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Inter simple sequence repeat (ISSR) markers and some physiological attributes of barley genotypes to drought and potassium nutrition

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Abstract: This study was designed to identify useful effects of potassium for drought tolerance in barley and determine the most tolerant genotypes to drought. Five barley genotypes were grown with two drought stress levels; 50% and 30% field capacity and treated with potassium sulfate in two levels K1 (40) and K2 (80 mg/ kg). Increasing water deficit (30% field capacity) lead to reduce spike length, spike weight, grain number, grain weight as well as 1000 grain weight in Giza 130 and Giza 134 genotypes although using of K1 and K2, whereas both K applications increased yield parameters at 50% field capacity. In Giza 123, 126 and 133 genotypes, applications of K1 and K2 increased yield parameters under 30% and 50% field capacity. Based on the obtained results, Giza 126 genotype showed the highest and stable yield across normal and drought conditions. The epidermis cell number, stomata number and stomata index increased in the upper surfaces of the control leaves in comparison with their lower surfaces. Application of potassium to the drought stressed plants, generally decreased stomata number, stomata index and epidermis cell number. Potassium may have served to adaptation of barley plants to drought stress conditions by causing a decrease in stomata movements. SDS-PAGE analysis has revealed that plant grown under drought showed induction or suppression in the synthesis of few polypeptides. Giza 126 showed best performance in respect of appearance of new bands in protein profile. ISSR-PCR technique was used to detect some molecular markers associated with drought tolerance in the five genotypes. Five ISSR primers were used and revealed 78% polymorphism. The primers produced 12 bands, which could be used as molecular markers and could be useful in breeding programs of barley.

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1. Introduction

One of the most important environmental stresses is drought stress which affecting agricultural productivity and may result in considerable yield reductions. Drought stress is most important a biotic stress that limit plant growth and development. Apart from the effect of drying soil on the transport of nutrients to plant roots, the morphological and physiological mechanisms involved in cellular and whole plant responses to water stress (Neumann, 1995). Drought stress reduces both nutrient uptake by the roots and transport from roots to the shoots, due to restricted transpiration rates and impaired active transport and membrane permeability (Yuncai & Schmidhalter, 2005).

Drought stress reduced dry matters by reduction in the area of the leaf, height of plant and lateral stem number (Aliabadi et al., 2009). Reactive oxygen species (ROS) are the byproducts of many degenerative reactions in crop plants, under drought stress which will affect the regular metabolism by damaging the cellular components (Foyer & Noctor, 2002). Drought resistance refers to a plant's ability to grow under drought conditions, and drought acclimation is plant's ability to modify its structure and function so that it can better tolerate drought. Water stress tolerance by many mechanisms such as osmotic adjustment, water storage tissues, deep or fast growing roots and water conductance. Upon exposure to a biotic stress conditions, plants undergo a variety of changes from physiological adaptation to gene expression (Shinozaki & Yamaguchi-Shinozaki, 2007).

The expression of many genes is induced by drought, and their gene products function directly in stress tolerance and regulation of gene expression and signal transduction in stress responses (Zhao et al.,

osmotic potential and water uptake and had impact on stomata closure which increases tolerance to water stress and also is osmotic in maintaining low water potential of plant tissues, so that, K+ is accumulated in response to soil water deficits may play an important role in water uptake along a soil–plant gradient. Potassium (K) is a soil aggregating agent which is known to have a positive effect on soil physical properties and subsequently crop yields (Hamza & Anderson, 2003). Moreover, it is involved in activating a wide range of enzyme systems which regulate photosynthesis, water use efficiency and movement nitrogen uptake and protein building. Potassium plays a vital role in: photosynthesis, protein synthesis, control of ionic balance, regulation of plant stomata and water use, activation of plant enzymes and, many other processes (Reddya et al., 2004). Also, potassium application improves the water content in the broad bean leaves and the plants showed more tolerance to drought stress. Potassium was found to be a crucial factor in the plants' ability to manage water shortage (Parsons et al., 2007). Çakmak (2005) reported that the improvement of K-nutritional status of plants might be of great importance for the survival of crop plants under environmental stress conditions, such as drought, chilling, and high light intensity. There is increasing evidence that plants suffering from environmental stresses like drought have a larger internal requirement for K. The reason for the need for K by plants suffering from environmental stresses appears to be related to that K is required for maintenance of photosynthetic CO_2 fixation (Cakmak & Engels, 1999). Potassium is important ion in the growth of plants and in the physiology of plant water relations.

The objective of this study was to test effectiveness of potassium application in alleviation of drought stress adverse effects. Understanding the physiological, anatomical and biochemical responses under different amounts of water and nutrients is imperative for efficient management of agronomical inputs (irrigation and nutrient). It also can be used as screening basics for drought tolerance in breeding programs.

2. Materials and Methods

In this study, five barley genotypes (*Hordeum vulgare* L. Giza 123, Giza 126, Giza 130, Giza 133 and Giza 134) were used. The seeds were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Pot experiment was established in 25 cm diameter clay pots, each filled with about 4 kg of loam based garden soil. Barley grains were surface sterilized by immersing in 70 % ethanol for 2 min. then in 0.2 % sodium hypochlorite (NaoCl) for 3 min. and were washed for several times with sterile distilled water. Fifteen seeds were placed into each clay pot. The seeds were sown at 2-3 cm depth in each pot and when emergence was complete (~7days) the seedling density was reduced to 10 seedlings / pot. The experiment was conducted under natural conditions (day length 12 - 14 hrs, at 20 – 22°C and 70% humidity). Pots were divided into five groups; the 1st group of pots was irrigated regularly with 100% hold water capacity (serve as control). In the 2nd and 3rd groups, plants were subjected to two levels of drought stress (50% and 30% hold water capacity respectively) and treated with potassium in the form of potassium sulphate (40 mg/kg soil) added to the soil (starting on the third week after sowing); the 4th and 5th groups were treated in the same manner of the 2nd and 3rd groups but by using 80 mg/kg of potassium sulphate. After 120 days of sowing the plant samples were collected to determine certain morphological characters (spike length, spike weight, grain number, grain weight and 1000 grain weight) in addition to some anatomical and biochemical measurements as follow:

2.1. Anatomical Observations

The leaf specimens including the midrib were taken from the second leaf from plant top by a microtome, in 6–7 µm thickness. Specimens were taken on day 45th of planting. Epidermis imprints were used to count stomata, and the imprints were later removed using transparent adhesive tape and were placed on a microscope slide. Staining was made by using safranine, cleared in xylol and mounted in Canada balsam (Ruzin, 1999). Stomata and epidermis cells in a 1-mm^2 unit area were counted using a light microscope with a 40 x 10 magnification lenses. These counts were made both in the lower and upper surfaces of each leaf 10 times as 3 replicates and the averages were calculated. After stomata number per unit area and epidermal cell number were determined, stomata index was estimated according to Meidner and Mansfield (1968):

stomata index = stomata number in unit area/ (stomata number in unit area + epidermis cell number in unit area) × 100

2.2. Biochemical Measurements:

2.2.1. Protein Preparation for SDS-PAGE

After 15 days of different NaCl treatments, plants were harvested and then soluble protein extracted by grinding one gram freeze dried sample with pestle and mortar in liquid nitrogen and 4 ml buffer solution (1.0 M tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 25 mM EDTA, 0.5 % (w/v) SDS 10 mM - mercaptoethanol). SDS_PAGE was performed by the methods described previously (Laemmili, 1976).

2.2.2. DNA extraction and PCR amplification conditions

Total genomic DNA was extracted from 100 mg young leaf tissue by using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). Quality and quantity of genomic DNA was assessed by Nano drop spectrophotometer ND-1000 (Thermo Scientific, GA). Five ISSR primers (XXIDT Integrated DNA Technologies Int., Coralville, IA) were used for standardization of optimum annealing temperature. All the PCR components used in this study were purchased from Fishersci, Georgia. PCR amplification was performed in an Eppendorf thermal cycler (Eppendorf North America, Inc.). PCR was performed in a 25 µl mixture containing 25-50 ng DNA, 2.5 µl10×Taq buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 1.6 µM primer, and 1U Taq DNA polymerase. The PCR consisted of an initial denaturation at 94°C for 3mins, 40 cycles comprising denaturation at 94°C for 30s, annealing at 72°C for 1 min, extension at Tm for 50sec, and a final extension step at 72°C for 10mins. Amplified products were separated by electrophoresis on 2.0% agarose gel in TAE (1×) buffer stained with ethidium bromide for 20 mins and photographs were taken using Gel documentation system (Bio-Rad Corporation, USA).

Statistical Analysis

The data were statistically analyzed using F-test and L.S.D. at 5% and 1% levels of probability according to SAS-Programme (1982).

3. Results

3.1. Yield parameters

Table 1: Yield and yield components of barley genotypes as affected by drought stress and potassium application.

Treatments	Genotype	Spike length (cm)	Spike wt. (gm)	Grain number / spike	Grain wt. / spike (gm)	1000 grain wt. (gm)
Field capacity 100% (control)	Giza 123	13.61	6.13	20.16	5.00	200.62
	Giza 126	14.25	6.72	22.74	6.10	210.50
	Giza 130	12.76	5.15	17.63	4.00	168.60
	Giza 133	13.57	5.74	19.54	4.32	176.34
	Giza 134	12.43	4.38	16.18	3.65	160.42
50% field capacity + K1	Giza 123	18.67**	8.61**	25.24**	5.43**	239.54**
	Giza 126	19.33**	9.35**	28.31**	6.91**	248.01**
	Giza 130	17.85**	6.92**	21.37**	4.92**	235.80**
	Giza 133	18.16**	7.04**	24.63**	5.16**	236.01**
	Giza 134	15.17**	6.45**	19.51**	4.51**	233.97**
30% field capacity + K1	Giza 123	17.54**	6.60**	23.26**	5.14*	221.42**
	Giza 126	18.63**	7.10*	25.41**	6.37**	227.61**
	Giza 130	10.54**	4.45**	15.50**	3.12**	156.21**
	Giza 133	17.00**	5.81^{ns}	21.37**	5.00**	186.80**
	Giza 134	9.80**	3.95**	14.16**	2.77**	136.32**
50% field capacity + K2	Giza 123	22.46**	10.65**	30.28**	7.14**	268.25**
	Giza 126	26.84**	11.24**	35.61**	8.53**	272.55**
	Giza 130	18.79**	7.62**	24.00**	5.56**	250.69**
	Giza 133	20.16**	9.23**	26.74**	6.91**	258.41**
	Giza 134	16.56**	6.65**	20.18**	5.50**	244.08**
30% field capacity + K2	Giza 123	19.58**	7.41**	24.18**	5.25**	230.16**
	Giza 126	21.60**	8.94**	27.16**	6.41**	231.67**
	Giza 130	11.61**	4.90*	16.92*	3.50**	160.98*
	Giza 133	19.50**	6.54**	22.60**	5.00**	226.89**
	Giza 134	10.21**	4.00**	15.12**	3.10**	152.21*
LSD at 0.05		0.49	0.22	0.5	0.14	5.08

Levels of significance are represented by at * = P < 0.05; ** = P < 0.01 and ^{ns} = non-significant (P >0.05).

The efficiency of potassium fertilizer on water stressed plants is much more than well watered plants. The obtained results were showing that potassium application to drought stressed plants significantly increased yield parameters (spike length, spike weight, grain number, grain weight and 1000-grain weight) of Giza 123, Giza 126 and Giza 133 genotypes at both levels of drought stress while these parameters were decreasing in Giza 130 & Giza 134 genotypes at 30% field capacity accompanied by the application of K1 and K2 when compared with respective controls. Table (1) reveals that spike length and spike weight were significantly influencing by potassium. There were significant differences ($p<0.01$) among genotypes for spike length, Giza 126 produced tallest spikes at 50% field capacity and the

application of K2 while the genotype Giza 134 produced the most shortest spikes under normal and 30% field capacity plus K1. Moreover, the utilization of potassium increased grain number per spike in Giza 123, 126 and 133 at both levels of drought stress particularly by applying K2 whereas, this parameter was decreased in Giza 130 and Giza 134 only at the severe level of drought stress (30% field capacity). There were significant differences ($p<0.01$) among the used genotypes for grain number per spike. The highest number (35.61) was recorded for the genotype Giza 126 grown under 50% field capacity and treated with K2 while, the lowest one (14.16) was recorded for the genotype Giza 134 at 30% field capacity and treated with K1. The weight of grain is an important yield component and made major contribution towards grain yield of barley. The 1000-grain weight is greatly influencing by drought stress and soil nutrients. Potassium had significant effect on 1000-grain weight. There were significant differences ($p<0.01$) among the five genotypes for 1000-grain weight. Maximum 1000- grain weight (272.55 g) was obtaining under 50% field capacity and application of 80 mg/kg potassium (K2) in the genotype Giza 126. The minimum (136.32 g) 1000- grain weight was obtaining from Giza 134 at 30% field capacity and application of 40 mg/kg potassium (K1).

In general potassium application significantly increased yield parameters of the drought stressed plants with the exception of the reduction in these parameters which observed in Giza 130 and Giza 134 subjected to 30% field capacity. In addition, the most effective treatments on yield were showing at 50% field capacity and treated with K2 in all genotypes while, the lowest at 30% field capacity and treated with K1. Moreover, there were significant different between genotypes in their responses to drought stress conditions as a result of potassium application as compare with to respective controls. Results of the study suggested that genotypes plants Giza 126, Giza123 and Giza 133 were responding best to drought stress.

3.2. Anatomical Observations

Table 2: Stomata movements in the leaves of under-study barley genotypes as influenced by drought stress and potassium application.

Treatments	Cultivars	Epidermis cell number		Stomata number		Stomata index	
		upper	lower	upper	lower	upper	lower
Field capacity 100% (control)	Giza 123	19.1	18.3	4.7	4.4	19.75	19.38
	Giza 126	17.2	16.1	4.3	4.0	20.0	19.90
	Giza 130	22.9	21.4	5.6	5.2	19.65	19.55
	Giza 133	21.3	20.1	5.2	4.9	19.62	19.60
	Giza 134	23.5	22.3	6.1	5.7	20.61	20.30
50% field capacity + K1	Giza 123	14.6**	16.4**	3.3**	3.9**	18.43**	19.21**
	Giza 126	13.4**	14.2**	3.1**	3.4**	18.79**	19.32**
	Giza 130	16.6**	19.3**	4.0**	5.0**	19.41**	20.57**
	Giza 133	14.8**	18.1**	3.4**	4.4**	18.68**	19.55 ^{ns}
	Giza 134	19.3**	20.0**	5.0**	5.6**	20.57 ^{ns}	21.87**
30% field capacity + K1	Giza 123	15.4**	17.4**	3.6**	4.2**	18.95**	19.44**
	Giza 126	14.0**	15.3**	3.3**	3.8**	19.08**	19.90 ^{ns}
	Giza 130	23.2 ^{ns}	21.5 ^{ns}	5.9**	5.2 ^{ns}	20.27**	19.47 ^{ns}
	Giza 133	15.7**	20.0 ^{ns}	3.8**	5.3**	19.48**	20.95**
	Giza 134	24.8**	22.8**	6.8**	5.7 ^{ns}	21.52**	20.00**
50% field capacity + K2	Giza 123	14.2**	15.7**	3.2**	3.8**	18.39**	19.49**
	Giza 126	13.0**	13.9**	2.9**	3.2**	18.24**	18.71**
	Giza 130	16.2**	18.2**	3.9**	4.9**	19.40**	21.21**
	Giza 133	14.7**	17.8**	3.3**	4.2**	18.33**	19.09**
	Giza 134	19.2**	19.5**	4.9**	5.4**	20.33**	21.69**
30% field capacity + K2	Giza 123	15.0**	16.9**	3.5**	4.1**	18.92**	19.52**
	Giza 126	13.6**	14.8**	3.2**	3.8**	19.05**	20.43**
	Giza 130	23.3 ^{ns}	21.9**	5.9**	5.3**	19.93**	19.49 ^{ns}
	Giza 133	15.1**	18.7 ^{ns}	3.4**	4.5**	18.37**	19.40**
	Giza 134	24.4**	22.6*	6.6**	5.7 ^{ns}	21.29**	20.14*
LSD at 0.05		0.37	0.17	0.11	0.04	0.08	0.09

Levels of significance are represented by at * = $P < 0.05$; ** = $P < 0.01$ and ^{ns} = non-significant ($P > 0.05$).

The findings related with effects of potassium on the stomata movements of barley genotypes are representing in Table (2). The epidermis cell number and stomata number per unit area in control plants (100 % field capacity) of barley genotypes were higher in the upper than in the lower surface. The stomata index of controls of the under study genotypes was higher in the upper than in the lower surface. Exposing the plants to drought stress levels (50 & 30% field capacity) accompanying by the application of potassium to the soil result in a change in stomata number, epidermis cell number and the value of stomata index of the barley leaves. Potassium treatment decreased stomata number, epidermis cell number and stomata index of the upper surface of all genotypes at 50 % hold water capacity. At 30% field capacity, these parameters were decreasing in the upper surface of Giza 123, Giza 126 and Giza 133 genotypes whereas, the same parameters were increased in the genotypes Giza 130 and Giza 134 of the upper surface as compared with the lower surface. Although potassium treatment increased stomata number, epidermis cell number and stomata index (upper) in the cultivars Giza 130 and Giza 134 at 30% field capacity, it had no effect on stomata number and epidermis cell number of the lower surface as compared with the respective controls.

Both treatments of potassium mostly decreased the stomata index in the upper surface at 50% hold water capacity as compared with the lower surface. We obtained the lowest stomata index (18.24) in the upper surface of the genotype Giza 126 treated with K2 and grown under 50% hold water capacity, whereas for the control plants this index was (20.0). The decrease in the values of stomata index in the plants treating with potassium and 50% field capacity occurred primarily because of a decrease in the number of stomata per unit area with a slightly increased in the number of epidermal cells. Moreover, treating the plants with potassium helped them to complete their growth under severe drought stress conditions (30% hold water capacity). In other words, potassium treatment alleviated the inhibitory effect of drought stress through the ecological adaptation of plants.

The results of the study showed that Giza 123, Giza 126 and Giza 133 genotypes have more tendencies to adapt stressful environment than others do particular at 30% field capacity.

3.3. SDS-PAGE protein banding pattern

In an attempt to understand the molecular basis of drought tolerance, SDS-PAGE was analyzing to identify protein patterns involved in drought stress response in the five barley genotypes as shown in table 3(a & b) and figure 1 (a & b). The total protein bands were 24 detected with different molecular weights ranging from 8 KD to 235 kDa, which were not necessarily being present in all genotypes. Among such bands, seven protein bands were clearly observing in all barley genotypes under study (monomorphic bands), while the other 17 bands (polymorphic) were varying in some distinctive genotypes under drought stress concentrations (Table 3a). According to SDS-PAGE of protein, in Giza 123-genotype one protein band with MW 98 kDa was inhibiting under both 50 and 30% field capacities accompanying by application of K1. Moreover, three bands with MWs (81, 45 and 41) were not expressed under only 50% field capacity and three bands were disappeared under only 30% field capacity with MWs (111, 49 and 19) kDa. On the other hand, three and one newly protein bands were appeared under 50% and 30% field capacity respectively accompanied by the application of K1 as comparing to control. In Giza 126, one protein band with MW of 98 kDa was disappeared and six bands (95, 81, 66, 43, 31 & 19 kDa) were newly appeared under the two levels of drought stress plus K1 treatment compared with the non-stressed plants. In Giza 130, two protein bands (98 & 87 kDa) were not expressed and three protein bands (95, 66 & 45 kDa) were expressed under both levels of drought stress plus K1 treatment compared with control. In case of Giza 133, one protein band was disappeared with MW 71 kDa accompanied by the appearance of two newly protein bands at MWs (106 and 66 kDa) under both levels of drought stress plus the application of K1. In Giza 134, three bands with MWs of 71, 49 & 19 kDa were not expressed, whereas the protein bands which having the molecular weights of 66, 53 & 45 were expressed under both levels of drought stress as compared with the non-stressed plants. In general, the result revealed that drought stress resulted in an increase of some proteins and a decrease of others.

Furthermore, drought stress induced in all genotypes the appearance of one new protein band with molecular weight 66 kDa compared with the non-stressed plants. Moreover, one band with molecular weight 98 kDa was disappeared in genotypes Giza 123,126 and 130 when they exposed to both levels of drought stress. The SDS-PAGE results revealed that exposing the plants to drought stress levels and the application of both concentrations of potassium resulted in an increasing in the total number of the detected protein bands (Table 3b) particularly in the more tolerant genotypes (Giza123 & Giza 126). Giza 126 genotype showing the highest number of protein bands, this indicated that the accumulation of proteins might relate to drought tolerant- genotype 126. These changes in protein expression suggest that these induced proteins play a role in plants response to drought stress.

Table 3 (a): Effect of K1 application on the protein patterns of the leaves of under-study barley genotypes grown under drought stress conditions.

MW. (kDa)	Field capacity 100% (control)					50% field capacity + K1					30% field capacity + K1				
	G. 123	G. 126	G. 130	G. 133	G. 134	G. 123	G. 126	G. 130	G. 133	G. 134	G. 123	G. 126	G. 130	G. 133	G. 134
219	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
133	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
111	+		+	+		+	+		+						
106		+	+		+		+		+	+	+	+	+	+	+
98	+	+	+												
95				+	+	+	+	+	+	+	+	+	+	+	+
91	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
87	+	+	+			+	+		+		+	+			
81	+			+	+		+	+	+	+	+	+		+	+
71		+	+	+	+							+	+		
66						+	+	+	+	+	+	+	+	+	+
61		+	+	+		+	+		+			+	+		
53	+	+				+	+			+	+	+		+	+
49	+	+	+	+	+	+	+	+	+		+		+		
47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45	+	+					+	+		+	+	+	+	+	+
43			+	+	+	+	+	+	+	+	+	+			
42	+	+									+	+	+	+	+
38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31			+	+	+	+	+	+				+		+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+			+	+	+	+					+		+	
15		+				+		+	+	+		+			
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
T.No of bands	16	17	16	16	15	18	20	15	17	15	17	21	15	16	15

Orange colour → monomorphic bands, Green colour→ disappeared bands, Yellow colour → newly appeared bands

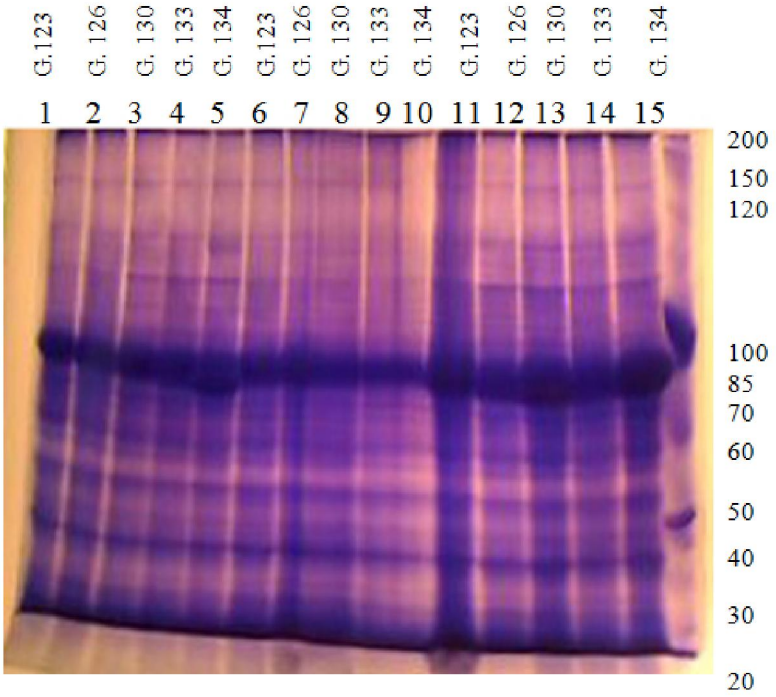


Fig. 1 (a): Protein Profile of the leaves of barley genotypes as affected by drought stress and K1 application.

Table 3 (b): Effect of K2 application on the protein patterns of the leaves of under-study barley genotypes grown under drought stress conditions.

MW. (kDa)	50% field capacity + K2					30% field capacity + K2				
	G. 123	G. 126	G. 130	G. 133	G. 134	G. 123	G. 126	G. 130	G. 133	G. 134
235	+	+	+	+	+	+	+	+	+	+
199	+	+	+	+	+	+	+	+	+	+
175										+
127						+	+			+
122	+	+	+	+	+	+	+	+	+	+
115	+	+	+	+	+	+	+	+	+	+
111							+	+	+	
106	+	+	+		+				+	
101		+		+		+	+	+	+	+
94	+	+	+	+	+	+	+	+	+	+
87		+	+	+	+	+	+	+	+	
75	+					+	+	+	+	+
70	+	+	+	+	+	+	+			
56	+	+		+	+	+	+	+	+	
61	+	+	+				+		+	+
57	+	+	+	+	+	+	+	+	+	+
55		+	+	+	+	+	+	+	+	
51	+	+	+		+	+	+	+	+	+
47	+	+	+	+		+	+			
43	+	+		+	+	+	+	+	+	+
39	+	+	+	+	+	+	+	+	+	+
33	+	+								
29	+	+	+	+	+	+	+		+	+
25	+		+	+	+		+			+
21		+	+			+	+	+		
16				+	+	+	+	+	+	+
Total No. of bands	18	20	17	17	17	20	23	17	19	17

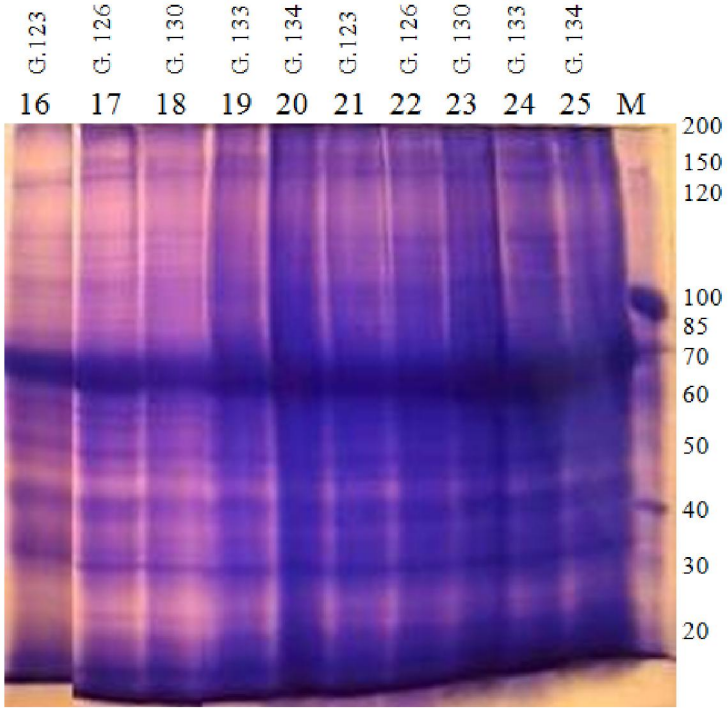


Fig. 1 (a): Protein Profile of the leaves of barley genotypes as affected by drought stress and K2 application.

3.4. Molecular markers by using ISSR analysis

Five oligonucleotide primers were used to establish ISSR-PCR fingerprints of the five barley genotypes sown under drought stress to detect molecular markers for drought tolerance. These primers were HB09, HB11, HB12, HB13 and HB15. Both the number and size of the amplified products varied considerably with the different primers. The results of ISSR-PCR of the studied barley genotypes are given in table (4). From this table it is clear that 52 polymorphic bands were generated by these primers in samples under study with a percentage of polymorphism 78%. A total of 12 unique bands were identified of them.

Table 4: List of primers, their sequence, numbers and size of the amplified fragments (bands) generated by ISSR primers in barley.

Primer code	Sequence (5′ to 3′)	Mono-morphic bands	Polymorphic bands		Total bands	Percent Polymo-rphism	Size range (bp)
			Shared bands	Unique bands			
HB09	-GTGTGT GTGT GTGC-	2	7	2	11	82	200-1145
HB11	- GTGTGTGT GTGTTGTCC-	3	9	2	14	78	177-928
HB12	- CACCAC CACG C -	5	7	3	15	67	79-1443
HB13	- GAGGA GGAGGC -	2	8	2	12	83	176-886
HB15	-GTGGT GGTGGC-	3	9	3	15	80	161-1441
Total		15	40	12	67	78	

Monomorphic Bands → Same Bands (similar Bands)
Polymorphic Bands → Different Bands (present in few but absent in others /not present in all)

Figures (2-6) and Table (5) exhibited the ISSR profile produced by the **primer HB09** with a percentage of polymorphism 82%. The size of the amplified fragments generated by this primer ranged from 1145 to 200 bp. Two monomorphic bands were detected in this profile. Also, two unique bands were scored in the tolerant genotype (Giza 123) at molecular sizes of 488 and 401 bp. These could be considered as a positive marker for this genotype. **Primer HB11** produced fourteen bands, the size of the amplified fragments generated by this primer ranging from 928 to 177 bp. Two unique bands were detected in this primer, one of them was recorded only in the moderately tolerant genotype (Giza 133) at molecular size of 553 bp. This band could be used as a positive marker for this genotype. While, the other band with molecular size of 818 bp was recorded in the least tolerant genotype (Giza 130) and this band could be used as marker assisted selection (MAS) for this genotype. **Primer HB12** gave fifteen bands with the percentage of polymorphism 67%. Three unique bands were detected in this primer, two bands with molecular sizes of 1069 and 438 bp were recorded in the most tolerant genotype (Giza 126) under severe drought stress condition (30% field capacity). These bands could be used as positive marker for this genotype. While, the third band was recorded in the genotype Giza 126 at molecular size of 79 bp under control conditions (100% field capacity). **Primer HB13** produced twelve bands with the percentage of polymorphism 83%. Two monomorphic bands and two unique bands were detected in this profile. The unique bands were found only in the sensitive genotype (Giza 130) under moderate drought stress condition (50% field capacity) at molecular sizes of 401 and 176 bp. Therefore, these bands could be used as marker assisted selection for this genotype. **Primer HB15** produced fifteen bands with a percentage of polymorphism 80%. Three monomorphic bands and twelve polymorphic ones were recorded in the profile by this primer. Three unique bands were recorded, from them two bands were detected in the most tolerant genotype (Giza 126) at the molecular sizes of 886 and 195 bp under severe drought stress conditions. Thus, these bands could be considered as a positive molecular marker for this genotype and could be used as marker assisted selection for this genotype. The third unique band was characteristic for the least tolerant genotype (Giza 134) at the molecular size of 161 bp under control condition.

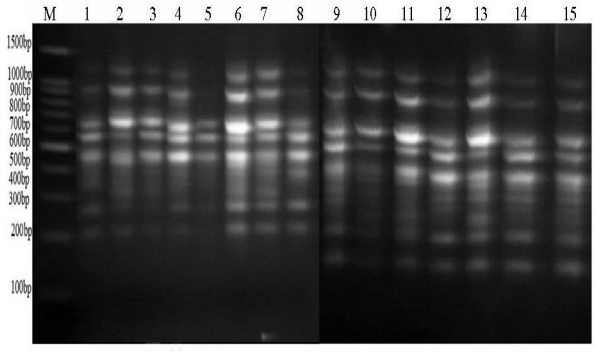
The obtained results revealed that the primer HB12 and HB15 have amplified maximum number of bands, while the primer HB09 has amplified least number of bands. Such results indicate that primer HB12 and HB15 repeats are more frequent in barley genome than the HB09 repeats. The highest percentage of polymorphism (83%) was detected with the primer HB13, while the least one (67%) was recorded in the primer HB12. A total of 12 unique bands were identified among the total bands, and could be considered as marker assisted selection. Among these, 4 unique bands were characteristic for the most tolerant genotypes (Giza 126) and were detected by primer HB12 and HB 15 under severe

drought stress conditions. In addition, 3 unique bands were characteristic for the moderate tolerant genotypes (Giza 123 and Giza 133) under severe drought stress conditions, two of them were detected in Giza 123 by the primer HB09 while the third one was scored in Giza 133 by the primer HB11. Moreover, one unique band was characteristic for the least tolerant genotype (Giza 130) by primer HB11 at molecular size 818 bp. Furthermore, there were two shared bands could be used as markers and were found under severe drought stress conditions only in the two most tolerant genotypes Giza 123 and Giza 126 by primer HB12 at molecular size of 1344 bp and by primer HB15 at molecular size of 1441 bp.

DNA marker	Molecular size (bp.)	Field capacity (100%)					Field capacity (50%) + K1					Field capacity (30%) + K1				
		G.123	G. 126	G. 130	G. 133	G. 134	G.123	G. 126	G. 130	G. 133	G. 134	G.123	G. 126	G. 130	G. 133	G. 134
HB09																
1	1145	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0
2	1019	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
3	894	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
4	704	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
5	541	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
6	488	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
7	453	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	401	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
9	339	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1
10	278	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
11	200	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HB11																
1	928	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
2	860	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
3	818	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
4	731	0	0	1	1	1	0	1	0	0	1	0	1	0	0	0
5	694	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0
6	616	1	1	1	0	0	0	1	0	1	1	1	0	1	1	0
7	553	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
8	509	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0
9	443	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	368	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	311	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	248	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0
13	196	1	1	1	0	1	1	1	1	0	0	0	0	1	1	1
14	177	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
HB12																
1	1443	1	1	1	1	1	1	1	0	1	0	0	1	1	1	0
2	1344	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
3	1069	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
4	915	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1
5	700	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	543	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	438	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
8	348	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	275	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	253	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1
11	199	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
12	178	1	0	0	1	1	1	1	1	0	0	1	0	0	0	1
13	142	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	105	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
15	79	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

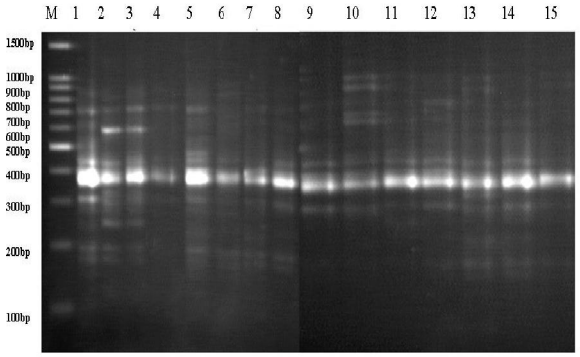
Table 5. Molecular weight base pairs of amplified DNA fragment that produced by using ISSR analysis with five primers.

HB13																
1	886	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0
2	810	0	0	0	1	1	1	0	0	0	1	1	1	1	0	1
3	679	1	0	0	1	1	0	1	1	1	0	0	1	0	0	0
4	614	1	1	1	1	0	1	0	0	1	1	1	1	1	0	1
5	542	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
6	504	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0
7	437	1	0	1	0	0	0	0	0	1	1	1	0	1	1	0
8	401	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
9	357	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	289	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	191	1	1	1	0	1	1	0	0	0	0	0	1	1	1	1
15	176	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
HB15																
1	1441	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
2	1096	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0
3	985	1	1	1	0	0	1	1	1	1	0	0	0	1	1	1
4	886	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
5	880	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1
6	781	1	1	1	0	0	0	0	0	1	0	1	1	0	0	0
7	687	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0
8	623	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
9	535	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	405	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	307	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	270	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0
13	233	1	1	0	1	1	1	1	1	1	0	0	0	0	1	1
14	195	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
15	161	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0



HB-09

Figure 2. The ISSR profile of 5 barley genotypes produced with primer HB-09 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).



HB-11

Figure 3.The ISSR profile of 5 barley genotypes produced with primer HB-11 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

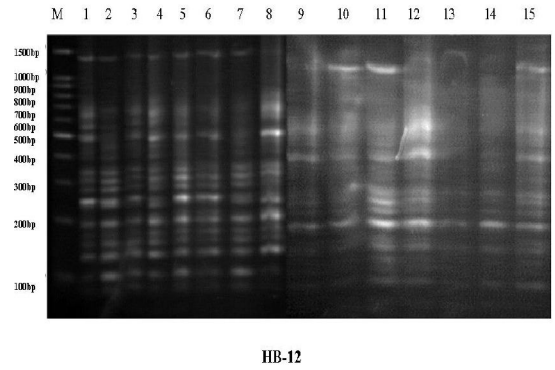


Figure 4. The ISSR profile of 5 barley genotypes produced with primer HB-12 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

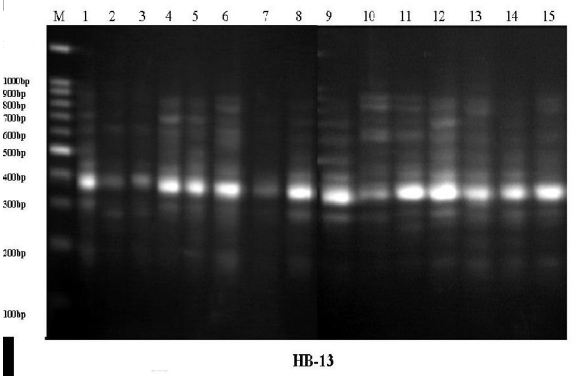


Figure 5. The ISSR profile of 5 barley genotypes produced with primer HB-13 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

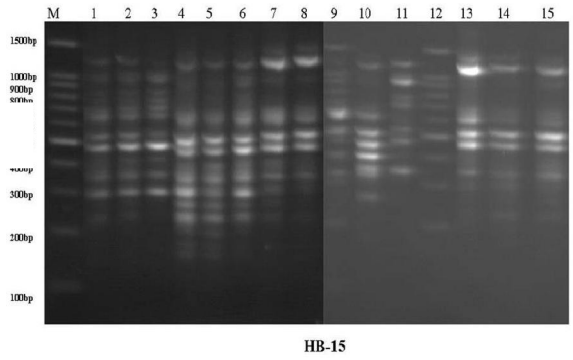


Figure 6. The ISSR profile of 5 barley genotypes produced with primer HB-15 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

4. Discussion

4.1. Yield parameters

The obtained results showed that, drought stress at 30% field capacity accompanied by the application of the lower concentration of potassium (40 mg/kg) caused significant decrease in yield parameters of barley plants of the genotypes Giza 130 and Giza 134 but caused significant increase in Giza 123, Giza 126 and Giza 133 genotypes as compared with well watered plants. These results are in harmony with many investigators who reported that wheat and other grain crops under water deficit substantially affect grain weight due to early plant senescence, cessation of grain filling and shortening of the grain filling period (Royo et al., 2000). Potassium is one of major nutrients essential for crop growth and yield development, although it is not an integral component of any cellular organelle or structural part of the plant. It is the most abundant cation in plants and is associated or involved in many of the physiological processes supporting plant growth and development. Numerous studies have shown that the application of K fertilizer mitigates the adverse effects of drought on plant growth (Sangakkara et al., 2001). In field experiments conducted in Egypt, it was found that decreases in grain yield resulting from restricted irrigation could be greatly eliminated by increasing K supply (Abd El-Hadi et al., 1997). Our results revealed that potassium application increased the grain number per spike under stress conditions. Similar results were observed by Hasina et al. (2011) who showed that application of potassium increase grain yield of wheat plants. Also, Pettigrew (2008) pointed to the positive role of adequate K supply in raising both yields and quality of various crop plants particularly under drought. Yadov (2006) had described K as the “quality element. Material transition in phloem vascular effected transition of growth stimulation material and increased cell division grains number (Tabatabaii et al., 2011). Potassium increased grain number by provide nutrients and increase the available moisture in the soil (Brar et al., 2001). Potassium has important role in water use efficiency and improves in growth plant condition and cell division and make of hydrocarbon, protein and quick transportation toward grain (Marschner, 1995). Under water-deficit conditions, K nutrition increases crop tolerance to water stress by utilizing the soil moisture more efficiently than in K-deficient plants (Waraich et al., 2011).

4.2. Anatomical Observations

Our results showed that potassium pretreatment reduced stomata number (upper), epidermis cell number and the value of stomata index in the leaves of barley grown under drought stress conditions particularly in Giza 126, Giza 123 and Giza 133

genotypes, while increased these parameters on lower surfaces. Furthermore, potassium pretreatment overcame the inhibitory effects of drought stress. In this concern, several reports indicated that potassium is a primary osmoticum in maintaining low water potential of plant tissues. Therefore, for plants growing in drought conditions, accumulating abundant K+ in their tissues may play an important role in water uptake along a soil–plant gradient. In general, K+ is accumulated in response to soil water deficits (Glenn et al., 1996). The accumulation and release of potassium by stomatal guard cells lead to changes in their turgor, resulting in stomatal opening and closing. Fusheing (2006) has revealed that lower water loss of plants well supplied with K+ is due to a reduction in transpiration which not only depends on the osmotic potential of mesophyll cells but also is controlled to a large extent by opening and closing of stomata. Stomata affect leaf resistance by way of stomatal density and stomatal activity. High stomatal density has a role in enhancing leaf conductivity mainly under well watered conditions. As stress develops, stomatal closure becomes the main controls of resistance. Stomata guard cells can sense environmental signals and they function as motor cells within the stomatal complex. Stomatal movements are controlled by the stomatal guard cells. In water stressed plants, increased abscisic acid (ABA) levels are known to stimulate the release of potassium from guard cells, giving rise to stomatal closure (Assmann & Shimazaki, 1999). Chao-Yi Lin and Der-Ming Yeh (2008) reported that the percentage of opening stomata decreased with increase in K concentration. Potassium regulates the stomatal functioning under water stress conditions and enhances photosynthetic rate (Kant & Kafkafi, 2002). Potassium application increases the plant's drought resistance through its functions in stomatal regulation, osmoregrulation, energy status, charge balance, protein synthesis and protect chloroplasts from oxidative damage (Sangakkara et al., 2000). The more K^+ requirement of plants under different abiotic stresses appears to be related to the inhibitory role of K^+ against reactive oxygen species (ROS), production during photosynthesis and NADPH oxidase (Cakmak, 2005).

4. 3. SDS-PAGE protein banding pattern

The appearance and disappearance of some protein bands means that drought stress resulted in an increase of some proteins and a decrease of others (Amini et al., 2007). The appearance of new protein bands under drought stress levels suggests that these proteins may be the cause of induction the resistance to drought in different barley genotypes (Zoro et al., 2006). One possible explanation for disappearance of some protein bands under drought stress is that the genes responsible for proteins synthesis had been completely suppresses because of stress. Therefore, the developed tissue had lost their ability to synthesis these proteins under stress. It is also possible that the genes not been completely suppressed but inhibited as the result of stress and complete recovery of the inhibition was not achieved (Amal, 2005). It seems that the most stable genotypes (Giza 123 and Giza 126) regard to inhibit or express bands. A limited number of genes were controlling the expression of protein or that gene expression is more stable under drought condition in Giza 126 (Amini et al., 2007). Other explanation, it can attribute to many mRNA may not be transcribed or that change in the protein level or enzyme activity can occur without any detectable changes in transcript (Amini et al., 2007). Therefore, our results suggested that the quantitative and qualitative changes in protein synthesis in the five barley genotypes may contribute to stress tolerant or stress injury mechanisms as compatible cytoplasm solutes in osmotic potential of the cytoplasm with the vacuoles under drought stress. The mechanisms by which drought stress may induce the appearance of some polypeptides significantly accumulated in drought-stressed plants. These polypeptides called osmotin was unique in tobacco cells because it was synthesizing and accumulated by cells undergoing gradual osmotic adjustment to desiccation stress (Amal, 2005). Wood and Goldsbrough (1997) reported that drought-induced expressions of some genes in both drought-tolerant and drought-sensitive cultivars of sorghum. Moreover, drought regulation of gene expression was observing in both drought-tolerant and drought-susceptible cultivars (Zoro et al., 2006). The soluble protein concentrations increased with the application of KNO_3 irrespective to the plant growth under stress conditions. This may be due to the direct involvement of K in several steps of translation process, including the binding of tRNA to ribosomes. The exogenous application of KNO_3 is relating to increased NO_3- absorption, its reduction and assimilation (Ruiz & Romero, 1999). Potassium is required for the major steps of protein synthesis. The expressing of the genetic code in plant cells to produce proteins and enzymes that regulate all growth processes would be impossible without adequate K. As plants are deficient in K, proteins are not synthesizing despite an abundance of available nitrogen (N). Protein was precursors for amino acids, amides and nitrate accumulate. K is likely responsible for its activation and synthesis nitrate reductase catalyzes the formation of proteins, and (Ruiz & Romero, 1999).

4.4. Molecular markers by using ISSR analysis

ISSR markers have been used to evaluate genetic variation within collections of cultivated plants (Sonante & Pignone, 2001). The polymorphisms

generated by ISSR were enough to differentiate accessions. The use of ISSR markers is obviously advantageous in differentiating closely related genotypes and has been used for cultivar identification in numerous plant species (Zhao et al., 2006). Applications of ISSR technique in gene tagging and marker assisted selections are becoming more popular. The results showed that the ISSR primers are informative markers which can be examined to correlate banding patterns and agronomic characteristics. However, this necessitates effective collaboration between plant breeders and molecular biologists to tag the gene of interest. The unique bands as produced by the primers may serve as unique identifier phenotype for drought tolerance. However, this needs to be further investigated using more number of primers. These fingerprints could be cultivar specific markers which can be exploited in planning the barley crosses and consequently it may enhance barley germplasm management and conservation. This concept has been advocated by several investigators who stated that molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant geneticists. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular marker linked to that trait (Negussie & Pretorius, 2012). Durán and Vega (2004) reported that, both RAPD and ISSR markers contribute a significant number of polymorphic markers which could be useful in identifying lentil genotypes, contributing to saturate genetic maps and in marker-assisted selection. The present study showed high genetic diversity in the studied barley genotypes. Intra-population improvement programs should, therefore, target selection of individual plants with desirable traits from these populations. On the other hand, the genetic distance between genotypes is a valuable parameter for germplasm improvement programs. Hybridization/crossing between any related genotypes is expected to yield more heterotic and vigorous plants constituting much of the different traits contained in the two parental lines. Therefore, hybridization between distantly related genotypes of the present study, like Giza 123 and Giza 126, could be an appropriate strategy for inter-population landrace improvement programs. The inheritance studies on resistance/tolerance to biotic and abiotic stresses is useful in designing appropriate breeding methodology based on the regional requirements. Molecular tagging of genes for resistance against biotic and abiotic stresses should receive first priority in order to exploit them in practical breeding programs with increased selection efficiency and better precision.

Conclusion

This study analyzed the physiological and biochemical markers associate with drought stress in five barley genotypes. Results of the study suggested that genotypes plants Giza 126, Giza123 and Giza 133 were responding best to drought stress in presence of potassium. The results also indicated a direct or indirect role for some drought-induced proteins in cellular adaptations to stress. These proteins in the five contrasting barley genotypes would aid in further understanding of the molecular detection the changes in gene expression of barley genotypes under drought stress and regulation of drought tolerance and sensitivity in plant cultivars. In conclusion, drought stress induced changes in protein synthesis. The accumulation of proteins was detecting in the drought-stressed plants of barley genotypes, which could protect plants from further dehydration damage. ISSR revealed more genotypic variations but more primers are needed for further studies, along with botanical descriptors. However, results of this study provide some ISSR molecular markers associated with barley genotypes productivity. They could be used to enhance breeding programs aimed to improve its drought tolerance by the aid of marker-assisted selection. At least, the ISSR developed from this study can consequently be used in any further study to identify stress-tolerant genotypes in barley or any other field crop.

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