) and stressed 50% FC (Y_s) conditions with mean productivity (MP), geometric mean productivity (GMP), tolerance index (TOL), yield stability index (YSI) and drought susceptibility index (DSI), in wheat genotypes.

Variable	Y_p	Y_{is}	Y_s	MP	GMP	TOL	YSI	DSI
y_p	1.00	-0.21^{ns}	0.07^{ns}	0.77^{*}	0.78^{*}	0.67^{*}	0.74^{*}	0.13^{ns}
y_{is}		1.00	0.70*	0.86*	0.83*	-0.74*	0.73*	-0.51*
y_s			1.00	0.93*	0.97*	-0.90*	0.98*	-0.94*
MP				1.00	0.93*	-0.79*	0.88*	-0.75*
GMP					1.00	-0.79*	0.91*	-0.87*
TOL						1.00	-0.97*	0.93*
YSI							1.00	-0.96*
DSI								1.00

RAPD analysis

In order to investigate the genetic differences between the genotypes Random amplified polymorphic DNA (RAPD) analysis was performed. Nine primers used in the present study resulted in the appearance of PCR products with a variable number of bands Fig. (4). RAPD analysis has been successfully used for detection the genetic diversity in diploid, tetraploid and hexaploid wheat Sivolap *et al*. (1999) and Pakniyat and Tavakol (2007).

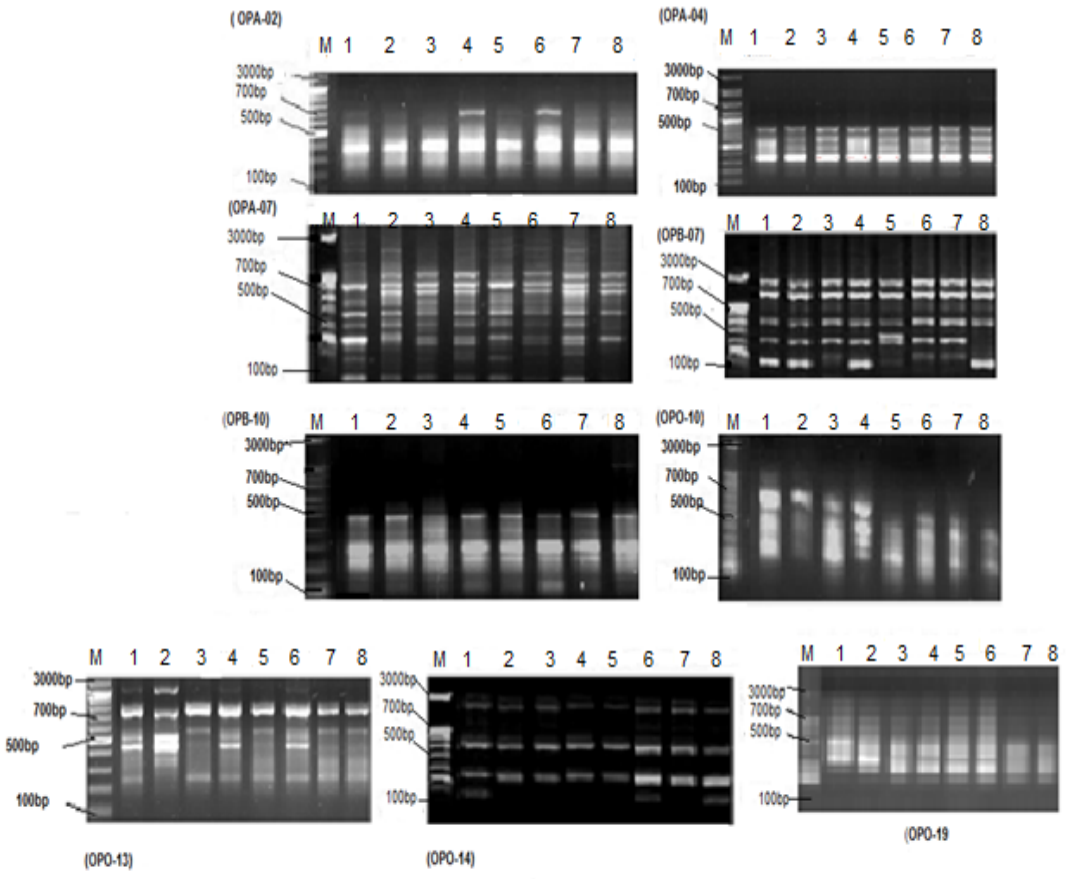

Fig. (4): RAPD pattern of wheat genotypes using primers OPA-02, OPA-04, OPA-07, OPB-07, OPB-10, OPO-10, OPO-13, OPO-14 and OPO-19 DNA fragments.

These primers generated 91 different bands were detected among the eight wheat genotypes of which, 19 monomorphic bands with 20.88% from total ratio, 60 polymorphic bands, 12 unique bands and the polymorphism percentage was 79.12%. These bands can be considered as useful RAPD markers for discriminating between the eight wheat genotypes which used in the present study (Table 6).

Primer OPA-07 produced the largest number of bands (13) followed by primer OPO-10 produced 12 bands, while primer OPO-19 produced the smallest number of bands (6). RAPD technique has a great potential to find DNA based polymorphism between the genotypes of same species. These identified polymorphic bands can be considered as potential markers to identify drought tolerant cultivars for marker assisted selection (MAS) in wheat breeding programs Deshmukh *et al*. (2012).

All producing fragments were used to calculate the similarity values and illustrated in the matrix (Table 7).

Table (6): Total number, monomorphic, polymorphic of bands and percentage of polymorphism as revealed using nine RAPD primers of eight wheat genotypes.

Primer	Total bands	Monomorphic bands	Polymorphic bands	% polymorphism
OPA- 2	9	2	7	77.78%
OPA-04	9	2	7	77.78%
OPA-07	13	3	10	76.92%
OPB-07	11	2	9	81.82%
OPB-10	11	2	9	81.82%
OPO-10	12	3	9	75%
OPO-13	9	2	9	77.78%
OPO-14	11	2	9	81.82%
OPO-19	6	1	5	83.33%
Total bands	**91(100%)**	**19(20.88%)**	**72(79.12%)**	**79.12%**

Table (7): Proximity matrix

Case	Gemmiza10	S1	S2	Sids13	S3	H1	H2	Maser 2
Gemmiza10	1.000							
S1	.780	1.000						
S2	.000	.003	1.000					
Sids13	.070	.292	.342	1.000				
S3	.260	.145	.429	.444	1.000			
Maser2	.255	.067	.012	.273	.295	1.000		
H1	.464	.505	.014	.475	.444	.402	1.000	
H2	.188	.128	.694	.440	.699	.152	.440	1.000

Table (7) showed the similarity and relationships between the genotypes whereas the highest value of similarity (0.78) was detected between Gemmiza 10 and S1 while, the lowest value (0.00) was detected between Gemmiza 10 and S2. Genotypes with the lowest similarity indices had the highest score for genetic variation in most of the morphological and agronomical traits Abd-El-Haleem (1999). On the other hand, PCR fragments produced with all primers used in detect dendrogram or the phylogenetic tree between eight genotypes constructed using the UPGMA differentiated into six clusters Fig. (5).

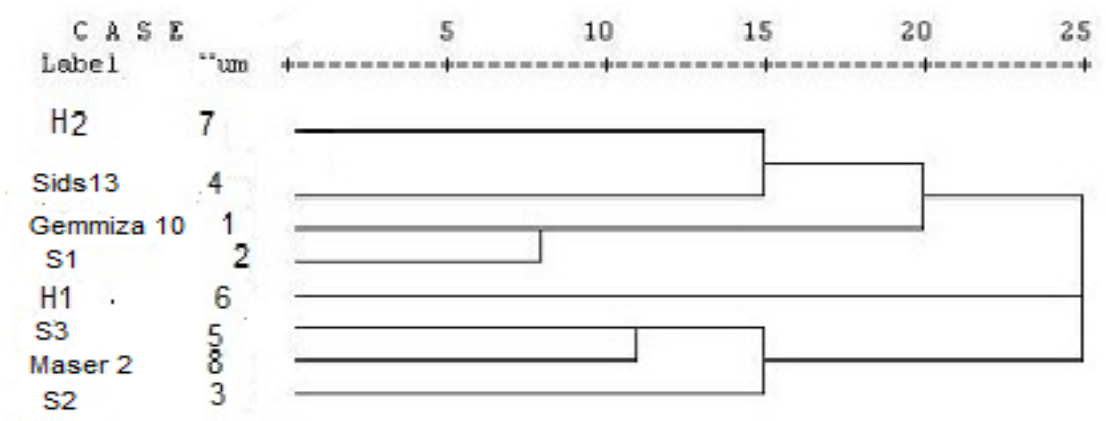

Fig. (5): Dendrogram using Average Linkage (Between Groups) Rescaled Distance Cluster Combine for eight bread wheat genotypes.

Where; H1= (H1xGiza167) and H2= (H1xSakha93).

Four clusters contained one genotype for each of them, while two clusters contained tow genotypes for each (Gemmeza10 & S1) and (S3 & Maser 2) respectively. These results confirmed the results of growth and yield parameters.

The expression of a number of drought-related genes takes place in water deficit conditions. Using nine primers in RAPD analysis, the results revealed 12 unique bands with (13.19%) as markers for drought stress whereas, it was detected two with OPA-02, OPA-04, OPB-07 and OPO-14. While, it was detected one marker with OPA-07, OPB-10, OPO-10 and OPO-13 respectively, genotypes S3 and Sids13 scored three markers each. On the other hand, Gemmiza 10 and S2 scored two markers. While, one unique band was detected in L1, H1 (S1 x Sakha 93) and H2 (S1xGiza167), (Table 8).

Table (8): Markers bands for drought stress of eight wheat genotypes using RAPD-PCR

Primers	Genotypes / No. of bands	Gemmiza10	L1	L2	Sids13	L3	H1	H2	Maser2
OPA-02	(3)	0	0	0	1	0	0	0	0
	(7)	0	1	0	0	0	0	0	0
OPA-04	(3)	0	0	0	0	1	0	0	0
	(7)	0	0	1	0	0	0	0	0
OPA-07	(11)	1	0	0	0	0	0	0	0
OPB-07	(1)	0	0	0	1	0	0	0	0
	(10)	0	0	0	0	1	0	0	0
OPB-10	(2)	0	0	1	0	0	0	0	0
OPO-10	(7)	1	0	0	0	0	0	0	0
OPO-13	(3)	0	0	0	1	0	0	0	0
OPO-14	(2)	0	0	0	0	1	0	0	0
	(5)	0	0	0	0	0	0	1	0

RAPD technology is a powerful tool in quickly identifying markers related to drought tolerance in wheat (Pakniyat and Tavakol, 2007**).** For early discovering of drought tolerant genotypes to be cultivated in suitable area of lower water supply and temperature increases, seven positive markers indicated in bread wheat, while sensitive ones appeared only in one negative RAPD marker Ameen (2013).

CONCLUSION

Breeding for drought tolerance is a challenging task because of the complexity of drought responses, environmental factors, and their interactions. Conventional breeding approaches have been successful, but progress has been slow. The indices DSI, GMP, TOL, YS and MP were used to identify tolerant genotypes that produced high yield under both irrigated and drought stress conditions. Based on the results, S1,

H1 (S1 x Sakha 93) and H2 (S1 x Giza167) were selected as tolerant genotypes and Gemmiza 10 and Sids 13 genotypes as sensitive to water stress. The expression of a number of drought-related genes takes place in water deficit conditions. Using nine primers in RAPD analysis, the results revealed 12 unique bands with (13.19%) as markers for drought stress. Therefore, the recent advances in genome mapping and functional genomics technologies could provide new powerful tools for the genetic dissection of drought tolerance components. It is anticipated that molecular genetics research is provide high-throughput DNA marker systems for marker-assisted selection that can be more efficient and effective in combing out favorable drought tolerance traits in breeding programs. It is also lead to a better understanding of the molecular basis of the genes underlying drought tolerance, which can be used in a genetic engineering program for drought tolerance improvement.

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تأثير الجفاف على بعض الصفات المورفوفسيولوجية والمعلمات الجزيئية للتراكيب الوراثية في القمح

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يعتبر نقص الماء من أهم المشاكل التي تواجه النبات والتي تؤثر على المحصول وتسبب تدهورا غير متوقع للإنتاجية الزراعية ، لذلك فان التقدم في التربية لتحسين التحمل للجفاف يكون مرتبطا بالحساسية للعوامل البيئية ، نقص كفاءة التوريث و الحجم الجينومى للقمح. في هذه الدراسة استخدمت ثمانية تراكيب وراثية من القمح لغربلتها تحت ثلاثة مستويات من الري (١٠٠%، ٧٥% و ٥٠% من السعة الحقلية) بمزرعة كلية الزراعة جامعة قناة السويس. تم استخدام خمسة دلائل للمقاومة للجفاف هي متوسط الإنتاجية، دليل التحمل، دليل الحساسية للجفاف، دليل ثبات المحصول و المتوسط الهندسي للإنتاجية والتي حسبت لكل تركيب وراثي بناء على محصول الحبوب تحت ٥٠ % من السعة الحقلية مقارنة بالكنترول. وجد أن هناك ارتباط معنوي بين هذه الدلائل و التحمل للجفاف. قدر المحتوى الكلوروفيللى وتراكم البرولين وكذلك التعبير الجينى للنباتات عند مرحلة طرد السنابل. أحدث الإجهاد المائي انخفاض ملحوظ في نمو النباتات والمحتوى الكلوروفيللى بينما زاد محتوى البرولين. تم استخدام المعلمات الجزيئية (طريقة RAPD) بواسطة ٩ بوادئ أنتجت ٩١ حزمة من المشابهات الاليلية بين التراكيب الوراثية بنسبة ٧٩.١٢% من التشابه وأن معظم هذه النسبة من التشابه اكتشفت بواسطة البادئ OPA-07 (١٣ حزمة) بينما اظهر البادئ OPO-19 اقل عدد من الحزم (٦) بنسبة تشابه ٨٣.٣٣ %. كما اظهر ارتباط بين ١٢ حزمة unique و بين تحمل الجفاف.