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Physiological Studies and Tolerance Indices of Six Bread Wheat Genotypes under Siwa Oasis and Ashmon Habitats

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ONE OF THE possible ways to ensure future food needs of an increasing world population involves development of crop genotypes more tolerant to stress. In order to study the response of six genotypes of bread wheat to different habitat conditions, an experiment was conducted in a randomized complete block design with three replications under Siwa Oasis and Ashmon habitat conditions during, 2012-2013 cropping season. Pigments content, total carbohydrates, protein and proline were varied in different genotypes between the two habitats. Eleven drought tolerance indices including stress tolerance index (STI), stress susceptibility index (SSI), tolerance index (TOL), harmonic mean (HM), geometric mean productivity (GMP), mean productivity (MP), modified stress tolerance index for Ashmon (adequate) environment (K_1STI), modified stress tolerance index for Siwa Oasis (stressed) environment (K_2STI), yield index (YI), yield stability index (YSI), and stress susceptibility percentage index (SSPI) were calculated based on biological yield under the two habitats condition. Biological yield was positively correlated with STI, GMP, MP, HM, YI, K_1STI and K_2STI and negatively correlated with SSI and YSI. Results of this study showed that the indices K_1STI, K_2STI, SSPI, SSI and YSI can be used as the most suitable indicators for screening stress tolerant cultivars. Cluster analysis classified the genotypes into tolerant, susceptible and semi-tolerant to Siwa Oasis conditions. Therefore Misr2 is the tolerant genotype, which may be recommended to cultivate it under Siwa Oasis habitats.

Keywords: Wheat, Stress, Adequate, Metabolism, Indices.

Wheat is the most important cereal crop, its stable diet for more than one third of the world population and contributes more calories and protein to the world diet than any other cereal crop (Abd-El-Haleem *et al*., 2009). Drought and salinity is the most severe stress and the main cause of losses in growth and productivity of crop plants (Passioura, 2007). Plant response to stress includes morphological, biochemical and later loss of plant parts (Sangtarash, 2010).

Physiological traits are ideal selection criteria for stress adaptation in wheat breeding programs, because of their relative contribution to grain yield (Araus *et al*., 2002 and Reynolds *et al.*, 2005). Genotypes with physiological traits conferring higher grain yield potential usually perform better under stress conditions (Olivares-Villegas *et al.*, 2007). Breeders also need to release cultivars which are adapted to different conditions, so identifying physiological traits that may confer simultaneously high grain yield potential and tolerance to stresses would be essential. These traits must allow the plants to capture more resources or to use them more efficiently (Slafer and Araus, 2007). A number of physiological traits have been reported to be associated with grain yield under both heat and drought stressed conditions (Gurmani *et al*., 2014). These traits include remobilization of carbohydrates (Moayedi, *et al.*, 2011)), canopy temperature (Blum *et al*., 1989 and Reynolds *et al*., 1998), ground cover (Richards *et al*., 2002) and chlorophyll protection or stay-green (van Herwaarden *et al*., 1998 and Reynolds *et al.,* 2000). Another common physiological response to stress in many plants is the accumulation of the amino acid proline (Mafakheri *et al*., 2010).

In addition to this, it has an adaptive role in plant stress tolerance (Verbruggen and Hermans, 2008). Proline has repeatedly been shown to increase under water stress and is potentially an important contributor to osmotic adjustment (Mattioni *et al*., 1997).

Stress indices which provide a measure of stress based on loss of yield under stress conditions in comparison to normal conditions have been used for screening tolerant genotypes (Mitra, 2001). Stress resistance is defined by Bartels and Sunkar (2005) as the relative yield of a genotype compared to other genotypes subjected to the same stress. The susceptibility of a genotype is often measured as a function of the reduction in yield under stress, whilst the values are confounded with differential yield potential of genotypes (Ramirez and Kelly, 1998). Several selection criteria have been proposed to select genotypes based on their performance in stress and non–stress environments. Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between stress and irrigated environments and mean productivity (MP) as the average yield of genotypes under stress and non-stress conditions. The geometric mean productivity (GMP) is often used by breeders interested in relative performance, since stress can vary in severity in field environments over years (Moghaddam and Hadizadeh, 2002).

Fischer and Maurer (1978) suggested the stress susceptibility index (SSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. Clarke *et al,* (1992) used SSI to evaluate drought tolerance in wheat genotypes and found year-to-year variation in SSI for genotypes and could rank their pattern. In spring wheat cultivars, Guttieri *et al,* (2001), using SSI, suggested that an SSI > 1 indicated above-average susceptibility to drought stress. The yield index (YI) suggested by Gavuzzi *et al,* (1997) and yield stability index (YSI) suggested by Bouslama and Schapaugh (1984) in order to evaluate the stability of genotypes in the both stress and non-stress conditions. Stress tolerance index (STI) was a useful tool for determining high yield and stress tolerance potential of genotypes (Fernandez, 1992). To improve the efficiency of STI a modified stress tolerance index (MSTI) was suggested by Farshadfar and Sutka (2002) which corrects the STI as a weight. Moosavi *et al*. (2008) introduced stress susceptibility percentage index (SSPI), stress non-stress production index (SNPI) and abiotic tolerance index (ATI) for screening drought tolerant genotypes.

The present study was therefore undertaken (i) to screen drought tolerance criteria and (ii) selection of the best tolerant genotypes of bread wheat in Siwa Oasis and Ashmon habitats of Egypt.

Material and Methods

Plant materials

In the present investigation six genotypes of breed wheat (M1, M2, Sh1, AL1, AL2 and AL3) kindly by provided by Afiah *et al.* (2014). Names, pedigree and/or selection history of the genotypes tested are described in Table 1 by Afiah *et al.* (2014).

TABLE 1. Origin, pedigree and/ or selection history of the six bread wheat varieties and lines used in the present study.

Sym bol.	**Genotype name**	**Origin**	**Pedigree and/or selection history**
M1	Misr-1	Egypt	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOOYO 1881T-050M-030Y-030M-030WGY-33M-0Y-0S.
M2	Misr-2	Egypt	SKAUZ/BAV 92. CMSS96M03611 S-1M-010SY-010M-010SY-8M-0Y-0S.
Sh1	Shandaweel-1	Egypt	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH.
AL1	Nesr	CIMMYT / ICARDA#	ICW85-0024-06AP-300AP-300L-1AP-0AP
AL2	S8 / 17*	Egypt	R8 tissue culture regenerated double haploid plant
AL3	Line – 606*	Egypt	Atlas 66/Nap Hall//(NE 70117) Skores Pelka 35/2*RCB – 61 Su 606 – 13 Su -2 Su – 5 Su – 0 Su

CIMMYT: Centro International de Mejoramiento de Maize Y Trigo (Mexico)
= International maize and wheat improvement center.
ICARDA: International Center for Agricultural Research in the Dry Areas.
Newly bred lines released through Desert Research Center wheat breeding program.

Grains of the selected genotypes were sown under Siwa Oasis and Ashmon conditions in Nov., 2012. Grains were sown at an adjusted rate of 50 viable grains /m^2 in three replications. Normal agronomic practices were performed and relevant metrological parameters were obtained from the observatory at each research station and daily minimum and maximum temperature and rainfall were recorded. Pigment content, relative water content (RWC), were estimated on the first fully expanded leaf (third from top) at vegetative stage.

Analysis of soil and water

Soil samples were taken from the two localities. Soil texture was determined using the sieve method, Piper (1947). Some physical and chemical analysis of soil and irrigation water as calcium carbonate, pH, EC, anions and cations were determined according to Jackson (1962).

Estimation of chlorophylls

Chlorophyll estimation was done by incubating 50 mg of the leaf material in 10 ml of dimethyl sulphoxide (Hiscox and Israelstam, 1979) for 4 hr at 65^{o}C. The absorbance of the clear solvent was recorded at 452, 663 and 645 nm (Arnon, 1949).

Water content

Water content (WC) was determined according to the following equation:

$$\frac{\text{fresh weigh of shoot- dry weigh of shoot}}{\text{fresh weigh of shoot}} \quad \text{X} \quad 100$$

Total soluble sugars

Total soluble sugars (TSS) of samples were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 6000 rpm. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water (Homme *et al*., 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol Spectrocolourimeter VEB Carl Zeiss (Yemm and Willis, 1954).

Total soluble proteins

Total protein was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard. An amount of 2 g of samples were grinded in mortar with 5ml of phosphate buffer (pH 7.6) and was then centrifuged at 8000 rpm for 20 min. 30µl of different samples were mixed with 70µl of distilled water then add 2.9 ml of Coosmassic Brillaint Blue solution and mixed thoroughly. The tubes were incubated for 5 min. at room temperature and absorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus Concentration (ug) of protein was calculated.

Proline

One g of samples was extracted with phosphate buffer (pH 7.6) and was then centrifuged at 8000 rpm for 20 min. Proline was assayed according to the method described by Bates *et al*. (1973). Two ml of extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Biological yield tested for stress tolerance indices

Genotypes were harvested at maturity and yield was recorded in both experiments.

Biological yield = yielded grains/plant +straw yield/plant

The condition tolerance indices were calculated as follows:

1. Tolerance index (TOL) and mean productivity (MP) as done by Rosielle and Hamblin (1981):

$$TOL = (Y_p - Y_s) \text{ and } MP = (Y_s + Y_p) / 2$$

2. Harmonic mean (HM) (Kristin *et al.*, 1997):

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

3. Stress susceptibility index (SSI) (Fisher and Maurer, 1978):

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

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

4. Geometric mean productivity (GMP) and stress tolerance index (STI) (Fernandez 1992; Kristin *et al.*, 1997):

$$GMP = (Y_p * Y_s)^{½} \text{ and } STI = (Y_p * Y_s) / (\hat{Y}p)^2$$

5. Yield Index (YI) (Gavuzzi *et al.*, 1997; Lin *et al.*, 1986):

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

6. Yield Stability Index (YSI) (Bouslama and Schapaugh, 1984):

$$YSI = Y_s / Y_p$$

7. Stress susceptibility percentage index (SSPI) as reported by Farshadfar and Sutka, (2002):

$$SSPI= [Y_p - Y_s /(2\ Y_p)] \times 100$$

8. Modified Stress tolerance index for Adequate Environment (K_1STI)

$$K_1STI= (Y_p)^2 / ({}^{-}{}_{Yp})^2$$

Modified Stress tolerance index for stressed Environment (K_2STI)

$$K_2STI= (Y_s)^2 / ({}^{-}{}_{Ys})^2$$

Where, Ys and Yp represent yield in stress and non-stress conditions respectively. Also, ${}^{-}{}_{\mathbf{Ys}}$ and ${}^{-}{}_{\mathbf{Yp}}$ are mean yield in stress and non-stress conditions respectively (for all genotypes).

Statistical analysis

Cluster analysis and dispersion of genotypes according to principal components method under Siwa Oasis and Ashmon conditions, depending on stress tolerance indices of biological yield were performed by SPSS ver.14.

Results and Discussion

Climate

The climate of the study area falls in a transitional zone between the Monsoon and Mediterranean climatic types. The data obtained in Table 2 show the range of climatic conditions at Siwa Oasis during 2011/12 and 2012/13 growing seasons.

It is clear that January is the coolest month (mean temperature 12.72 and 12.81°C) and air temperature rises in February and such rise continued till May. Relative humidity exhibits considerable monthly variations. The maximum wind speed recorded in April. The total amount of rainfall ranged from 2.73 mm / month in May to 53.82mm/month in December. In general climate is dry warm desert for most climatic classifications. It may be concluded that climate is warm and humid according to the basis of Blair (1971) because the temperature not less than 10°C and the relative humid is more than 40 % for most months (Trewartha and Horn, 1980).

TABLE 2. Monthly average weather data during 2011/12and 2012/13 growing seasons at Siwa Oasis site.

Month	**T.† (C°)**	**R.H.• %**	**W.S.♦ at 2mm/sec.**	**Amount of Rainfall (mm)**
2011/12season				
Nov.2011	17.74	57.64	2.00	18.82
Dec.2011	13.89	59.74	2.24	53.82
Jan.2012	12.72	54.95	2.30	25.03
Feb.2012	13.14	59.43	2.54	53.13
March.2012	14.45	55.85	2.87	23.84
April.2012	16.76	54.95	3.29	14.60
May.2012	19.83	51.67	2.93	5.56
2012/13 season				
Nov.2012	20.32	67.47	2.29	19.87
Dec.2012	15.92	69.93	2.57	48.24
Jan.2013	12.81	64.32	3.40	57.41
Feb.2013	13.43	69.56	3.75	50.95
March.2013	15.37	82.58	4.24	11.29
April.2013	18.17	81.25	4.86	18.64
May.2013	22.60	76.40	4.33	2.73

†T. = Temperature,• R.H. % = Relative humidity percentage,♦ W.S. = Wind speed.

Soil

Soil of Siwa Oasis habitat is sandy loam texture, saline (EC, 12.3 dsm^{-1}) calcareous (17.5% $CaCO_3$) and pH of 7.9. Deep artesian well irrigation water of EC about 3.96 dsm^{-1} and pH 7.3 was used for supplying irrigations through the growing season (2012/2013). The soil of Ashmon habitat characterized by clay texture, non saline (EC, 1.8 dsm^{-1})with pH 7.5 and irrigated by Nile River water (EC 1.35 dsm^{-1} and pH 7.6). Na^+, K^+, Ca^{2+} and Mg^{2+} recorded more values in soil of Siwa Oasis than in soil of Ashmon. More details of soil and irrigation water chemical analysis are presented in Table 3.

TABLE 3. Chemical and physical analysis of soil and irrigation water in Siwa Oasis and Ashmon habitats.

Habitat	Type	Texture Class	$CaCO_3$ (%)	pH	EC (dsm^{-1})	Anions (meq/L.)			Cations (meq/L.)			
						Cl^-	HCO_3^-	SO_4^{--}	Na^+	K^+	Ca^{2+}	Mg^{2+}
Siwa Oasis	Soil	Sandy loam	17.5 ±.8	7.9±.3	12.3 ±.4	83.6 ±2	2.3 ±.1	36.2 ±1	68.9 ±1	1.60 ±.1	34.5 ±.9	17.4 ±.7
	Irrig. water	-	-	7.3 ±.2	3.96 ±.1	18.6 ±1	10.8 ±.5	7.48 ±.6	22.1 ±.7	0.45 ±.04	8.3 ±.5	8.7 ±.5
Ashmon	Soil	clay	1.6 ±.1	7.5 ±.3	1.8 ±.1	9.8 ±.8	1.15 ±.1	7.1 ±.5	8.7 ±.5	0.35 ±.01	5.7 ±.3	3.2 ±.2
	Irrig. water	-	-	7.6 ±.2	1.35 ±.1	1.87 ±.1	3.56 ±.2	6.92 ±.3	12.3 ±.4	0.32 ±.03	1.15 ±.1	1.18 ±.1

Biomass

The obtained data (Table 4) showed that bread wheat genotypes grow under Ashmon habitat (adequate habitat) have higher biomass (fresh and dry weight) than that of Siwa Oasis habitat (stress habitat). Drought, being the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor (Shao *et al.*, 2009). Plant produces their maximum biomass under adequate water supply, whereas moisture stress causes a marker decrease in plant biomass production (Ashraf, 1998).

The values of WC varied among genotypes in response to stress condition at Siwa Oasis habitat compared at Ashmon habitat. A decrease in the RWC in response to drought stress has been noted in wide variety of plants (Nayyar and Gupta, 2006). According to Almeselmani *et al.* (2011 &2006) RWC indicates the water status of the cells and has significant association with yield and stress tolerance. Abdel-Hady and El–Naggar (2007) reported that RWC of the leaves is a better indicator of water stress than other growth or biochemical parameters of the plants. RWC of the leaves is very responsive to drought stress and has been shown to correlate with drought tolerance (Colom and Vazzana, 2003). Differences in WC were observed in the present results between genotypes of bread wheat at two habitats. This deviation in WC may be attributed to differences in the ability of the genotypes to absorb more water from the soil and or the ability to control water loss through the stomata (Khakwani, *et al.*, 2013). It may also be due to differences in the ability of the tested genotypes to accumulate and adjust osmoticum to maintain tissue turgor and hence physiological activities.

Pigment contents

M2, Shl and AL1 have highest Chl *a* at Ashmon habitat; however AL2 and AL3 have highest Chl *a* (.38 mg/g FW) at Siwa Oasis habitat (Table 4). Chl *b* and carotenoids recorded higher values at Siwa Oasis habitat compared to Ashmon habitat. This trait has been used successfully by many workers for screening and selection of drought tolerance wheat cultivars (Almeselmani *et al*., 2011). According to Izanloo *et al.* (2008) water deficit leads to an increased depletion of chlorophyll and a decreased concentration of chlorophyll. Zaharieva *et al.* (2001) reported that leaf color and chlorophyll content were correlated, as expected, since chlorophyll loss is the main factor responsible for change in leaf color. According to Manivannan *et al.* (2007) chlorophyll is one of the major chloroplast components for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate and flag leaf chlorophyll content is an indicator of the photosynthetic activity and its stability for the conjugation of assimilate biosynthesis (Bijanzadeh and Emam, 2010).

Total soluble sugars

The soluble sugar contents of bread wheat genotypes recorded higher values at Ashmon habitat compared with that at Siwa Oasis habitat (Table 4)**.** Our results showed that the stress conditions at Siwa Oasis reduce soluble sugars content of bread wheat genotypes. Like other cellular constituents, starch are also affected by stress (Abdel-Nasser and Abdel-Aal, 2002 and Gurmani *et al*., 2014). Stress caused a marked reduction in glucose, fructose and sucrose content of grains of sensitive cultivar (Saeedipour, 2011). The present research confirms the fact that sugars seems to be a very sensitive and genotype related marker for stress tolerance improvement. There are contradictory results on the effect of stress on sugar accumulation by many research workers (Kerepesi and Galiba, 2000; Saeedipour, 2011). Wheat typically accumulate a significant reserve of water-soluble sugars when the photosynthesis/respiration ratio is favorable, which can be used for immediate growth or stored and remobilized later in development. A total of 12 QTLs for WSC were mapped in three wheat populations across multiple environments, indicating polygenic control of the trait (Rebetzke *et al.*, 2008). Xue *et al.* (2008) suggested MYB genes (TaMYB13) as positive regulators for controlling the expression of enzymes involved in the WSC synthetic pathway in wheat, while other work has indicated the feasibility of genetic manipulation of regulatory genes for WSC expression (McIntyre *et al.*, 2012).

TABLE 4. Mean biomass, pigments, total soluble sugars, total soluble proteins and proline content of six bread wheat genotypes grow under Ashmon and Siwa Oasis habitats.

Genotype	**Biomass**			**Pigments (mg/g)**			**Total soluble sugars (mg/g)**	**Total soluble protein s(mg/g)**	**Proline mg/100 gFW**
	F.Wt (g/plant)	**D.Wt (g/plant)**	**RWC %**	**Chl a**	**Chl b**	**Carot.**			
Ashmon habitat									
M1	26.2± **1.4**	3.08± **.4**	88.2± **3.4**	0.26± **.04**	0.09± **.01**	0.07± **.01**	37.0± **1.4**	6.09± **.6**	0.05± **.01**
M2	29.4± **1.1**	4.04± **.2**	86.2± **2.7**	0.19± **.01**	0.08± **.01**	0.05± **.01**	53.4± **1.8**	4.72± **.4**	0.05± **.01**
Sh1	32.9± **1.5**	4.12± **.3**	87.4± **1.9**	0.25± **.01**	0.06± **.01**	0.02± **.01**	49.9± **1.7**	3.23± **.2**	0.06± **.01**
Al1	15.3± **1**	1.92± **.4**	87.4± **1.8**	0.26± **.01**	0.10± **.01**	0.04± **.01**	54.3± **1.8**	4.16± **.2**	0.02± **.005**
Al2	18.7± **1.1**	2.32± **.2**	87.5± **2.4**	0.24± **.02**	0.13± **.01**	0.07± **.01**	67.8± **2.1**	4.86± **.3**	0.06± **.01**
Al3	25.9± **1.5**	3.2± **.2**	87.6± **2.3**	0.20± **.01**	0.08± **.01**	0.06± **.01**	53.7± **2.1**	7.80± **.6**	0.06± **.01**
Siwa Oasis habitat									
M1	21.2± **1.4**	2.48± **.1**	88.3± **1.5**	0.27± **.04**	0.14± **.01**	0.15± **.01**	26.10± **1.4**	4.38± **.3**	0.07± **.01**
M2	24.4± **1.3**	3.44± **.2**	85.9± **2.4**	0.14± **.01**	0.07± **.01**	0.08± **.01**	26.28± **1.2**	3.84± **.3**	0.05± **.01**
Sh1	30.7± **1.4**	4.0± **.1**	86.9± **1.8**	0.19± **.01**	0.18± **.01**	0.04± **.01**	25.25± **1.4**	2.53± **.1**	0.07± **.01**
Al1	14.0± **1.1**	1.56± **.1**	88.8± **2.2**	0.20± **.01**	0.11± **.01**	0.13± **.01**	27.19± **1.5**	3.23± **.1**	0.07± **.01**
Al2	17.7± **1.1**	2.10± **.1**	88.1± **1.6**	0.33± **.05**	0.18± **.01**	0.21± **.01**	26.45± **1.6**	3.74± **.2**	0.08± **.01**
Al3	22.9± **1.4**	3.0± **.1**	86.9± **1.9**	0.38± **.05**	0.23± **.02**	0.23± **.01**	23.45± **1.2**	7.58± **.5**	0.08± **.01**

Soluble protein content

The obtained results show that total soluble protein contents of bread wheat genotypes varied between tested genotypes as follow AL3 > M1> AL2 > M2>AL1> Sh1. The data cleared that total protein contents of bread wheat genotypes at Ashmon habitat higher than that of Siwa Oasis habitat. The reduction of protein content of bread wheat genotypes at Siwa Oasis habitat may be attributed to increase of soil salinity compared to that at Ashmon habitat. Salt and water stress induce a decrease in protein content (Al-Ahmadi, 2014). Parida, *et al.* (2004) reported that under high concentration of salts (NaCl) and the activities of both acid and alkaline protease increased, lead to an increase in free amino acids, also Ferrario-Mery *et al.* (1998) reported that water stress disrupted the nitrogen metabolism leading to proteins solubility and accumulation of amino acids. Al-Ahmadi (2014) found that amino acids concentration decrease because of decreasing nitrate reeducates enzymes responsible for transforming NO^{-3} to NO^{-2} in plants under drought stress.

The stress tolerance potential of tolerant genotype (M1) is associated with elevated level of osmolytes in leaf tissues. Increased production of compatible solutes under stress has already been reported in wheat (Din *et al*., 2008), maize (Kaya *et al.,* 2013), *Chenopodium quinoa* willd, (Prado *et al.,* 2000) and tomato (Amini and Ehsanpour, 2005). Afzal *et al.* (2006), reported that increases in leaf soluble protein contents of wheat plants occur regardless of their sensitivity to salt stress; however, the level of increase or decrease in soluble protein under stress conditions is genotype dependent (Amini and Ehsanpour, 2005).

Proline content

As shown in Table 4, the increase of soil and irrigation water salinity at Siwa Oasis increased the proline content in the leaves of bread wheat genotypes from 22% to 47% than that of plants at Ashmon habitat. The highest proline content was observed in the AL2 at Siwa habitat. Bayoumi *et al.* (2008) reported a similar positive relationship between grain yield and proline accumulation under stress conditions in wheat. This suggests that the high proline content in the genotypes is probably a positive adaptive mechanism for overcoming the stress conditions. It is well documented that accumulated proline plays a role as a compatible solute in plants, regulating and reducing water loss from the cell under water deficit conditions (Verbruggen and Hermans, 2008). In wheat, it has been reported that osmotic adjustment is an important factor explaining differences in genotype yield or yield stability (Errabll *et al.,* 2006).

Furthermore, Sankar *et al.* (2007) reported that high proline accumulation in plants could provide energy for growth and survival and thereby help the plant to tolerate stress. It is now well known that proline accumulation in plant leaf cells, as a compatible solute, plays an important role in regulating water loss from the cells under water deficit and osmotically stressful conditions (Bayoumi *et al.,* 2008). It is therefore reasonable to suggest that the selection of new stress tolerance genotypes based on high proline accumulation, can be advocated as a parameter for selection, as it can be effective in enhancing tolerance in plants (Silverira *et al.,* 2003; Jaleel *et al*., 2007).

Biological yield

The stress factors negatively affect plant growth and development and cause decrease of plants biological yield at Siwa Oasis habitat compared with that of Ashmon habitat. Biological yield per plant decreased under stressed environment has been also reported by Chandler and Singh (2008). Moreover Pan *et al.* (2002) reported that yield and yield components of twelve spring wheat varieties were significantly decreased when they received minimum annual precipitation. To investigate suitable stress resistance of bread wheat genotypes under Siwa Oasis condition, biological yields of genotypes under this condition and non-stress conditions (Ashmon) were studied. Eleven indexes were measured for calculating different sensitivity and tolerance genotypes (Table 5). A suitable index must have a significant correlation with biological yield under both the conditions (Mitra, 2001). Based on the stress tolerance index (STI) and grain yield, Misr 2 genotype is the most drought tolerance with highest STI and grain yield under Ashmon (non-stressed) condition as well as under Siwa (stressed) condition. Mevlut and Sait (2011) indicated that the genotypes with high STI usually have high difference in yield in two different conditions. They reported in general, similar ranks for the genotypes were observed by GMP and MP parameters as well as STI, which suggests that these three parameters are equal for selecting genotypes. Thus, in this study the highest GMP and MP were in the genotype Misr 2. Al3 genotype displayed the lowest amount of STI and biological yield under this condition. Other genotypes were identified as semi-tolerance or semi-sensitive to stress conditions (Table 5).

TABLE 5. Mean performance of Biological yield/plant (kg/plant) for six bread wheat genotypes grow under Ashmon and Siwa Oasis habitats, tested for stress tolerance indices.

Genotype	**Biological yield**			**SSI**	**MP**	**GMP**	**HM**	**TOL**	**STI**	**K_1STI**	**K_2STI**	**YI**	**YSI**	**SSPI**
	At Ashmon	**At Siwa**	**Comb**											
M1	1.032	.736	.884	0.20	0.884	0.872	0.859	0.296	0.081	0.092	0.064	0.09	0.072	1.53
M2	1.491	1.283	1.387	0.097	1.39	1.38	1.38	0.208	0.204	0.482	0.49	0.15	0.086	1.07
Sh1	.801	.728	0.765	0.063	0.765	0.764	0.763	0.073	0.062	0.042	0.048	0.08	0.091	0.37
Al1	.745	.617	.681	0.12	0.681	0.678	0.675	0.128	0.049	0.029	0.027	0.07	0.083	0.66
Al2	1.271	1.242	1.257	0.016	1.26	1.26	1.26	0.029	0.168	0.289	0.37	0.15	0.098	0.15
Al3	.472	.371	.4220	0.149	0.422	0.418	0.415	0.101	0.019	0.004	0.004	0.05	0.078	0.52
Mean	.969	.830	.899	-	-	-	-	-	-	-	-	-	-	-
Grand mean	.899		-	-	-	-	-	-	-	-	-	-	-	-
$LSD_{0.05}$ G:	.328	.576	.193	-	-	-	-	-	-	-	-	-	-	-
$LSD_{0.05}$ L:	-	-	0.076	-	-	-	-	-	-	-	-	-	-	-
$LSD_{0.05}$ GL:	-	-	.273	-	-	-	-	-	-	-	-	-	-	-

SSI: Stress susceptibility index**, MP:** Mean productivity**, GMP:** Geometric Mean productivity**, HM:** Harmonic mean, **TOL:** Tolerance index**, STI:** Stress tolerance index**, K_1STI:** Modified Stress tolerance index for Adequate Environment**, K_2STI:** Modified Stress tolerance index for stressed Environment, **YI:** Yield index**, YSI:** Yield stability index**, SSPI:** Stress susceptibility percentage index.

The highest tolerance index (TOL), stress susceptibility index (SSI) were recorded at genotype Misr1 the most relative tolerant genotypes and genotype Al2 the least relative tolerance, respectively. Soorninia, *et al.* (2012) reported that stress tolerance index and mean productivity were defined as the difference in yield and the average yield between stress and non-stress conditions, respectively. The highest HM, K_1STI, K_2STI and YI were at M2 genotype. Ilker *et al.* (2011) concluded that HM, K_1STI, K_2STI and YI values are convenient parameters to select high yielding wheat genotypes in both stress and non-stress conditions whereas relative decrease in yield, TOL and SSI values are better indices to determine tolerance levels.

Cluster and principal component analysis

In order to determine tolerant and susceptible genotypes, cluster analysis based on the Ward method with Euclidian distance related to all stress indices was performed (Fig. 1). The cluster analysis grouped genotypes into four main clusters including Sh1, AL1 and AL3 in the first cluster and one genotype in the other three clusters. The highest similarity was found between Sh1 and AL1 genotypes. Since genotypes fallen in the cluster1generally had lowest values of mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress tolerance index (STI), modified Stress tolerance index for adequate environment (K_1STI), modified stress tolerance index for stressed environment(K_2STI) and yield index(YI). It has been shown that the first cluster composed a group of susceptible genotypes to Siwa Oasis conditions (Fig. 1 and 2). Najafian *et al.* (2011) also concluded that growing of cultivars with intermediate features, might reduce the yield gap between stress and non-stress wheat, to some extent, and could enhance of the average wheat grain yield in terminal drought prone areas. The third and fourth clusters occupied by Misr2 and Al2 were tolerant genotypes and those associated with high values of stress tolerance index (STI), modified stress tolerance index for adequate environment (K_1STI) and modified stress tolerance index for stressed environment (K_2STI).

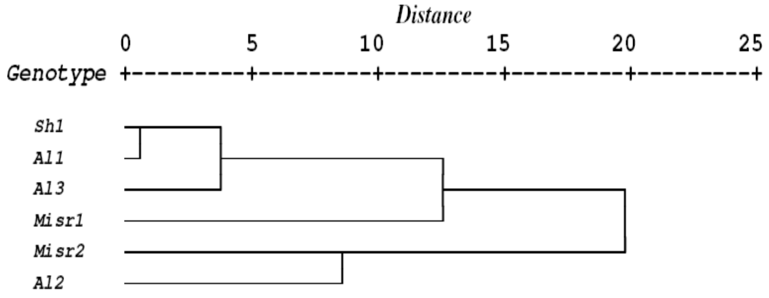

Fig. 1. Achieved dendrogram form biological yield analysis using Ward method showing classification of six bread wheat genotypes under Ashmon and Siwa Oasis habitats, based on eleven of stress tolerance indices.

Figure 2 shows dispersion of under-study genotypes according to principal components method. Similar to the results of cluster analysis, dispersion of genotypes clearly separated four locations. Since tolerance indices had positive correlation with the two components, while the susceptibility indices had negative and vice versa. Genotypes with high values related to first or second components were tolerant genotypes and those with low values were susceptible

(Fig. 2). Genotype Misr1 in second cluster had high values of second component and low of second component, it was near to susceptible indices vectors, indicating their susceptibility to stress, however, genotype Al 2 in the fourth third cluster had high value for first component and low value for second component and so it was near to the most tolerant genotype.

Misr2 had the highest value for component 1 and component 2 with the highest yield under Siwa Oasis condition comparing to other genotypes (Table 4). Similar analysis has been used for introducing tolerant and susceptible genotypes by Farshadfar and Sutka (2002) in maize, Golabadi *et al.* (2006) and Talebi *et al*. (2009) in durum wheat, and Jamaati-e-Somarin and Zabihi-e-Mahmoodabad (2012) in lentil. Cluster analysis for grouping stress indices showed that STI, K_1STI, K_2STI and YI could be the best indices for screening genotypes for stress tolerance. Jamaati-e-Somarin and Zabihi-e-Mahmoodabad (2012) reported that the positive correlations between STI and yield (at normal condition) and the negative correlation between TOL and yield under stress (at Siwa Oasis habitat).

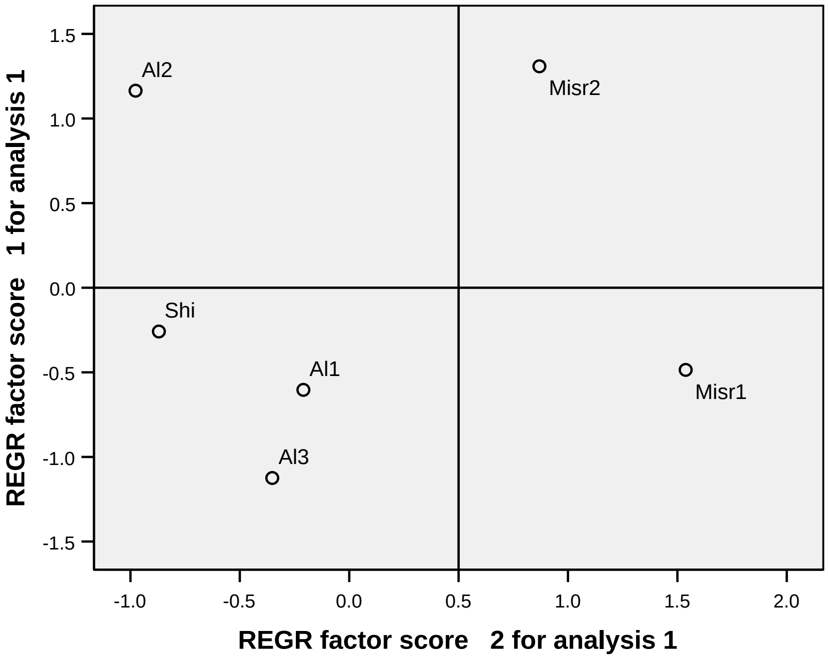

Fig. 2. Dispersion of under-study genotypes according to principal components method of six bread wheat genotypes under Ashmon and Siwa Oasis habitats, based on eleven stress tolerance indices of biological yield analysis.

Conclusions

Growth and photosynthesis are two of the most important processes abolished, partially or completely, by stress and both of them are major cause of decreased crop yield. The best option for crop production, yield improvement, and yield stability under adverse conditions is to develop stress tolerant crop varieties. The principal component and cluster analysis based on stress resistance grouping durum genotypes into four groups. STI, K_1STI, K_2STI and YI exhibited strong correlation with Ys and Yp, therefore, they can discriminate stress tolerant genotypes with high yield at the same manner under stress and non-stress conditions. With regard to these indices and cluster analysis, Misr2 genotype was the most tolerant genotype for Siwa Oasis habitat.

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دراسات فسيولوجية ومؤشرات التحمل لبعض الطرز الوراثية من قمح الخبز لظروف البيئية لواحة سيوه ومنطقة أشمون

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يعتبر انتاج سلالات من قمح الخبز تتحمل ظروف الاجهاد البيئي من اهم الخيارات الممكنة للتغلب على مشكلة نقص الغذاء العالمي الحالي ولضمان الاحتياجات الغذائية المتزايدة لسكان العالم في المستقبل. ولذلك تم دراسة مدى استجابة بعض الطرز الجينية من قمح الخبز لظروف بيئية مختلفة للوقوف على مدى تحملها للاجهاد البيئي. حيث أجريت تجربة حقلية باستخدام ست طرز جينية من قمح الخبز بثلاث مكررات تحت الظروف البيئية لكل من واحة سيوة و منطقة أشمون أثناء موسم ٢٠١٢-٢٠١٣ وقد تفاوتت محتويات الطرز الجينية من الاصباغ والكربوهيدرات والبروتين و البرولين في الطرز الجينية المختلفة بين البيئتين. وتم أختيار عدة مؤشرات لتحمل الاجهادوهي: مؤشر قابلية الإجهاد و مؤشر متوسط الإنتاجية و مؤشر الوسط الهندسي لاانتاجية و مؤشر تحمل الاجهاد ومؤشر التسامح و مؤشر التسامح للبيئة الملائمة ومؤشر مؤشر العائد ومؤشر الاستقرار والمؤشر المئوي لقابلية الإجهاد. واظهرت النتائج ارتبط انتاجية الطرز الجينية بإيجابية مع مؤشر التسامح للبيئة الملائمة ومؤشر مؤشر العائد والمؤشر المئوي لقابلية الإجهاد. وبذلك يمكن أن تستخدم كمؤشرات أكثر ملاءمة للتعرف على الطرز الجينية من قمح الخبز الاكثر تحملا للإجهاد البيئي.