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Screening of Some Egyptian Wheat Cultivars under Low Soil Moisture and Molecular Characterization Using Drought Specific Markers and DREB1 Gene Markers

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Drought stress during seed germination may act as selection criteria for drought tolerance in wheat. An experiment was done at University of Nebraska-Lincoln (UNL) under a controlled condition to investigate germination of ten Egyptian wheat varieties under a stressful amount of water, and to verify for the presence of drought-resistant genes in the studied Egyptian cultivars using specific molecular markers for drought and DREB1 Gene markers. A sample from Nebraska soil was used to construct the moisture retention curve, and to select soil moisture stressful levels. Wheat cultivars showed different significant variations in the percentages of germination ($p<0.01$) as well as there were a significant effects of water stress treatments and days on germination ($p<0.01$. The most responsive Egyptian cultivars on the more stressful condition (7.5 ml) were Sids13 (18.33%) followed by Giza168 (16.66%). Among the Egyptian cultivars Giza168 and Sids12 showed the highest mean of germination responses (42.50%) to the two water treatments followed by Sids13 (40.83%). In the present study, 22 drought specific molecular markers were used to screen the ten wheat cultivars from Egypt. The PIC ranged from 0.00 to 0.90 with a mean PIC of 0.38. The generated Dendrogram revealed three main clusters. Cluster 1 contains three cultivars (Sids 12, Giza 168 and Wesley), cluster 2 includes three cultivars (Gemmiza 10, Gemmiza11 and Sakha 93)

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and cluster 3 contains (Misr1 and Misr2). Screening for dreb1 gene specific to dehydration responsive element binding protein's was made using four primers, results showed two primers (P18 and P22 primers) were approximately matched the expected band size products. This study may add valuable information for further wheat improvement programs and selection and hybridization for drought tolerance. Tolerant cultivars can be grown under rainfall condition in the north coastal region of Egypt.

Keywords: Egyptian wheat cultivars; water retention curve; drought and DREB1 specific markers.

1. INTRODUCTION

Wheat is the main field crop in Egypt, vertical and horizontal expansion in cultivated wheat is currently the main demand to increase the production and reduce the amount of imported wheat. Cultivation of field crops in Egypt is faced by the limited amount of irrigated water, inadequate rainfall and also the competition of the other winter crops upon the cultivated land. Another challenge facing Egypt is the growth of its population that is expected to reach 170 million by 2050 [1]. The consequences of water declination may result in limitation of the crop production and expansion in cultivated land. Climatic changes may contribute to limitation of water resulted in a drought which may affect the quality and the quantity of crops yield, also at the molecular level gene expression may influence plant development and growth [2,3,4,5].

Delay in germination under the insufficient amount of water increases the chance of infection with pathogens, and the missing spots will appear in the field. Moreover, seed germination may be completely stopped if water deficiency is presented [6,7]

The response of plant during water stress conditions during its different development stages have become an important research topic particularly in regions affected by water stress. Seed germination and seedling development under water deficiency status are considered as an early stage test which may help in identifying and selection of drought tolerant grass crop like wheat and also monitoring their recovery after stress. Many investigators prefer to carry germination test to obtain early results about drought tolerant genotypes [8,9,10,11,12]. Genetic differences among wheat cultivars in germination characters and seedling parameters were reported in various studies [13,14,15,16].

A precise protocol used to detect water stress as a limiting factor for seed germination is to construct the moisture retention curve and to select soil moisture stressful level [17]. Wheat crop is one of the few plants that able to evolve and start germination at an adequate degree in environments of lower than negative 1500 kilopascal of soil water potential [18].

Phenotypic evaluation for drought tolerance, when coupled with specific molecular marker which may act as markers-assisted selection (MAS), can be a good tool and fulfill strategy which facilitates screening germplasm of many crops under water stress condition, at both phenotypic and genotypic level [19].

The objectives of the present study were: (1) Screening the response of some Egyptian wheat cultivars for seed germination under two stressful water levels. (2) Verification for the presence of drought resistant genes in the studied Egyptian cultivars using specific molecular markers for drought.

2. MATERIALS AND METHODS

2.1 Plant Materials

This study was carried out during 2014-2015 at laboratory of agronomy and horticulture, University of Nebraska-Lincoln (UNL) to compare germination of ten wheat cultivars, represent the commercial cultivars grown throughout Egypt and two drought tolerance winter wheat cultivars as check cultivars (Anton and Wesley) under water stress conditions (Table 1). Fresh seeds of ten wheat cultivars were obtained from the Agricultural Research Station (ARS), Egypt. Seed were multiplied in the greenhouse of Nebraska University (during the season 2014-2015) together with two check varieties were obtained from the Seed Lab University of Nebraska Table 1.

2.2 Soil Water Energy Curve and Water Treatment Selection

A sample from Nebraska soil was used to construct the moisture retention curve, and to select soil moisture stressful level. Soil moisture

Table 1. Pedigree of the ten studied bread wheat genotypes

Genotypes	Pedigree
Misr-1	OASIS/SKAUZ//4*BCN/3/2*PASTOR
Misr-2	SKAUZ/BAV 92
Sids-12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S" /6/MAYA/VUL//CMH74A.63014*SX
Sids-13	ALMAZ.19=KAUZ"S"// TSI/ SNB"S"
Gemmisa-9	ALD'S'/HUAC'S'//CMH74.630/5X
Gemmisa-10	Maya 74
Gemmisa-11	BOW"S"/ KVS"S"// 7C/ SERI 82/3/ GIZA 168/ SAKHA 61
Sakha93	Sakha 92/TR 810328 S 8871-1S-2S-1S-0S
Sakha94	OPATA/RAYON//KAUZ. CMBW90Y3280-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.
Giza168	MRL/BUC//Seri. CM93046-8M-0Y-0M-2Y-0B-0GZ
Anton USA	WA-691213-27/N-86-L-177//PLATTE[3486][3814][3889]
Wesley	KS-831936-3/NE-86501[2501]

curve was determined for a silt loam soil (43.8% sand, 41.2% silt, and 15% clay) following standard procedure [20]. The relation between soil moisture tension (kPa) and soil moisture content (g of water per g of soil) is called a moisture retention curve or soil moisture characteristic or energy curve [17]. In order to construct the moisture retention curve of the soil and derive the water treatments for the germination test, the moisture content of the sample was measured by setting the near saturated soil at a succession of known water potentials, namely -10, -33, -50, -100, -500, -1500 KPa, until equilibrium was attained at each (Fig. 1). The gravimetric soil moisture retained after equilibrium was then determined from a subsample following equation 1 below. The soil water contents lower than -1500 kPa were estimated using the soil texture and the Saxton model [21].

Water content by mass (g water/g soil) = (Moist Soil -Dry Soil)/Dry soil [1]

For this experiment, drier soil conditions representing lower soil water potentials below the wilting point of -1500 kPa were selected. Experimental water potentials were -1900 kPa, and -2000 kPa, which corresponded to water treatments of 8 and 7.5 ml, respectively. Based on preliminary tests, water treatments, -1900 kPa and -2000 kPa, were selected because there was more germination at -1900 kPa and less germination at -2000 kPa.

2.3 Germination Test under Water Stress

In order to study the effects of water stress on the germination of some commercial Egyptian wheat cultivars, an experiment was done in 12x2x2 factorial form (cultivars × water treatments x days of germination), using a completely randomized design with three replications. The least significant difference (LSD) tests were carried if the F values of ANOVA analysis are significant. In this study twelve wheat genotypes (Table 1) with two water stress treatments 8 and 7.5 ml were performed, the selected water treatment based on the construct of the moisture retention curve. Each of the water treatment and 70 g of soil was mixed for one to two minutes before being placed into the Petri dishes. The amount of water added was adjusted to account for the water absorbed by each seed (17 mg per seed) [22], (Table 2). One piece of filter paper was set on top of the soil and seeds were placed on top of the filter paper in order to see the development and to avoid interference with germination [23]. Twenty dried seeds were then placed on the filter paper, covered with the lid, and incubated at 21^{o}C. The light was turned on in the incubator for eight hours per day. The Petri dishes were placed lid side down in the incubator in order for the radical to grow towards the lid and easier to see [23]. Seeds were grown and observed for up to 25 days [22]. However, as wheat normally germinates in three to four days, many of the treatments were fully germinated and scored before 14 days. Germination was scored on the 5th day and 25th day after watering. When a 5-millimetre root or shoot sprouted from the seed, it was considered as germinated [22]. The total number of seeds germinated per petri dish by the end of the experiment was used to measure germination. This indicated if the rate of germination was affected by the water and cultivar treatments. Germination percentage out of 20 seeds was calculated for each petri dish experimental unit. Analysis of variance for

Table 2. Soil water tension and corresponding soil water content of the two water treatments used to test germination of five wheat cultivars

Soil water potential (kPa)	Soil water content (g water/g soil)	Water added per 70 g of dry soil (ml)	70 grams of soil + water absorbed by seed (ml)*
-1900	0.115	0.115x70=8.05	8.05+0.34=8.39 ≈ 8
-2000	0.102	0.102 x70=7.14	7.14+0.34=7.48 ≈ 7.5

**Seed water absorption 17 mg/seed*

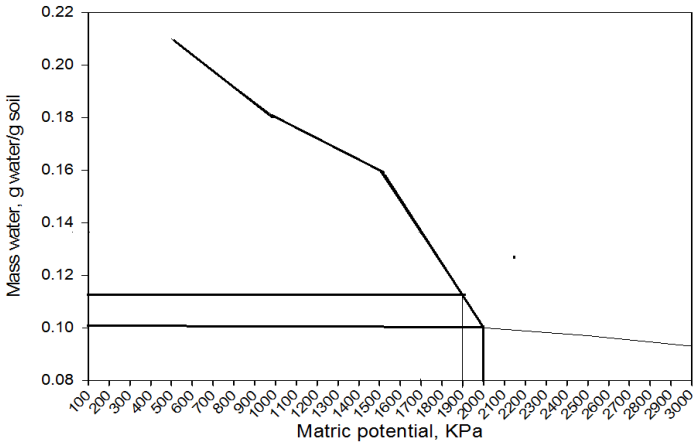

Fig. 1. Displays the soil water release curve, or soil moisture retention curve, of a silt loam soil, which pinpointed the two soil water potentials and mass water content (g of water/g of soil) under low water treatments

germination test was conducted by the PROC GLM procedure of SAS (SAS Inst. 1999). Treatments means were considered significantly different at P<0.05. The germination percentage data were previously subjected to arcsine transformation The differences between the means were compared by the least significant difference (LSD) test (p≤0.05).

2.4 Molecular Characterization of Drought Tolerance

2.4.1 DNA extraction

DNA was extracted from young leaf tissue of wheat cultivars using Plant DNAzol (Promega, Madison, WI). The DNA was then re-suspended in 200 ml of TE buffer, and DNA concentration was quantified by spectrophotometry (TKO100 Fluorometer, Hoefer Scientific Instruments, Holliston, MA).

2.4.2 Allele specific molecular markers analysis

Twenty-two drought specific molecular markers for wheat (Table 3), previously identified to be associated with drought tolerance genes in wheat [24,25,26] were screened for amplification and polymorphism in the 12 wheat cultivars. The PCR reaction mixture (25 µl total) consisted of 50 mM KCl and 10 mM Tris–HCl (pH 8.8), 2 mM MgCl2, 125 mM of dNTP, 50 ng of each primer, 1.0 unit of Taq polymerase (Promega, Madison, WI), and 20 ng of genomic DNA. Amplification was carried out in C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA), using a program that consisted of initial denaturation for 1 min at 94 °C, followed by 32 cycles of 30 s at 94 °C, 50 s at 53 °C, 50 s at 72 °C and final extension for 5 min at 72 °C. The amplified PCR products were gel fractionated on 2.5% Agarose gel. Gel images and marker data were processed using Quantity One Software v. 4.0.1 (Bio-Rad Laboratories, Hercules, CA).

2.4.3 Molecular markers data scoring and analysis

Each marker band was scored as present (1) or absent (0), each of which was treated as an independent character. The similarity between the cultivars was analyzed on the basis of the scores. A dendrogram was constructed based on Jaccard's similarity coefficient [27] using the markers data for all wheat cultivars following the unweighted pair group method (UPGMA) [28].

Table 3. The used specific molecular markers for screening drought tolerance in ten Egyptian wheat cultivars

Primers	Chromosome location	Total No. of alleles	No. of polymorphic alleles	PIC
Wmc603	7A	1	0	0.00
Wmc9	7A	3	3	0.56
Wmc596	7A	1	0	0.00
gwm540	5B	2	2	0.49
psp3200	6D	1	0	0.00
Xgwm484	2D	1	1	0.71
Xgwm601	4A	1	1	0.50
Xgwm111	7B	3	3	0.84
Xgwm157	2D	1	1	0,41
Xgwm186	5A	1	1	0.76
Xgwm257	2B	2	2	0.43
Xgwm260	7A	3	3	0.30
Xgwm296	4A	1	1	0.88
Xwmc323	7B	3	3	0.90
Xgwm356	2A	2	2	0.05
Xwmc420	4A,2B	2	2	0.43
Xgwm11	1B	3	3	0.50
Xgwm44	7D	1	0	0.00
Xwmc48	4B,4D	1	0	0.00
Xwmc73	5B	1	1	0.16
Xwmc89	4A	1	1	0.3
Barc108	7A	1	0	0.00

Table 4. Specific primers for amplification of wheat Dreb1 genes in Egyptian wheat cultivars responsible for drought tolerance

Primers	Sequences (5′ → 3)	Chromosome location	Expected size (bp)	Ann. temp. (°C)	Obtained band size (bp)
P18F	CCCAACCCAAGTGATAATAATCT	3B	717	50	700
P18R	TTGTGCTCCTCATGGGTACTT				
P20F	TCGTCCCTCTTCTCGCTCCAT	3D	1193	63	-
P20R	GCGGTTGCCCCATTAGACATAG				
P21F	CGGAACCACTCCCTCCATCTC	3A	1113	63	200 & 300
P21R	CGGTTGCCCCATTAGACGTAA				
P22F	CTGGCACCTCCATTGCCGCT	3D	596	63	600
P22R	AGTACATGAACTCAACGCACAGGACAAC				

2.4.4 Dreb1 genes amplification

Four primer pairs (Table 4) specific to Dehydration-responsive element binding protein (DREB1) gene [29] were used for amplification. The PCR was performed in a total volume of 25 µl volume containing 20 ng/µl of genomic DNA, 1X PCR buffer, 1.5 mM MgCl2, 0.25 mM each dNTP, 2 µM of each primer and 1 U TaqDNA polymerase. (Promega, Madison, WI). The PCR amplifications were initiated by denaturation at 94°C for 3 min; 35 cycles of 94°C for 1 min, an annealing step at variable annealing temperatures depending on the primer pairs for 1 min, 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR products were electrophoresed on 1.5% (w/v) agarose gels and the products sizes were detected using 100 bp DNA ladder (Promega, Madison, WI).

3. RESULTS AND DISCUSSION

Percentages of germinated seeds were significantly different among the Egyptian wheat cultivars (Table 5). There was a significant effect of water stress treatments on germination ($p<0.01$, 8 ml (66.66a%), 7.5 ml (14.58%), Table 5 and 6). There was a significant difference in germination percentage between the fifth day after watering and the 25th day after watering ($p>0.01$) Table 5). In addition, interaction between cultivars and days were non-significant in germination percentage (Table 5). There was a significant interaction of cultivar by water stress treatment ($p>0.01$), also interaction between days, genotypes and waters stress treatment was highly significant.

Among the Egyptian cultivars Giza168 and Sids12 (42.50%), showed the highest mean of germination responses to the two water treatments followed by Sids 13 (40.83%), Gemmiza 10 and Sakha 94 both gave (40.41%), where Gemmiza-9 had the lowest germination mean (17.91%) (Table 6).

The most responsive Egyptian cultivars on the more stressful condition (7.5 ml) were Sids13 (18.33), followed by Giza168 (16.66), Gemmiza 10 (6.66%) and Sid12 and Sakha94 both had (3.33 (Table 6). In contrast Misr-1, Misr2, Gemmiza 9 and Sakha 93 showed no germination (0.00%) on the more stressful treatment 7.5ml (Table 5). In addition, Sids12 showed faster germination which had the highest germination on the fifth day (32.5%) among the Egyptian cultivars followed by Sakha 94 (27.50%) mean germination on the fifth day. For the 25th day, Giza168 was the highest (58.33%) in germination among the Egyptian cultivars followed by Gemmiza 10 (55.83%) mean germination on the 25th day (Table 6).

Seed germination for any crop is considered as a critical stage especially if water deficiency is presented [29]. Water stress during germination may result in reducing germination percentage or even inhibit germination [30,31]. Abdelghany et al. [31] concluded that Sids13 was the most tolerant cultivars under water stress condition in the northwestern coast in Egypt, while Mekkei et al. [32], determined that Sids 12 cultivar was more tolerant for drought stress compared with other studied wheat cultivars (Gemmiza 9, Sakh93 , Misr 1 and Misr 3). Farhat [33] recommended that Sakha 94 and Sakha 93 are good cultivars to be cultivated in the area characterized by water deficiency. Screening crop genotypes during the germination stage can give a preliminary indication of the tolerance performance of the plant genotypes [34] and early selection for tolerance may base on germination percentage [35].

Table 5. Analysis of variance for germination at five days and germination at 25 days of the 12 wheat cultivars during in vitro drought stress induced by 1900 kPa and 2000 kPa

Source of variation	d.f.[a]	Mean square
Water stress	1	97656.25 **
Cultivars	11	4469.89**
Days	1	27225 **
Water stress * Cultivars	11	1139.58**
Water stress *Days	1	8100 **
Cultivars *Days	11	438.64ns
Water stress*Cultivars *Days	11	901.52**
Error	96	261.11

*[a] d.f. =degrees of freedom. ** Highly Significant (p <0.01).*

Table 6. Mean percent germination for cultivars, water treatments, and days after watering

Water treatment cultivars	8ml (at -1900 kPa)	7.5ml (at -2000 kPa)	Mean	5th day after watering	20th day after watering
Misr-1	56.66 efgh	0.00 k	28.33 def	10 ij	46.66 cdef
Misr-2	58.33 efgh	0.00 k	29.16 def	10 ij	48.33 cdef
Sids-12	81.66 abcd	3.33 K	42.5 c	32.5 fgh	52.5 cde
Sids-13	63.333 defg	18.33 jk	40.83 cd	16.66 hij	65 bc
Gemmiza-9	35.83 ij	0.00 k	17.91 f	1.66 j	34.166 efgh
Gemmiza-10	74.16 bcde	6.66 k	40.41 cde	25 ghi	55.83 cd
Gemmiza-11	45 ghi	1.66 k	23.33 f	9.166 ij	37.5 defg
Sahka93	55 fgh	0.00 k	27.5 ef	20.83 ghi	34.166 efgh
Sahka94	77.5 bcd	3.33 k	40.41 cde	27.5 ghi	53.33 cd
Giza168	68.33 cdef	16.66 k	42.5 c	26.66 ghi	58.33 c
Anton	87.5 ab	83.33 abc	85.41 a	84.16 a	86.66 a
Wesley	96.66 a	41.66 hi	69.16 b	58.33 c	80ab
Mean	66.66a	14.58 b		26.9 b	54.4 a

Data in the same column with different letters indicate a significant difference at P < 0.05

3.1 Analysis for Drought Tolerance with Specific Molecular Markers

Screening with the 22 drought specific molecular markers, showed that 36 alleles in total were detected, with an average of 1.63 alleles per locus. The PIC ranged from 0.00 to 0.90 with a mean PIC of 0.38. Five markers had a PIC higher than 0.70 (Table 3). A dendrogram based on Jaccard's similarity values was constructed using the UPGMA cluster with cophenetic correlation 0.94 (Fig. 3). The generated dendrogram revealed 3 clusters. Cluster 1 contains 3 cultivars (Sids 12, Giza 168 and Wesley), cluster 2 includes 3 cultivars (Gemmiza 10, Gemmiza 11 and Sakha 93) and cluster 3 contains (Misr1 and Misr2). The similarity matrix values ranged from 0.25 between Misr 2 and Sids12 to 0.85 between Sakha 93 and Gemmiza 10. Screening for dreb1 gene specific to dehydration responsive element binding proteins with four primers, two primers were approximately matched the expected band size products P18 and P22 primers (700, 600, respectively) and this agrees with the findings of [36] Wei et al. (2009), P22 was found in all the studied cultivars, while P18 presented in all cultivars except for Sids 13, Gemmiza 9 and Anton (Table 4 and Fig. 2). Primers P21 gave amplified product didn't match with the size of the expected band size reported by Wei et al (2009) [36] (Table 4 and Fig. 2). Moreover, P20 didn't give amplification products. Varietals selection under drought or normal condition is mainly based on morphological traits. One of the major limits of the conventional plant of water deficient traits [37]. Drought tolerance traits are quantitative traits influenced by complex genetic and phenotypic interactions. Which are very difficult to analyze, thus, using MAS (molecular marker assisted selection) can facilitate selection for any trait of interest [38]. Molecular markers are extensively used in wheat breeding programs to discover the location of genes responsible for tolerance of drought. Gene Identification studies revealed that primers located on wheat chromosome 7A, like wmc9, wmc596, wmc603 and barc108 were found previously to be associated with cell membrane stability after exposure to severe water stress condition [39], and these primers can be used for screening progenies with better tolerance under water deficient condition in a wheat genetic improving program [39]. Important drought specific molecular markers in wheat are found to be located on chromosome 5B, 4B and 7B which including the important genes for drought tolerance [40]. In a study to detect molecular markers associated with a QTL for grain yield in wheat under drought condition using drought tolerant wheat genotypes, Xwmc89 marker was found to be located on chromosome 4A which was significantly associated with water stress tolerance [25]. According to numerous studies using SSRs (simple sequence repeats) markers with wheat, SSRs were found to be effective marker techniques to characterize the genetic variability among advanced wheat breeding generations [41].

DREB known as dehydration responsive element binding proteins formed a big group of transcription factors that are prompted during the exposure to abiotic stresses conditions. DREB regulate a great number of genes linked to much abiotic stress like drought, high level of salinity and cold temperature. The DREB transcription factors could be separated into DREB1 and DREB2, which are functioned in two distinct signal transduction pathways [42]. Wei et al. [36]

and Huseynova and Rustamova [29] were reported the location of drought QTLs in chromosome 3A of their wheat cultivars. Screening selected Egyptian wheat cultivars in the present study with specific primers of DREB1 like P18 and P22 give similar results with Wei et al. [36]. Hassan et al. [43] studied the expression of dehydrin genes in wheat using two wheat cultivars Gemmiza 10 and Sids 4 under severe water stress, they found high expression of dreb in leaves of the tolerant genotype Sids 4 under low water condition than Gemmiza 10. This study may add valuable information about the presence of DREB1 in the ten studied Egyptian wheat cultivars and allow further work in pyramiding tolerant genes for drought.

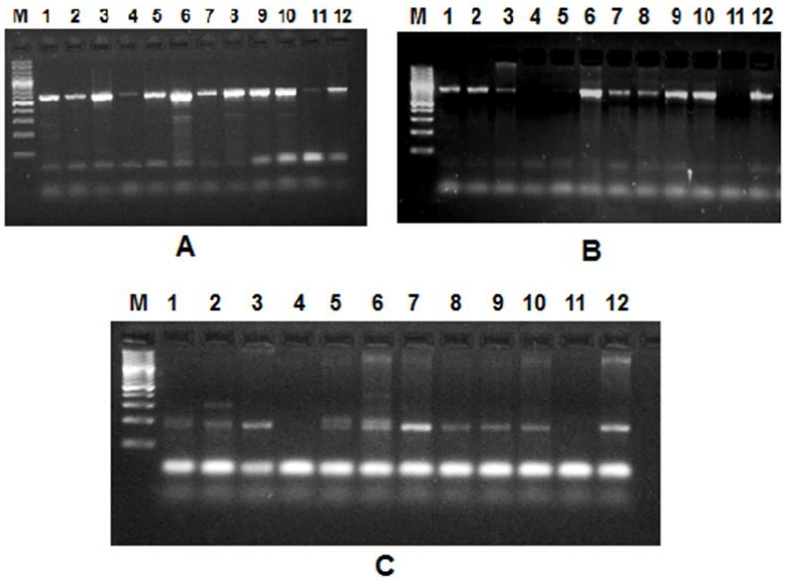

Fig. 2. Amplification products for wheat Dreb1 genes in Egyptian wheat cultivars:. 1= Misr 1, 2= Misr 2, 3= Sids 12, 4= Sids 13, 5= Gemmiza 9, 6= Gemmiza 10, 7= Gemmiza 11, 8= Sakha 93, 9= Sakha 94, 10= Giza 168, 11= Anton , 12= Wesley and M= 100 bp ladder size marker. A, B and C represents primers, P22F, P18 and P21 respectively

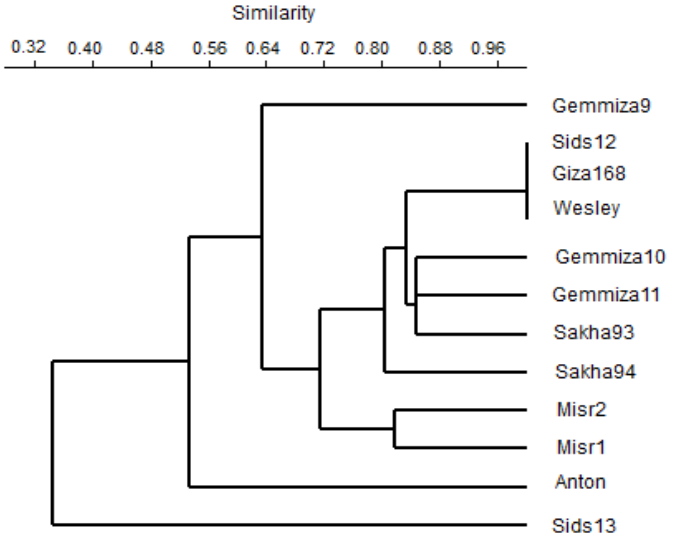

Fig. 3. Dendrogram of 10 Egyptian wheat cultivars and two check cultivars (Anton and Wesley) based on 26 drought specific markers. Values in the *X*-axis correspond to Jaccard's coefficients of similarity

4. CONCLUSION

Three techniques were used in this study, water retention curve to calculate and select the precise amount of the stressful water treatment, germination test and drought specific markers. On the more stressful condition (7.5 ml) Sids13 showed the highest germination among the Egyptian cultivars followed by Giza168, while Giza168 and Sids12, showed the highest mean of germination responses (42.50%) to the two water treatments followed by Sids13 (40.83%). Specific molecular markers used in this study grouped the cultivars according to their genetic similarity. Cultivars with less similarity could be tested in future for hybrid production. Screening for dreb1 gene specific to dehydration responsive element binding protein's added valuable information about their occurance in the studied cultivars for further wheat improvement programs and gene pyramiding for drought tolerance. Tolerant cultivars can be grown under rainfall condition in the north coastal region of Egypt.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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