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INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS AND SOME PHYSIOLOGICAL ATTRIBUTES OF BARLEY (*HORDEUM VULGARE* L.) 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. Five genotypes were grown in pot experiment with drought stress levels; 50% and 30% field capacity and potassium sulfate in two levels K1 (40) and K2 (80 mg/ kg). 30% field capacity lead to reduce yield parameters in Giza 130 and Giza 134 genotypes although using of K1 and K2, whereas both K applications increased yield parameters at 50% FC. In Giza 123, 126 and 133, applications of K1 and K2 increased yield parameters under drought stress levels. Based on the obtained results, Giza 126 genotype showed the highest and stable yield across normal and drought conditions. Potassium generally decreased stomata movements of the upper surface of the leaves in the drought stressed plants. SDS-PAGE analysis 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. Five ISSR primers were used and revealed 78% polymorphism. The primers produced 12 bands, which could be used as molecular markers in barley breeding programs.

Key words: Drought, Yield, ISSR. Proteins, Anatomical responses.

INTRODUCTION

One of the most important stresses is drought stress affecting agricultural productivity and may result in considerable yield reductions. Drought stress is more important for a biotic stress that limits plant growth and development (Foyer and Noctor, 2002). Drought stress reduces both nutrient uptakes by the roots and transport from roots to the shoots, due to restricted transpiration rates and impaired active transport and membrane permeability (Yuncai and 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 substances (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 and Noctor, 2002). Water stress tolerance is achieved by many mechanisms such as osmotic adjustment, water storage tissues, deep or fast growing roots and water conductance. Upon exposure to biotic stress conditions, plants undergo a variety of changes from physiological adaptation to gene expression (Shinozaki and 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*., 2010). Potassium (K) is essential in the maintenance of osmotic potential and water uptake and has an impact on stomata closure, which increases tolerance to water stress so that K^+ accumulates 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 subsequent crop yields (Hamza and 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 (Nguyen *et al*., 2002). 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). In addition, 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 shortages (Parsons *et al*., 2007). Cakmak (2005) reported that the improvement of K-nutritional status of plants might be important for the survival of crop plants under stress conditions, such as drought, chilling, and high light intensity. There is increasing evidence that plants suffering from stresses like drought have a larger internal requirement for K. Potassium is required for maintenance of photosynthetic CO_2 fixation particularly in plants suffering from environmental stresses (Cakmak and Engels, 1999). Potassium is an important ion in the growth of plants and in the physiology of plant water relations. The use of molecular markers assists the discrimination and selection of the closely related genotypes for future breeding and genetic engineering programs. Inter simple sequence repeats or ISSR is one of the common molecular markers used for this purpose especially in drought-tolerant crops (Zhao *et al.,* 2006; Durán and Vega, 2004).

The objective of this study was to test the effect 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 agronomic inputs (irrigation and nutrient); Discriminating the most tolerant varieties by using the ISSR markers which can be used as screening basis for drought tolerance in breeding programs.

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 30 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 (NaClO) for 3 minutes and washed for several times with sterile distilled water. Fifteen seeds were placed in 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 - 22ºC and 70% humidity). Pots were divided into five groups; the 1st group of pots was irrigated regularly with 100% hold water capacity (serve as controls). 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 sulfate (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 sulfate. 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 follows:

Anatomical Observations: The leaf specimens including the midrib were taken from the second leaf from the 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 1mm^2 unit area were counted using a light microscope with a 40 x 10 magnification lens. These counts were made both in the lower and upper surfaces of each leaf 10 times as three replicates and the averages were calculated. After a 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.

Biochemical Measurements:

Protein Preparation for SDS-PAGE: Electrophoretic protein profile of barley leaves were analyzed according to SDS-PAGE technique in which 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).

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 were assessed by NanoDrop 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 buffers, 2.5 mm MgCl2, 0.2 mm dNTP, 1.6 µM primer, and 1U Taq DNA polymerase (Sigma, USA). The PCR consisted of an initial denaturation at 94°C for 3 min, 40 cycles comprising denaturation at 94°C for 30 sec, annealing at Tm for 50sec, extension at 72°C for 1 min, and a final extension step at 72°C for 10 mins. Amplified products were separated in 2.0% agarose gel in 1x Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer by electrophoresis at 100V for 3, 5 h. Gels were stained with ethidium bromide for 20 mins and digitally photographed using Gel documentation system (Bio-Rad Corporation, USA).

Statistical Analysis: The data were statistically analyzed using an F-test and L.S.D. at 5% and 1% levels of probability according to SAS-program (Der and Everittt, 2009).

RESULTS AND DISCUSSION

Yield parameters: 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 and 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 significantly influenced 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 contributions towards grain yield of barley. The 1000-grain weight is greatly influenced by drought stress and soil nutrients. Potassium had a significant effect in 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 obtained 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 obtained from Giza 134 at 30% field capacity and application of 40 mg/kg potassium (K1).

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 a significant decrease in yield parameters of barley plants of the genotypes Giza 130 and Giza 134 but caused a 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 the 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 (Pettigrew, 2008). Our results revealed that potassium application increased the grain number per spike under stress conditions. Hasina *et al*., (2011) who showed that potassium increase grain of wheat observed similar results. In addition, Pettigrew (2008) pointed to the positive role of adequate K supply in raising both yield and quality of various crop plants particularly under drought. Yadov (2006) had described K as the “quality element. Material transition in phloem vascular effected a transition of growth stimulation material and increased cell division grains number (Tabatabaii *et al*., 2011). Potassium increased grain number by provides nutrients and increase the available moisture in the soil (Brar *et al*., 2001). Potassium has an 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 (Tabatabaii *et al*., 2011). Underwater-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).

Anatomical Observations: The findings related to effects of potassium on the stomata movements of barley genotypes are represented 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 understudy genotypes was higher in the upper than in the lower surface. Exposing the plants to drought stress levels (50 and 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 the 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 particularly under 30% field capacity.

In this concern, several reports indicated that potassium is a primary osmotic 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 stomata guard cells lead to changes in their turgid, resulting in stomata opening and closing (Fletcher *et al.*, 2007). 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 largely by opening and closing of stomata. Stomata affect leaf resistance by way of stomata density and stomata activity. High stomata density has a role in enhancing leaf conductivity mainly under well-watered conditions. As stress develops, stomata closure becomes the main controls of resistance. Stomata guard cells can sense environmental signals and they function as motor cells within the stomata complex (Fletcher *et al.*, 2007). Stomata movements are controlled by the stomata 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 stomata closure (Assmann and 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 stomata functioning under water stress conditions and enhances photosynthetic rate (Kant and Kafkafi, 2002). Potassium application increases the plant's drought resistance through its functions in stomatal regulation, osmoregrulation, and energy status, charge balance, protein synthesis and protects chloroplasts from oxidative damage (Sangakkara *et al.*, 2001). 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).

SDS-PAGE protein banding pattern: In an attempt to understand the molecular basis of drought tolerance, SDS-PAGE was used to identify protein patterns involved in drought stress response in the five barley genotypes as shown in table 3 (a & b) and figure (1). The total protein bands were 24 detected with different molecular weights ranging from 8.22 KD to 218.57 kDa, which were not necessarily being present in all genotypes. Among such bands, seven protein bands were clearly observed 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, 126 and 130 genotypes one protein band with MW 98 kDa was inhibiting and one protein band with MW 95.36 kDa was appeared under both 50 and 30% field capacities accompanying by application of K1 as compared with controls (Table 3a). Moreover, two bands with MWs (71.10 and 41.64) were not expressed under only 50% field capacity and one band was disappeared under only 30% field capacity with MWs 110.52 kDa. Furthermore, drought stress induced in all genotypes the appearance of one new protein band with molecular weight 65.79 kDa compared with the non-stressed plants. In Giza 126, one protein band with MW of 98 kDa was disappeared and six bands (95.36, 81.48, 65.79, 43.12, 31.23 & 19.44 kDa) were newly appeared under the two levels of drought stress plus K1 treatment compared with the non-stressed plants. In Giza 130, three protein bands (110.52, 98.00 & 86.71 kDa) were not expressed and three protein bands (95.36, 65.79 & 45.08 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 71.10 kDa accompanied by the appearance of two newly protein bands at MWs (106.32 and 65.79 kDa) under both levels of drought stress plus the application of K1. In Giza 134, three bands with MWs of 71.10, 48.55 & 19.44 kDa were not expressed, whereas the protein bands which having the molecular weights of 65.79, 52.70 & 45.08 were expressed under both levels of drought stress as compared with the non-stressed plants. Application of K2 to the drought stressed plants 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.

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 the disappearance of some protein bands under drought stress is that the genes responsible for protein synthesis had been completely suppressed because of stress. Therefore, the developed tissue had lost their ability to synthesise 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). Another 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 might 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 synthesized and accumulated by cells undergoing gradual osmotic adjustment to desiccation stress (Amal, 2005). Wood *et al.* (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 observed 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 the translation process, including the binding of tRNA to ribosomes. The exogenous application of KNO_3 is related to increased NO_3- absorption, its reduction and assimilation (Ruiz and 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 of amino acids, amides and nitrate accumulate. K is likely responsible for its activation and synthesis nitrate reductase catalyzes the formation of proteins, and (Ruiz and Romero, 1999).

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 with a percentage of polymorphism 78. Twelve unique bands were identified of them. Figures (2-6) and table (5) exhibited the ISSR profile produced by five primers**.** The obtained results revealed that the primer HB12 and HB15 have amplified the maximum number of bands, while the primer HB09 has amplified the least number of bands. Such results indicate that primer HB12 and HB15 repeats are more frequent in the 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. Twelve unique bands were identified among the total bands, and could be considered for 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.

ISSR markers have been used to evaluate genetic variation within collections of cultivated plants (Sonante and 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 (Joshi *et al*., 2000; Hou *et al*., 2005; Zhao *et al*., 2006). Applications of the 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 a 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. Several investigators who stated that molecular markers have several advantages over the traditional phenotype markers that were previously available to plant geneticists have advocated this concept. 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 and 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, 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 an 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.

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

Treatments	**Genotype**	**Spike length (cm)**	**Spike weight (gm)**	**Grain number / spike**	**Grain weight / spike (gm)**	**1000 grain weight (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 ($P > 0.05$).

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 ($P > 0.05$).

Table 3 (a & b). Effect of potassium applications on the protein patterns separated by SDS-PAGE of the leaves of under-study barley genotypes grown under drought stress conditions.

M. W. (kDa)	Field capacity 100% (control)					50% field capacity + K_1					30% field capacity + K_1				
	Giza 123	Giza 126	Giza 130	Giza 133	Giza 134	Giza 123	Giza 126	Giza 130	Giza 133	Giza 134	Giza 123	Giza 126	Giza 130	Giza 133	Giza 134
218.57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
132.51	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
110.52	+		+	+		+	+		+						
106.32		+	+		+		+		+	+	+	+	+	+	+
98.00	+	+	+												
95.36				+	+	+	+	+	+	+	+	+	+	+	+
90.80	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
86.71	+	+	+			+	+		+		+	+			
81.48	+			+	+		+	+	+	+	+	+		+	+
71.10		+	+	+	+							+	+		
65.79						+	+	+	+	+	+	+	+	+	+
61.07		+	+	+		+	+		+			+	+		
52.70	+	+				+	+			+	+	+		+	+
48.55	+	+	+	+	+	+	+	+	+		+		+		
46.69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45.08	+	+					+	+		+	+	+	+	+	+
43.12			+	+	+	+	+	+	+	+	+	+			
41.64	+	+									+	+	+	+	+

37.78	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31.23			+	+	+	+	+	+				+		+	+
24.67	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19.44	+			+	+	+	+					+		+	
14.67		+				+		+	+	+		+			
8.22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total No. of bands	16	17	16	16	15	18	20	15	17	15	17	21	15	16	15

M. W. (kDa)	**50% field capacity + K_2**					**30% field capacity + K_2**				
	Giza 123	**Giza 126**	**Giza 130**	**Giza 133**	**Giza 134**	**Giza 123**	**Giza 126**	**Giza 130**	**Giza 133**	**Giza 134**
235.42	+	+	+	+	+	+	+	+	+	+
198.75	+	+	+	+	+	+	+	+	+	+
175.00										+
127.22						+	+			+
122.31	+	+	+	+	+	+	+	+	+	+
114.83	+	+	+	+	+	+	+	+	+	+
110.83							+	+	+	
106.25	+	+	+		+				+	
101.38		+		+		+	+	+	+	+
94.37	+	+	+	+	+	+	+	+	+	+
87.34		+	+	+	+	+	+	+	+	
75.20	+					+	+	+	+	+
69.99	+	+	+	+	+	+	+			
56.32	+	+		+	+	+	+	+	+	
61.22	+	+	+				+		+	+
57.19	+	+	+	+	+	+	+	+	+	+
54.52		+	+	+	+	+	+	+	+	
50.51	+	+	+		+	+	+	+	+	+
46.86	+	+	+	+		+	+			
43.00	+	+		+	+	+	+	+	+	+
38.75	+	+	+	+	+	+	+	+	+	+
33.24	+	+								
28.89	+	+	+	+	+	+	+		+	+
24.71	+		+	+	+		+			+
21.18		+	+			+	+	+		
16.05				+	+	+	+	+	+	+
Total No. of bands	18	20	17	17	17	20	23	17	19	17

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′)**	**Monomorphic bands**	**Polymorphic bands**		**Total bands**	**Percent polymorphism**	**Size range (bp)**
			Shared bands	**Unique bands**			
HB09	-GTGTGTGTGTGTGC-	2	7	2	11	82	200-1145
HB11	- GTGTGTGTGTGTTGTCC-	3	9	2	14	78	177-928
HB12	- CACCACCACGC -	5	7	3	15	67	79-1443
HB13	- GAGGAGGAGGC -	2	8	2	12	83	176-886
HB15	-GTGGTGGTGGC-	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)

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

DNA marker	Molecular size (bp.)	Field capacity (100%)					Field capacity (50%) + K1					Field capacity (30%) + K1				
		Giza 123	Giza 126	Giza 130	Giza 133	Giza 134	Giza 123	Giza 126	Giza 130	Giza 133	Giza 134	Giza 123	Giza 126	Giza 130	Giza 133	Giza 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
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

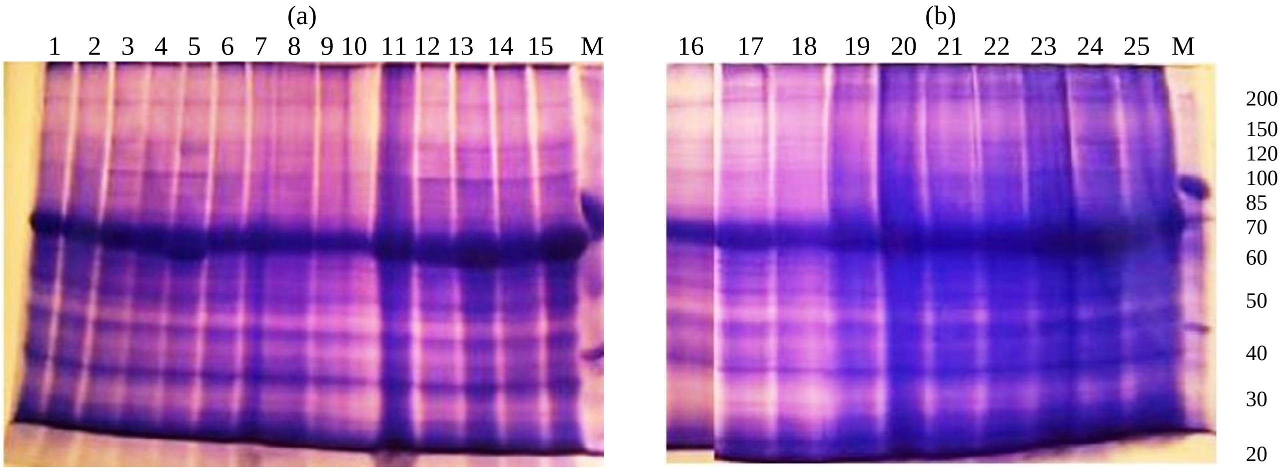

Figure 1. (a and b): Protein Profile on SDS-PAGE of the leaves of barley genotypes as affected by drought stress and potassium application. where: (a): drought stress levels + K1; and (b): drought stress levels + K2.

(a)	(b)
Lanes (1-5) = 100 % field capacity (control)	
Lanes (6-10) = 50% field capacity + K1	Lanes (16-20) = 50% field capacity + K2
Lanes (11-15) = 30% field capacity + K1	Lanes (21-25) = 30% field capacity + K2

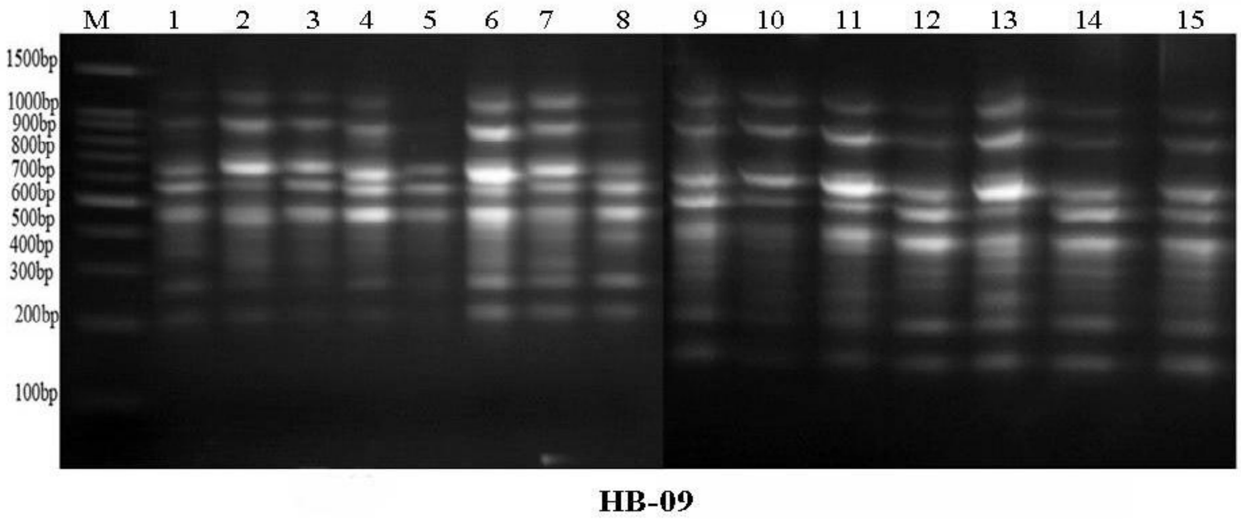

Figure 2. The ISSR profile of 5 barley genotypes produced with primer HB-09 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

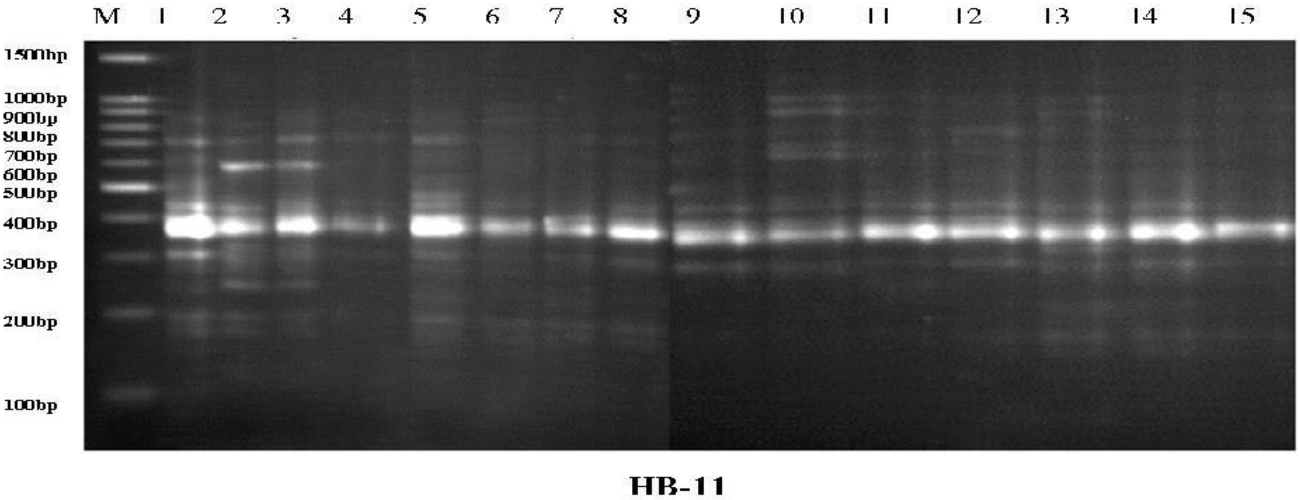

Figure 3. The ISSR profile of 5 barley genotypes produced with primer HB-11 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

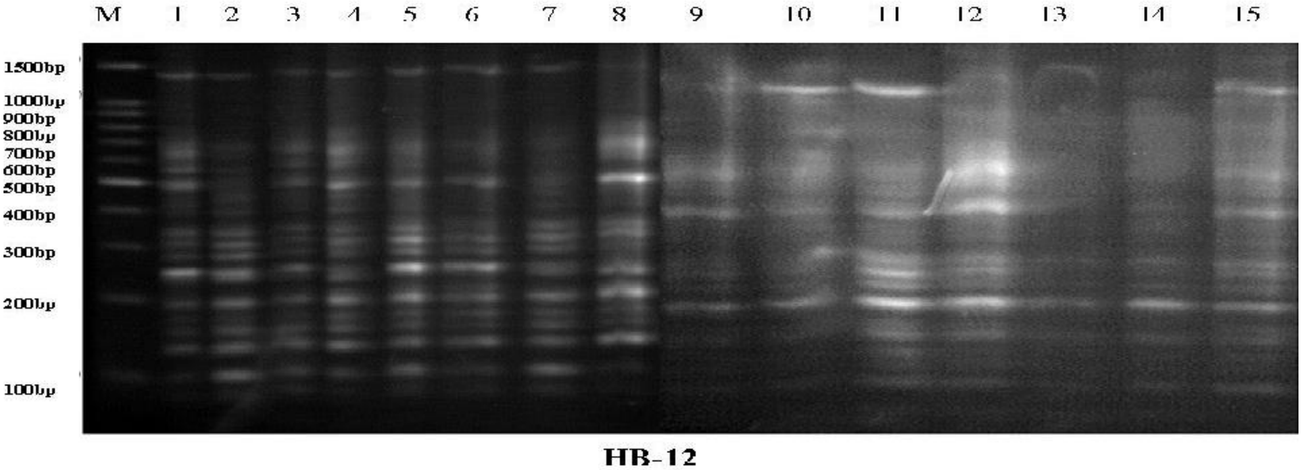

Figure 4. The ISSR profile of 5 barley genotypes produced with primer HB-12 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

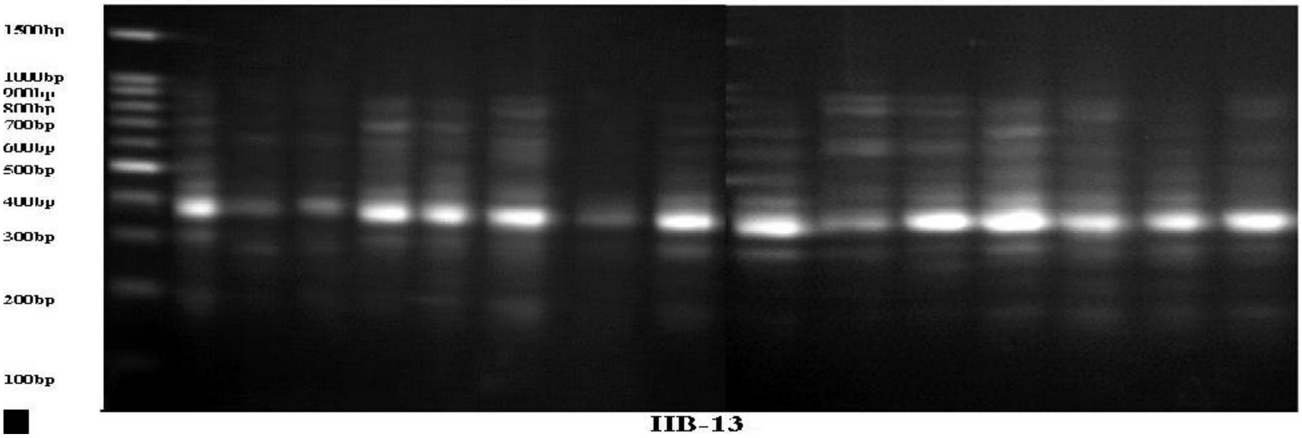

Figure 5. The ISSR profile of 5 barley genotypes produced with primer HB-13 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

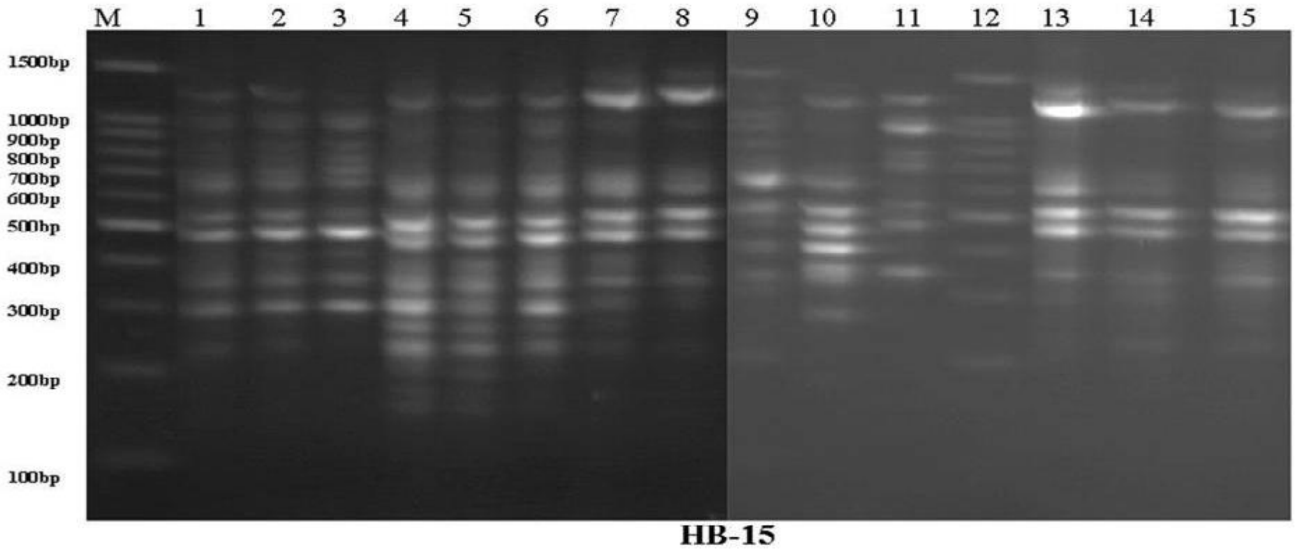

Figure 6. The ISSR profile of 5 barley genotypes produced with primer HB-15 (lane M is 1 kb DNA ladder, lanes 1 to 5 represent 100% field capacity (control), lanes 6 to 10 represent 50% field capacity + k1 and lanes 11 to 15 represent 30% field capacity + K1).

Conclusion: This study analyzed the physiological and biochemical markers associate with drought stress in five barley genotypes. The detailed data here might be the potential key to significant progress in highlighting the effects of potassium on the physiological and biochemical responses of plants subjected to drought at different levels. The results of the study suggested that genotype plants Giza 126, Giza123 and Giza 133 were responding best to drought stress in the 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 detected in the drought-stressed plants of barley genotypes, which could protect plants from further dehydration damage. ISSR revealed more genotypic variations but more primers need for further studies, along with botanical description. However, the 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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