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Cellular and physiological responses to drought stress in *Aegilops tauschii* genotypes

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Abstract: Drought stress is one of the most important limiting factors in crop yield through impact on the cellular and physiological functions of the plant. Therefore, the study of physiological responses of plants can help to better understanding the drought tolerance mechanisms. In this experiment, 125 wild diploid wheat genotypes of *Aegilops tauschii* were evaluated for the physiological responses under rainfed and supplemental irrigation conditions. The physiological characteristics such as leaf relative water content (RWC), excised leaf water retention (ELWR), relative water loss (RWL), chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, ion leakage, membrane stability index (MSI) and proline content were measured. The results showed that the higher proline content, lower chlorophyll degradation rate and low amount of the membrane stability index (MSI) may inhibit the grain yield reduction under rainfed conditions. It was also found that the lower ion leakage due to the low cell membrane damage may led to the higher yield under rain-fed conditions. The results of regression analysis in both rainfed and supplemental irrigation conditions showed that proline content and total chlorophyll were introduced into the model, and explained the most variation in the grain yield. So, considering the above traits, the genotypes 16, 22, 43, 66 and 106 seems to be more drought tolerant and could be exploited in wheat breeding programs after further assessments.

Key words: Cluster analysis, Drought stress, Principal component, Regression analysis, Wild wheat.

Introduction

Drought stress is one of the main causes of damage to crops worldwide by reducing the average yield by 50% (1-4). Almost all plant species show tolerance to water stress but this capacity varies among species and even in the cultivars of each each species (5-8). Therefore, significant efforts have been made to improve the plants efficiency under water stress. So far, much basic research on the plant physiological responses to drought stress has been conducted, while the difference between plants yield under normal and stress conditions and relative stability of yield is an important problem. Therefore, the production of sustainable drought-tolerant cultivars is an important strategy for ensuring food security for future generations (9-13). As a consequence, the physiological aspects of drought tolerance must be studied and the desired physiological characteristics determined and used in breeding programs (2, 14). Drought tolerance is a quantitative and complex trait with diverse aspects. It is the results of a combination of morphological and physiological characteristics related to chlorophyll content, proline accumulation, and other parameters (2). The plants response to drought stress is accompanied by some of their physiological properties.

One of these responses is proline accumulation. proline is an effective regulator in K^+, ion penetrating channels. In addition, it has a direct effect on K^+/Na^+ intracellular homeostasis (15) and oxygen activity levels decline during osmotic stress (16). Jiang and Huang (2001) (17) stated that the leaf relative water content (RWC) and chlorophyll content decreased with the persistence of dryness and heat which the decrease in these traits depends on the species and duration of stress.

The *Aegilops* is considered to be one of the bread wheat ancestors, which is the origin of the D genome in wheat (18). This plant is particularly important in the conventional (classic) and advanced plant breeding as well as a source of traits and genes of tolerance to biotic and abiotic stresses (19).

The genus *Aegilops* plays an important role in the development of wheat cultivars. Two genomes of three genomes of the wheat are related to this genus, whose D genome is derived from the *Aegilops tauschii* (19). With total homology of D genome of *A. tauschii* and wheat, its specific botanical status, wide ecological adaptation, high diversity in such traits and easy crossing with

wheat, *A. tauschii* has become a very important source for gene transfer and breeding of wheat (18).

Different populations of *A. tauschii* have developed for a wide range of important agricultural traits such as disease and pest resistance, quality endospermic proteins and physiological characteristics (18). Iran is one of the centers of wheat diversity in the Middle East and the Fertile Crescent, so that the existence of natural habitats of the *Aegilops* and *Triticum* species in these regions has led to the creation of the richest wheat gene pool in the region. *Aegilops* species are the closest wild relatives of wheat, originating from the semi-arid regions of western and central Asia and widespread in Iran (20). *Aegilops* grows in both the Mediterranean and Irano-Turanin regions (21). The purpose of this study was to investigate and identify drought-tolerant *Aegilops tauschii* genotypes and use them in future for wheat breeding programs after further investigations.

Materials and Methods

Experimental design and plant materials

The present study was conducted in 2017 at the Faculty of Agriculture, Razi University, Kermanshah, Iran. To conduct this research, 125 *A. tauschii* genotypes were obtained from the Cereal Seed Bank of Ilam University and Department of Genetics and National Plant Gene Bank of Iran (NPGBI). Experimental samples were collected from Europe, Turkey, Caspian littoral states, Iran (the Caspian Sea region and the Zagros region as the main source of these species and East Asian regions) (Table 1). The present study was carried out as an augmented design with three replications and six genotypes as controls under supplementary irrigation and rainfed conditions. In order to seed germination and grow uniformly and simultaneously, in both experiments, irrigation was performed immediately after seed planting . Under the supplementary irrigation conditionirrigation was carried out three times during stem elongation , flowering and seed filling stages. The weeds were manually controlled.

The Cellular**,** physiological and biochemical traits were estimated as the following:

Relative Water Content (RWC)

The relative water content of the leaf was calculated according to Formula 1. Thus, at the time of application of drought stress, 10 leaf samples from each genotype were randomly selected and the following RWC was calculated for each genotype.

RWC = [(FW-DW) / (TW-DW)] × 100

Where FW, DW, and TW are the weight of the fresh leaves, dried leaves in the oven and the saturated leaf weight, respectively (22).

Excised Leaf Water Retention (ELWR)

The Excised Leaf Water Retention (ELWR) of genotypes during the stress period was calculated using formula 2. For the calculation of this trait, each genotype was randomly sampled and ELWR was calculated for each genotype using the following formula.

(ELWR) = [1- (FW-ADW) / FW] × 100

Where FW and ADW are the weight of the fresh leaves and the withered leaves, respectively (23).

Relative Water Loss (RWL)

To calculate this trait, the leaf sample was separated from each genotype in three replicates and immediately weighed in a laboratory precision balance (FW). Then the leaves stored for 5 hours under laboratory conditions and weighed (WT) (23).

Relative Water Lose (RWL) was calculated using the following formula:

RWL=[(FW-WT)/FW] ×100.

Membrane Stability Index (MSI)

In order to estimate the membrane stability index, 3-5 young and developed leaves were selected and the following formula was used (24).

MSI= [1-(C1/C2] ×100

C1 and C2 indicate electrical conductivity at 40 °C and 100 °C, respectively.

Chlorophyll content

In order to measure the chlorophyll, 0.5 g of the fresh leaf stamen samples were being homogenized in 5 ml of acetone. After addition of 3 ml of ether, the absorption level of the samples obtained was read using spectrophotometer at 663 and 645 nm wavelengths and their chlorophyll concentration was measured on the basis of following equations (25).

Chl a (mg/g fresh weight) = 12.7 (absorption at 663 nm) -2.69 (absorption at 645 nm) × extracted sample size/500

Chl b (mg/g fresh weight) = 22.9 (absorption at 645 nm) -4.69 (absorption at 663 nm) × extracted sample size/500

Chl a + b (mg/g fresh weight) = 20.2 (absorption at 645 nm) +8.02 (absorption at 663 nm) × extracted sample size/500

Carotenoids

In order to measure the carotenoids, 0.5 g of the fresh flag leaf samples were being homogenized in 5 ml of acetone. After adding 3 ml of ether, the absorption level of the samples obtained was read using spectrophotometer at 470 nm wavelengths and Carotenoid concentration was being measured based on the following formula (25).

Carotenoids (mg/gr fresh weight) = [(85.02) (Chl b content) -1.8 (Chl a content)]– [1000 (absorbance at 470 nm)].

Proline content

Proline content was measured based on Bates et al (1973) (26) method. About 0.1 g of powdered dried leaves with 10 ml of 3% sulfuric acid was mixed, and then 2 ml of the obtained extract with 2 ml of nine hydrine and 2 ml of acetic acid for 1 hour was placed in the hot water bath at a temperature of 100 ° C. Then 4 ml toluene was add and mix well for 20 seconds. In the test tube, two phases were formed: the pink organic phase is above and the colourless, clear blue phase, on the bottom. The organic phase was used for colourimetry in a spectrophotometer and a wavelength of 520 nm. For drawing the standard curve, pure proline was used at concentrations of 0, 50, 100 and 250 µm and all steps were done on the ice. Then the standard proline curve was plotted and the amount of dissolved proline was

obtained by this graph.

Statistical analysis

Analysis of variance, Principle component analysis, correlation and regression analysis were performed using SPSS 16.0 and SAS 9.1 software.

Results

Analysis of variance

Analysis of the variance of traits showed (Table 2.) the genotypes were significantly different ($P<0.01$) for grain yield, RWC, ELWR, RWL, MSI, chlorophyll a, b and total (Chl a, Chl b and Chl t respectively), Carotenoids (CAR) and proline (Pro) content. It indicates the genetic diversity of genotypes in terms of these traits. Since diversity is the basis for choosing the best genotypes, the understudied genotypes can provide an appropriate diversity to select the best.

Principal component analysis

Principal component analysis for physiological traits was performed on 125 genotypes of *Aegilops tauschii* in rainfed and irrigation conditions (Table 3). Based on the results, the first four components accounted for the highest diversity and had an eigenvalue more than one.

Table 1. List of genotypes used in this study.

No	Genebank Code	Origine	No	Genebank Code	Origine
1	IUGB-00020	Iran/Ardabil-Sarein Road	43	IUGB-00305	Iran/unknown
2	IUGB-00039	Iran/unknown	44	IUGB-00306	Tajikistan
3	IUGB-00051	Azerbaijan	45	IUGB-00307	Iran/unknown
4	IUGB-00080	Iran/Salman Shahr	46	IUGB-00308	Azerbaijan
5	IUGB-00107	Iran/Gilan- Kooch Esfahan Road	47	IUGB-00309	Iran/unknown
6	IUGB-00108	Iran/ unknown	48	IUGB-00310	Iran/unknown
7	IUGB-00141	Iran/ unknown	49	IUGB-00311	Sweden
8	IUGB-00143	Iran/ unknown	50	IUGB-00312	Iran/unknown
9	IUGB-00144	Tajikistan	51	IUGB-00313	Iran/unknown
10	IUGB-00151	Iran/ Ardabil-Sarein Road	52	IUGB-00314	Azerbaijan
11	IUGB-00157	Iran/ Sadraldin Village	53	IUGB-00315	Iran/unknown
12	IUGB-00164	Iran/ Astara-Ardabil Road	54	IUGB-00325	Iran/Karaj-Chaloos Road
13	IUGB-00193	Iran/Ahar-Kelibar	55	IUGB-00362	Armenia
14	IUGB-00196	Iran/Ramsar	56	IUGB-00365	Iran/Mazandaran-Amol
15	IUGB-00198	Iran/Voroodi –Zanjan Road	57	IUGB-00366	Iran/Ghaemabad-Lahijan Village
16	IUGB-00223	Iran/ Salmanshahr-Ramsar Road	58	IUGB-00367	Iran/30km Ahar-Kelaibar
17	IUGB-00224	Iran/Gilan-Koochsfehan Road	59	IUGB-00369	Iran/Gilan-Kalachay
18	IUGB-00238	Iran/Karaj-Chaloos,	60	IUGB-00370	Iran/unknown
19	IUGB-00245	Iran/Karaj-Chaloos	61	IUGB-00371	Iran/unknown
20	IUGB-00247	Iran/Mazandaran-Amol	62	IUGB-00374	Iran/5km ta Astaneh Ashrafyeh
21	IUGB-00249	Iran/Salmanshahr-Mazandaran Road	63	IUGB-00375	Iran/Dashte Moghan
22	IUGB-00260	Iran/Gilan-3km Astara	64	IUGB-00383	Iran/Abbasabad –Ramsar
23	IUGB-00261	Iran/Karaj- Chaloos Road	65	IUGB-00386	Iran/30km Ahar-Kelaibar
24	IUGB-00263	Iran/Mazandaran-Amol	66	IUGB-00396	Iran/Mazandaran-Noshahr
25	IUGB-00269	Iran/Rasht	67	IUGB-00400	Iran/20km Chalooos Road
26	IUGB-00273	Iran/km5 Sarein-Ardabil Road	68	IUGB-00401	Iran/shahrestan Noor
27	IUGB-00274	Iran/Chaloos	69	IUGB-00402	Iran/shahrestan Noor
28	IUGB-00276	Iran/ Galoogah Bandar Behshahr	70	IUGB-00404	Iran/Rasht- Talesh,
29	IUGB-00279	Iran/10km Ahar-Tabriz Road	71	IUGB-00405	Iran/Karaj-Chaloos
30	IUGB-00289	Afghanistan	72	IUGB-00429	Iran/unknown
31	IUGB-00290	Turkmenistan	73	IUGB-01746	Iran/unknown
32	IUGB-00291	Azerbaijan	74	KC-50006	Iran/unknown
33	IUGB-00292	Turkey	75	KC-50037	Iran/unknown
34	IUGB-00293	Japan	76	KC-50084	Iran/Azarbayjan sharghi
35	IUGB-00295	Turkey	77	KC-50133	Iran/Khorasan
36	IUGB-00296	Armenia	78	KC-50136	Iran/Khorasan
37	IUGB-00297	Iran/unknown	79	TN-01-0312	Iran/Azarbayjan gharbi
38	IUGB-00298	Iran/unknown	80	TN-01-0369	Iran/Azarbayjan sharghi
39	IUGB-00299	Afghanistan	81	TN-01-0562	Iran/Semnan
40	IUGB-00300	Iran/unknown	82	TN-01-0563	Iran/Gilan
41	IUGB-00302	Afghanistan	83	TN-01-0569	Iran/Gilan
42	IUGB-00303	Turkey	84	TN-01-0667	Iran/Azarbayjan sharghi

Table 1. (continue)-List of genotypes used in this study.

No	Genebank Code	Origine	No	Genebank Code	Origine
85	TN-01-0699	Iran/Golestan	106	IUGB-02061	Iran/Rezvanshahr
86	TN-01-0804	Iran/unknown	107	IUGB-02062	Iran/Rezvanshahr
87	TN-01-0836	Iran/Mazandaran	108	IUGB-02063	Iran/Rezvanshahr
88	TN-01-0945	Iran/unknown	109	IUGB-02064	Iran/Somesara
89	TN-01-1005	Iran/unknown	110	IUGB-02065	Iran/Astara
90	TN-01-1559	Iran/unknown	111	IUGB-02066	Iran/Somesara
91	TN-01-1695	Iran/unknown	112	IUGB-02067	Iran/Somesara
92	TN-01-1745	Iran/Semnan	113	IUGB-02068	Iran/Somesara
93	TN-01-1770	Iran/unknown	114	IUGB-02069	Iran/rasht-Feiz mahaleh
94	TN-01-1970	Iran/unknown	115	IUGB-02070	Iran/rasht-Feiz mahaleh
95	TN-01-1772	Iran/unknown	116	IUGB-02071	Iran/rasht-Feiz mahaleh
96	TN-01-2115	Iran/unknown	117	IUGB-02072	Iran/Ponel Road
97	TN-01-2120	Iran/unknown	118	IUGB-02073	Iran/Foman
98	TN-01-2207	Iran/unknown	119	IUGB-02074	Iran/Karaj
99	IUGB-02054	Iran/Rasht	120	IUGB-00205	Iran/Ardabil-Hayran Village
100	IUGB-02055	Iran/Rasht	121	IUGB-00205	Iran/Golestan- Aliabad_Ketol
101	IUGB-02056	Iran/Rasht	122	IUGB-00205	Iran/Ardabil-Sarein Road
102	IUGB-02057	Iran/unknown	123	IUGB-00205	Iran/3km to Astara
103	IUGB-02058	Iran/unknown	124	IUGB-00205	Iran/ebtedaye Harsin-Noorabad Road
104	IUGB-02059	Iran/unknown	125	IUGB-00205	Iran/unknown
105	IUGB-02060	Iran/Baraghan			

The eigenvalues of the first four components were 3.27, 1.87, 1.52 under stress conditions, and 1.29, and in total, these four components explained 66.39% of the variability among the traits to be evaluated. In the first component, which explained the most significant variation, chlorophyll a, chlorophyll b and total chlorophyll traits had the highest share in justifying a variation. In the second component, the highest contribution was to justify the diversity of grain yield, MSI and proline content. In the third component of RWC and carotenoids, more than other traits were involved in explaining the changes. In the fourth component, ELWR and RWL expressed the highest values of data variation.

Regression analysis

To determine the contribution of the cumulative effects of traits, a stepwise regression was used to justify the variation in grain yield. In stepwise regression analysis, grain yield was considered as a dependent variable against other traits. Under non-stress conditions (Table 4), the first trait introduced into the model was the proline content, with its coefficient equal to R^2 = 0.31, and showed that proline content justified 31% of the variation in grain yield. Then, the total chlorophyll traits and the membrane stability index were introduced into the model, which in total, these three attributes justified 54% of the variation in yield. Other traits had no significant effect on the regression model. The resulting regression equation is: $Y=-3.95+0.64X_1+0.21X_2+1.81X_3$

In the above relationship, Y is grain yield and X_1 to X_3 traits are membrane stability index, total chlorophyll and proline, respectively. The intercept of regression was estimated -3.95. Under rainfed conditions (Table 5), the first trait introduced into the model was proline, whose coefficient of R^2 was equal to 0.41, and showed that proline content justifies 41% of variation of yield. Subsequently, total chlorophyll and RWC traits were introduced into the model, which accounted for 57% of the variation in yield. Other traits had no significant effect on the regression model. The resulting regression equation is:

$Y=25.33+1.2X_1+0.31X2+017X_3$

In the above relationship, Y is grain yield and X_1 to X_3 traits are proline, RWC and total chlorophyll, respectively, and the intercept of the regression was calculated 25.33. The descriptive statistics of traits in studied *Aegilops* population are shown in Table 6.

Cluster analysis

In order to classify genotypes based on physiological traits and yield, cluster analysis using Ward method was used. As a result, the genotypes were divided into five different groups (Table 7). The first to fifth groups consisted of 7, 2, 59, 9 and 48 genotypes respectively. To show the value of each cluster in terms of the number of traits measured, the percentage of deviations from the average clusters was calculated from the total average (Table 7). These deviations may indicate to a certain extent the diversity of genotypes. So, it is better to use the genotypes that have been assigned to far clusters for hybridization.

In current study, the results of this analysis showed that under stress conditions, genotypes in the fourth group had the highest grain yield and performance-related attributes including proline, carotenoids RWC and ELWR. In this group, the lowest mean values of MSI, and RWL indices were observed.

Discussion

High RWC content of genotypes is due to two causes. First such genotypes can preserve their relative water content at a high level by closing their stomata and lower transpiration under drought stress conditions.

Table 2- Analysis of variance physiological traits under control and drought stress conditions

Source of variation	*df*	Mean Square											
		RWC	ELWR	RWL	C1	C2	MSI	Chl a	Chl b	Chl t	CAR	Pro	Yield
Condition (C)	1	718.2**	299.38^{ns}	76608.44**	3542.02**	8109.56**	692.87**	44866.75**	56.13**	41531.63**	1475604.518**	2903.34**	183.85*
Block (stress)	4	168.31	345.9	40.55	38.98	59.91	7.78	1.57	25.02	131.88	241197.3	2.311	12.72
Genotype (G)	124	375.62**	1012.04**	1874.6**	144.56**	198.71**	112.31**	1156.87**	202.84**	1170.42**	1857303.5**	41.68**	11.35**
C*G	124	38.14^{ns}	163.46^{ns}	1735.25^{ns}	26.22**	29.16**	36.71**	129.88**	101.46**	216.31**	828861.9**	16.40**	5.84^{ns}
Error	20	157.23	391.45	20272.04	1.14	0.58	3.32	0.232	0.559	1.66	4878.7	0.279	4.82
CV%		16.12	14.23	17.68	12.11	13.03	14.1	18.6	16.74	18.43	12.35	19.7	15.93

Where RWC: Relative Water Content, ELWR: Excised Leaf Water Retention, RWL: Relative Water Lose, C1: electrical conductivity at 40 °C , C2: electrical conductivity at 100 °C , MSI: Membrane Stability Index, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Chl t: total Chlorophyll, CAR: Carotenoids, Pro: Proline content
*: Significant at 5%, **: Significant at 1%, ns: non-significant

Table 3. The first four principal components for the 125 genotypes of *A.tauschii.*

Trait	Estimated factor loading for the 125 genotype							
	irrigation condition				Rain-fed condition			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
RWC	0.205	-0.169		).211	-0.203	-0.143	0.613	-0.318
ELWR	0.111	-0.316	-0.267	0.309	0.070	-0.280	-0.171	0.550
RWL	0.132	-0.395	0.261	0.339	-0.072	-0.541	-0.030	0.461
C1	0.327	0.059	0.508	0.176	0.345	-0.368	-0.171	-0.360
C2	0.382	0.076	0.222	0.100	0.246	-0.469	-0.154	-0.140
MSI	0.193	0.366	0.045	-0.186	-0.220	0.351	0.059	-0.106
Chla	0.433	-0.183	-0.331	0.280	0.361	0.100	-0.448	0.122
Chlb	0.401	-0.327	0.222	-0.358	0.376	0.081	0.372	-0.109
Chlt	0.304	-0.347	-0.270	0.467	0.445	0.089	-0.171	0.013
CAR	-0.405	-0.324	0.479	0.351	0.335	-0.027	0.488	-0.097
Pro	0.022	0.461	-0.199	-0.260	0.328	0.490	0.075	0.154
Yield	0.179	0.353	-0.188	-0.083	0.175	0.575	0.104	0.042
Eigenvalue	3.009	2.046	1.911	1.281	3.43	1.921	1.645	1.361
Percent of variance	25.072	17.050	15.928	10.675	27.683	15.862	13.127	11.863
Cumulative Percentage	25.072	42.123	58.050	68.725	27.683	43.545	56.672	68.535

Where RWC: Relative Water Content, ELWR: Excised Leaf Water Retention, RWL: Relative Water Lose, C1: electrical conductivity at 40 °C , C2: electrical conductivity at 100 °C , MSI: Membrane Stability Index, Chla:Chlorophyll a, Chlb: Chlorophyll b, Chlt: total Chlorophyll, CAR: Carotenoids, Pro: Proline(µg/g).

Table 4. Step wise regression analysis of plant, yield (dependent variable) and other traits (independent variables) in normal condition.

Trait	Coefficient regression	S.E	R^2	Pr
Pro	1.81	0.41	0.31	<0.0001
Chlt	0.21	0.08	0.43	<0.0001
MSI	0.64	0.29	0.54	0.0102

MSI: Membrane Stability Index, Chlt: total Chlorophyll, Pro: Proline (µg/g).

Table 5. Step-wise regression analysis of plant, yield (dependent variable) and other traits (independent variables) in stress condition.

Trait	Coefficient regression	S.E	R^2	Pr
Pro	1.2	0.308	0.41	0.0022
RWC	0.31	0.119	0.45	0.0243
Chlt	0.17	0.068	0.49	0.0208

RWC: Relative Water Content, Chlt: total Chlorophyll, Pro: Proline(µg/g).

Table 6. Descriptive statistics of traits in studied *Aegilops* population.

Trait	Minimum		Maximum		Range		Average		Standard Deviation		Coefficient of Variation	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
RWC (%)	1.97	0.154	93.6	92.85	93.44	90.87	27.45	30.65	16.07	13.82	58.53	45.08
ELWR (%)	1.85	1.43	165.67	160	164.24	158.14	62.99	57.13	29.99	25.67	47.61	44.93
RWL (%)	14.08	6.71	995.32	57.91	988.61	43.82	62.01	18.98	22.12	5.58	35.67	29.39
C1	24.2	1.1	143.73	156.66	119.53	155.56	51.85	56.58	14.56	9.94	28.08	17.56
C2	30.2	45.1	166.33	189.96	136.13	144.86	61.07	69.21	17.67	10.74	28.93	15.51
MSI (%)	5.08	5.88	42.61	98.24	37.53	92.36	15.69	17.95	7.81	10.4	49.79	57.93
Chla (mg/g Fw)	0.126	6.45	220.97	239.68	220.84	233.23	23.43	49.03	31.23	23.13	33.3	47.17
Chlb (mg/g Fw)	15.42	1.18	117.09	154.88	101.67	153.7	42.78	39.04	15.32	13.65	35.82	34.96
Chl (mg/g Fw)	17.07	12.85	311.83	319.18	294.75	306.33	66.21	87.93	35.16	27.12	53.1	30.84
CAR (mg/g Fw)	1634.48	312.82	11573.84	14802.96	9939.36	14490.13	4259.69	3825.44	1521.9	1251.83	35.72	37.72
Pro (µmol/g Fw)	0.508	0.64	42.22	59.33	41.71	58.69	5.6	12.32	4.63	6.39	82.71	51.86
Yield (g/p)	1.43	0.71	198.41	168.69	167.26	197.7	31.89	16.83	24.03	11.07	75.34	65.77

RWC: Relative Water Content, ELWR: Excised Leaf Water Retention, RWL: Relative Water Lose, C1: electrical conductivity at 40 °C, C2:electrical conductivity at 100 °C, MSI: Membrane Stability Index, Chla: Chlorophyll a, Chlb: Chlorophyll b, Chlt: total Chlorophyll, CAR: Carotenoids, Pro: Proline(µg/g).

Table 7- Grouping of 125 *Aegilops tauschii* genotypes base on grain yield and physiologic traits using the mean of distance in stress condition

Group	Genotypes		RWC (%)	ELWR (%)	RWL (%)	MSI (%)	Chl a (mg/g Fw)	Chl b (mg/g Fw)	Chl t (mg/gFw)	CAR (mg/g Fw)	Pro (µmol/g Fw)	Grain yield(g/p)
1	9, 13, 25, 27, 53, 80, 99	Mean	26.87	54.82	21.3	28.04	26.98	21.12	48.1	1748.35	5.17	6.8
		Deviation from total mean	-12.33	-4.04	12.22	56.21	-45.27	-45.9	-45.3	-54.3	-58.04	-59.6
2	42,63	Mean	32.4	66.49	17.72	22.24	28.71	3.79	32.5	520.12	8.34	14.9
		Deviation from total mean	5.71	16.38	-6.64	23.9	-41.76	-90.29	-63.04	-86.4	-32.31	-11.47
3	1,2,3,6,7,8,12,14,17,18,19,23,26 28,31,32,35,37,38,44,45,46,47,48 50,51,52,54,55,56,60,61,62,64,67 70,71,72,73,76,82,84,87,88,89,94 96,97,104,105,107,108,110,112 113,119,121,124,125	Mean	28.52	58.09	19	17.7	58.02	32.1	93.26	3163.46	12.67	15.43
		Deviation from total mean	-6.95	1.68	0.11	-1.39	17.69	-17.78	6.06	-17.3	2.84	-8.32
4	16,22,40,43,85,106,111,117,120	Mean	36.11	72.6	17.06	11.61	75.27	63.12	125.12	5944.86	21.34	31.51
		Deviation from total mean	17.81	27.08	-10.12	-35.32	52.68	61.68	42.3	55.4	73.21	87.23
5	4,5,10,11,15,20,21,24,29,30 33,34,36,39,40,49,57,58,59, 65,66,68,69,74,75,77,78,79 81,83,86,90,91,92,93,95,98 100,101102,103,109,114 115,116,118122,123,	Mean	32.71	53.01	19.05	17.8	37.83	47.15	82.54	4682.37	11.41	17.35
		Deviation from total mean	6.72	-7.21	0.37	-0.84	-23.27	20.77	-6.13	22.4	-7.39	3.09
Total Mean			30.65	57.13	18.98	17.95	49.3	39.04	87.93	3825.44	12.32	16.83

Second they can absorb water from the depths of the soil and transfer it to the shoots and maintain its relative water content at a high level due to a stronger root system. Therefore, genotypes with high water content under stress conditions cannot have a high tolerance to drought and other traits such as root depth, photosynthesis, chlorophyll content and membrane, and proline stability index should also be investigated. As it is known, genotypes that have higher levels of water content under stress conditions and can do higher photosynthesis under these conditions have higher yield and drought tolerance (27). The relative amount of leaf water naturally decreases during the late stages of the growing season. The potential effects of increasing wastewater can be as follows. When the RWC of leaves is between 70 and 100%, photosynthesis is reduced due to stomatal closure.

This mode is quickly reversible. When the relative water content of the leaves is between 35 and 70%, the photosynthetic capacity decreases at a high light intensity and only improves slowly with distilled water. The main reason for this decrease can be an optical obstruction. If the relative water content of leaves reaches less than 30%, due to damage to the cell membrane and chloroplast, an irreversible decrease in photosynthetic capacity is created and eventually the plant is lost. According to the results of this experiment, it seems that reduction in the yield in without irrigation treatment is related to the reduction of photosynthesis due to the optical suppression and electron transfer chain perturbation (28). The relative water content (RWC) of leaf has actually been introduced as an indicator of drought stress damages. Higher relative water content further increases the number of light products and yield under stress conditions (29). The results of this research showed that RWC effect on justifying the diversity between genotypes under stress conditions. The positive load of this trait and yield indicates a positive correlation between these two attributes. The genotypes number 16, 22, 43 and 106 had the lowest RWC in stress conditions and genotypes 13, 27 and 99 had the highest RWC. The results of the principal component analysis indicated that in the second component there was a negative correlation between the relative loss of leaf water (RWL) and performance, which was consistent with the results of Chandra and Islam in 2003 (30). The lower water loss from leaves show a higher ability to maintain water under stress condition. Chandra and Islam concluded that additive genes effects on water loss from incised leaves, and there is generally a negative correlation between the loss of water from incised leaves and yields. Therefore, selection of genotypes with the lowest RWL in drought conditions will be appropriate. In this study, genotypes number 16, 22, 43 had significantly the lowest RWL. Also, genotypes number 9, 13, 27, 80 and 99 had the highest RWL. The genotypes number 1, 16, 22, 43 and 106 had the highest ELWR. The genotypes 13, 27, 53 and 99 had the least of these traits. According to the results, drought stress can increase ELWR in resistant genotypes, which can be attributed to the mechanisms of water storage under drought conditions with leaf twist or leaf area reduction (31). According to Lonbani and Arzani (31) results, among the parameters related to water in the plant, ELWR can be the best traits for

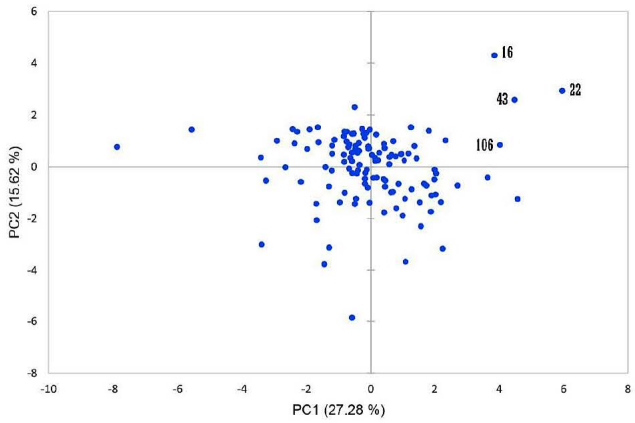

Figure 1. Distribution plot of the 125 genotypes of *Aegilops tauschii* under rain-fed condition according to the first two principal components

indirect selection of plant yield. This trait is heritable and can easily be estimated in large populations. The results of regression analysis showed that proline plays a significant role in plant performance in normal and stress conditions. Proline and RWC play a major role in water deficient resistance. Amini et al (32) reported that biological yields and harvest index are critical in drought stress condition in wheat. Di Fonzo et al. (33) concluded that spike length had the greatest effect on grain yield in both stress and non-stress conditions. They also argued that this effect may be the result of changes in the division of dry matter on the harvestable yield (grain yield). It seems that in normal and stress conditions the traits remaining in the regression can be used to improve the seed yield of the plant and make selections to achieve this goal (29). The results showed that highest levels of chlorophyll a, b and total under stress conditions found in genotypes 16, 22, 43 and 106 and the lowest level found in genotypes number 13, 27, and 99, respectively. The seed yield of genotypes 22, 16,43 and 106 were higher than the genotypes 13, 27, and 99. Then the reduction of the seed yield in some genotypes versus other genotypes could be related to low proline, higher percentage of cell membrane damage, relative water loss (RWL) or less RWC in stress conditions. Low chlorophyll concentration means reducing production potential and decreasing storage, which is important for decreasing the effects of drought stress on grain filling in wheat (34). Drought stress reduces all photosynthetic pigments, especially chlorophyll (28). Yang et al (35, 36) reported that flag leaf chlorophyll content in the filling stage decreased in the control treatments gradually, but a deficiency of water resulted in a decrease in chlorophyll content. Also, with increasing water stress, the amount of chlorophyll more rapidly decreased, which indicates that water stress increases the ageing rate of leaves. Sayar et al (37) reported a decrease in chlorophyll content due to drought stress in wheat, which is consistent with the current results. Also, Lonbani and Arzani (31) reported a decrease in chlorophyll content in some wheat and triticale genotypes due to drought stress.

The results showed that the level of carotenoids reduced due to stress. However, in drought tolerant genotypes, high yield under stress conditions, carotenoids level increased. In current research genotypes, 16, 22

and 43 had the highest levels of carotenoids in stress conditions and genotypes 13, 27 and 99 had the highest reduction in carotenoids. Carotenoid plays a fundamental role in response to water deficiency conditions and may help plants to tolerate drought stress. The increased carotenoid content is associated with the absorption of excessive light to avoid photo-oxidative damage to PSII (38) In this study, genotypes with higher seed yield under stress conditions had a higher proline content and also showed that proline content in stress conditions was higher than normal conditions. Mallick et al (39) observed increases in proline content in some wheat genotypes due to drought stress, which is consistent with the results of the present study. Genotypes No. 16, 22, 43 and 106 had the highest amount of proline in stress condition. There are four reasons for increasing the proline accumulation in the reaction of the plant to stress: 1. Stimulating proline synthesis from glutamic acid, 2. Reducing its transmission through the phloem, 3. Preventing its oxidation during stress, 4. Disturbing and damaging to the protein synthesis process (26, 40). Genotypes evaluation based on cell membrane stability (in percent damage to the membrane) showed that genotypes 16, 22, 28, 43, 55, 66 and 106 in stress condition had the least damage, so this genotypes had lower electrical conductivity and ion leakage under stress conditions than normal conditions and also had a relative appropriate seed yield. In this regard, in stress condition, the most damage to the cell membrane was related to 27, 62 and 99, and these genotypes were among the low-yielding genotypes, with increasing the cell wall resistance, the damage to the plant is reduced and the plant can be more productive. Koochau and Georgiyev (41) observed lower damages in cell membranes of drought tolerant cultivars in evaluating drought tolerance in barley cultivars. Under drought stress, cell membranes lose their stability and if the leaves fall in an aqueous medium, their soluble materials leak out, thus, the stability of the membrane is evaluated by the evaluation of ion leakage (42). It seems that the stability of cell membranes in tensions is related to the synthesis of heat shock proteins and the characteristics of the photosynthesis system, including key enzymes and thylakoid membranes (43). Maintaining cell membrane stability during stress has a critical role in increasing plant tolerance (44). It has been reported that cell membrane stability, even in the early stages of stress, is a good indicator of plant tolerance to stress. In addition, some studies have shown that cell membranes and organelles are the first places of damage to cells under stress conditions by reactive oxygen species (45). Based on Biplot resulting from of principle components analysis (PCA), the best genotypes were identified under drought stress. The PCA for 125 genotypes showed that the four top components justified 66.39% of the total variation in the measured physiological parameters. PC1 was strongly influenced by Chl a, Chl b and Chl t and was termed photosynthetic capacity, and PC2 was mainly explained by grain yield, MSI and proline was identified as the performance component. The third component was described with carotenoids and RWC. Finally, the fourth component was described with RWL and ELWR. According to this analysis, superior tolerant genotypes should be selected based on high PC1 and PC2 values. Therefore, genotypes No. 16, 22, 43 and 106 (Fig. 1) were recognized as superior drought-tolerant genotypes. Thus, these genotypes and the can be used in wheat breeding programs to improve and develop new high yielding varieties with drought resistance. Based on cluster analysis (Table6), Genotypes were classified into 5 groups, also resistant and susceptible genotypes were found in almost identical groups. Based on the stress condition genotypes number 13, 27 and 99, which had the lowest values for the above indicators, were in the same group (group 1). Genotypes No. 16, 22, 43 and 106 were assigned in group 4. In fact, cluster analysis showed that genotypes in group 4 had the highest seed yield and high values of performance related attributes such as chlorophyll, proline, carotenoid and ELWR in terms of stress, as well as the average of RWC traits, MSI index and RWL in this group, are lower than other groups. Therefore, these genotypes are introduced as candida genotypes for drought tolerance, which have the potential for further investigation for the identification and isolation of genes involved in the drought tolerance process for gene transfer or use for classical breeding

The results of this study showed that biochemical and physiological parameters, especially chlorophyll, RWC, carotenoids, membrane stability index and proline may be effective on grain yield and, considering the above traits, the genotypes 16, 22, 43, 66 and 106 seems to be more drought tolerant and after advanced assessments, could be exploited in wheat breeding programs.

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