PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOME EGYPTIAN BARLEY (*Hordeum vulgare* L.) CULTIVARS FOR SALT TOLERANCE

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Salinity stress is the most serious environmental challenge in cereal productivity in many arid and semi-arid regions (Clark and Duncan, 1993). It has reached 40% of the irrigated land and 2.1% of the globe and 25% of the irrigated land is affected by salt stress. The increase of salt-affected soils is referred to poor soil and water management in the irrigated areas, and become a great importance for agriculture production in this region (Sayar *et al*., 2010).

Barley (*Hordeum vulgare* L.) is the main crop which grown on a large scale in coastal regions such as the new reclaimed land and in soils with chemical problems (saline soil), total area of cultivated barley fluctuates from one year to another due to the rainfall amount and its distribution in Egypt. Cultivated production area in the Nile Valley decreased gradually; on the other hand, barley production area increased in the new reclaimed lands under different irrigation systems, (Walia *et al*., 2006).

Physiological and biochemical markers considered to be the most attractive ways to develop new cereals genotypes which are tolerant to salt stress (Araus *et al*., 2008). Chlorophyll content of leaf is an indicator of photosynthetic capability, light reflection from leaf was increased with increasing salt stress and chlorophyll content of leaf significantly decreased (Chaves *et al*., 2002; Schlemmer *et al*., 2005; Fotovat *et al*., 2007). Leaf area index is a major index for moderating water use and reducing injury under salt stress. Clarke *et al.* (1984) reported that the leaf area reduction is a common salt avoidance mechanism. While, Davidson and Chevalier (1987) reported that the difference in size of leaf area development between control and stress conditions was due to the reduction on leaf growth of the main shoot and primarily and secondary tillers.

Antioxidant enzymes are related to the tolerance to various abiotic stresses including salinity. In barley, the salt tolerant varieties have higher antioxidant enzyme activities than the salt sensitive varieties (Xiaoli *et al.*, 2009). To protect against oxidative stress, plant cells produce both antioxidant enzymes such as peroxidase (POX) and catalase (CAT) enzymes (Mittler, 2002). Yildiz and Terzi

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(2013) subjected two tolerant and sensitive cultivars of barely to salinity stress, significant positive correlation between increase of salinity levels were founded with increased activity of POX in tolerant than in sensitive cultivars. Moreover, proline accumulation is supposed to show adaptive roles in plant stress tolerance and used as a parameter of selection for stress tolerance. Salinity exposure was very effective in proline accumulation in leaves of many crop (Amirjani, 2010) including barley (Pirasteh-Anosheh *et al*., 2014). Thus, the proline content is a good indicator for screening salinity tolerant varieties (Bayoumi *et al*., 2008).

Identified genetic variations based on DNA polymorphism are profuse and independent of the environmental factors (Garland *et al*., 1999). Microsatellite markers are very useful for plant breeding and genetic diversity studies. In barley, more than 775 microsatellites have been studied and published (Varshney *et al*., 2007). The genetic maps based on microsatellites for all seven barley chromosomes were conducted (Saghai-Maroof *et al*., 1994; Ramsay *et al*., 2000).

Physiological and molecular markers are useful in screening different cultivars of barley for their tolerance against salt stress during breeding programs. Abdel-Hamid (2014). This investigation was carried out using large numbers of Egyptian barley cultivars, to study their salt tolerance in Egypt. The present study aimed to study relative importance of some physiological and molecular markers on 15 barley cultivars and to establish specific molecular markers associated with salt tolerance using SSRs to facilitate breeding programs for salt tolerance in barley, to increase the production in the new reclaimed lands under different irrigation systems

MATERIALS AND METHODS

***Plant materials*:** Fifteen Egyptian barley cultivars were used, namely, Giza 123, Giza 124, Giza 125, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130, Giza 131, Giza 132, Giza 133, Giza 134, Giza 135, Giza 136 and Giza 2000 which kindly provided by Sakha Barley Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt were used in field experiment.

Field Experiments: The used cultivars were grown in the field at two locations in the farm of aforementioned Institute, Sakha as a control and El-Hamrowy as a saline soil; (EC 10.2 ds/m^2, PH=8.5) condition in two seasons; 2012/2013 and 2013/2014. The fifteen cultivars were planted in a randomized complete block design with three replicates. Four yield related traits which related to salt tolerance were measured, those were; number of grains spike $^{-1}$, grain yield plant $^{-1}$ (g), total chlorophyll content and flag leaf area (cm^2) using chlorophyll meter (SPAD-502 Minolta Camera Co. ltd., Japan) at heading stage and flag leaf area which measured according to Muller (1991), using the following formula; Flag leaf area = maximum length x maximum breadth x 0.76**.**

Physiological studies

***Determination of Proline content*:** Proline content was determined according to Bates *et al.* (1973). Leaf tissue (0.5 g) was homogenized with 10 ml of 3% sulfosalicylic acid at 4°C. The extract was filtered through whatman No. 2 filter paper. 2 ml of extracted filtrate, 2ml of acid-ninhydrin and 2ml of glacial acetic acid were mixed. The mixture was incubated at 100°C for 1h. The reaction was terminated on ice and the reaction mixture was then extracted with 4ml of toluene. The absorbance at 520 nm was spectrophometrically determined with toluene as the blank.

Enzymes activity assay: Two antioxidant catalase (CAT) and peroxidase (POX) enzymes activity were determined under the investigation, which the CAT enzyme activity was determined in the homogenates by measuring the decrease in absorption at 240 nm in a 3 ml of reaction mixture containing 0.16 ml of 10% W/V H_2O_2 diluted to 100 ml with 0.067 M phosphate buffer and 0.1 ml of enzyme extract, according Sadasivam and Manickam (1999). POX activity was spectrophotometrically measured using guaiacol/H_2O_2 as substrate according Lobarzewski *et al.* (1990).

DNA Extraction and PCR Amplification for Microsatellite Markers

Genomic DNA of the 15 barley cultivars under investigation was extracted from leaves using CTAB method according Doyle and Doyle (1990). DNA concentration was measured using Nanodrop (ND-1000 Spectrophotometer). Polymerase chain reaction (PCR) amplification was prepared in volume of 25 µl using 40 ng of genomic DNA, 2 µmol dNTP., 25 mM of $MgCl_2$, 10 pmol of each primer (forward and reverse), and a 0.5 µl of 5U of *Taq* polymerase and 12 µl of 10X PCR buffer. PCR was carried out as the following program; one cycle at 95°C for 5 min., then 35 cycles was performed as follow: 1 min. at 95°C for denaturation, 45 sec. at 55°C for annealing and 30 sec. at 72°C for extension, then incubated at 72°C for 7 min. using nine microsatellite primers which were used for this study as listed in Table (1). Amplified products were separated using agarose gel electrophoresis (2%) according to Ben Naceur *et al.* (2012) in 0.5 x TBE buffer against 100 bp DNA Ladder as a size marker. Fragments were detected with ethidium bromide and documented on Gel Documentation UVITEC, UK.

Data Analysis: Data were statistically analyzed following the analysis of variance procedure (ANOVA) by using MSTAT-C according to Russel (1996). Least Significant Difference (L.S.D) test was used to compare means at 0.05 and 0.01 levels. Amplification of SSR profiles for test barley cultivars were compared with each other and DNA fragments were scored as a binary data. Each fragment was scored as present (1) or absent (0), and pairwise comparisons between individuals were made to calculate the Jukes-Cantor coefficient using PAST program (PAleontological Statistics Version 1.94b)

adapted by Hammer *et al.* (2001). Cluster analysis was performed to produce a dendrogram using unweighted pair-group method with arithmetical average (UPGMA). The polymorphism information content (PIC) was calculated according to the method of Anderson *et al.* (1993). $PIC_i = 1 - \Sigma(P_{ij})^2$, Where, n is the number of marker alleles for marker i and P_{ij} is the frequency of the j^{th} allele for marker i.

RESULTS AND DISCUSSION

The analysis of mean performance of field experiment

The results were showed in Table (2), the general mean of the four traits in tested cultivars under investigation during two seasons which revealed that the two cultivars, Giza 123, Giza 136 were the highest in number of grains $spike^{-1}$ with values of 72 and 66 followed by Giza 131 with values of 72 and 62 number grains $spike^{-1}$ in control and salt conditions, respectively. Similarly, results were observed in the aforementioned cultivars where these cultivars were the highest in grain yield $plant^{-1}$ in control and salt conditions. Regarding flag leaf area, the broadest flag leaf area was found in Giza 131 in control and stress conditions with value of 12.34 and 9.91 cm^2, respectively, followed by cultivar Giza 136 with values of 11.43 and 9.33 at control and saline conditions, similarly, the highest total chlorophyll content was found in cultivar Giza 131 with values of 53.97 and 50.05 in control and stress conditions, respectively.

The analysis of variance of field experiment

The combined data analysis in Table (3) showed a significant and highly significant differences for all effects of the interaction between locations (L) and cultivars (C) and the interaction between seasons (S) and cultivars (C) expect for No. of grains $spike^{-1}$ of cultivars showed elastic response to the change in season conditions where, the results represented insignificant in both of the interaction between season x cultivars and interaction location x cultivars, indicating that cultivars performance changed over salinity stress and seasons, grain yield of cultivars was varied, particularly under salt stress condition. This variation could be explained, in part, by the fact that traits suitable for a given environment with its own salinity level may be unsuitable in another environment the results were agreed with Bchini *et al*. (2012), Mariey (2013) and El-Akhader *et al*. (2016). In this present study, the results showed that the most of cultivars under salinity stress had lower means values more than plants under control condition for tested traits with variable decreasing ratios among the 15 cultivars under investigation. Based on the result, it was revealed that tolerant cultivars had the highest chlorophyll content, largest flag leaf area and high values of grain yield under salt stress so that, Giza

123, Giza 131, and Giza 136 could be considered as tolerant cultivars.

Analysis of physiological traits

Assessment of porline contents

Proline concentrations, in the 15 tested barley cultivars under investigation showed the highest relative increase in response to salt stress (Fig. 1) which the effect of salinity was substantially differed among the tested cultivars. Salt-tolerant cultivar, Giza 136 was the highest in porline accumulation among the other cultivars under salt stress. Proline content values in salt-tolerant cultivars under salinity were more than in non- saline cultivars. The proline accumulation may contribute to osmotic adjustment at the cellular level (Amirjani, 2010). Stress induced proline accumulation indicates a multifunctional defensive system, which in fact, this is the indicates for the plant's general response to negative environmental conditions during growth also could be, used as assist to improve their tolerance to stress conditions (Ashraf and Foolad, 2007).

Analysis of Catalase (CAT) activit

The results of activity studies in (Fig. 2) revealed that the activities of antioxidant CAT enzymes of the 15 tested barley cultivars were increased in leaves under salt stress. The responses of CAT activity in some tolerant cultivars such as Giza 136 and Giza 131 cultivars the salt tolerances were higher than its activity in salt sensitive cultivars under salinity stresses. This demonstrated that these cultivars were more ability to decompose H_2O_2 in stress conditions. These results were in agreement with (Xiaoli *et al.*, 2009). Shao *et al.* (2007**)** reported that catalase is an antioxidant enzyme that scavenges H_2O_2 in cells, therefore, the reduction of CAT activity was supposedly due to the inhibition of enzyme synthesis, change in the assembly of enzyme subunits, or protein degradation under salinity stress.

Analysis of peroxidase (POX) activity

The results in Fig. (3) showed that the highest activity of POX enzymes was observed in the tolerant cultivar and therefore, it possible to draw the conclusion that Giza 125, Giza 131 and Giza 136 cultivars possessed the highest tolerance to salinity stress among the 15 studied cultivars. Increase in POX activity under salt condition has been linked with protection from oxidative damage. Antioxidants are directly involved in the changes taking place in the plant under salt stress. From these results it possible to concluded that the CAT enzymes showed the highest rate of activity changes under salt stress in early growth period of barley seedlings in accordance with the experiments while the increases of POX activity were relatively low when compared with CAT activity in the leaves of salt stressed barley cultivars. This indicated the major role of CAT enzyme in the antioxidant defense of barley plants in salt stress conditions. These results were agreement with Khosravinejad *et al*. (2008), Dai *et al.* (2009), Unal *et al*. (2014) and Mohammad *et al*. (2015).

Analysis of Microsatellite Markers

Out of nine used primer pairs, three primers showed monomorphic fragment profiles (GBM 1459, GBM1405 and GBM 1221) which were discarded from analysis beside GBM 1464 primer had no amplification. The outstanding five primer pairs (Bmac 0032, Bmac 0040, EBmac 788, EBmac 775 and Bmag 770) generated clear fragment patterns with high polymorphism (100%) which were used to evaluate the genetic diversity of the 15 tested barley cultivars used primer pairs revealed a total of 13 alleles ranging from one to four alleles per locus with fragment sizes ranged from (120 to 290 bp). The five discriminatory primer pairs were used to evaluate the genetic diversity and association with salt tolerance in the 15 tested barley cultivars. The highest number of fragment was developed by the primers (Bmag770, 1H and Bmac0040, 6H) showed four fragment. Primer Bmag770 amplified specific allele with molecular size 260 bp found in the tolerance cultivars (Giza 123, Giza 125, Giza 128, Giza 131 and Giza 136) and was absent in other cultivars as a positive marker for salt tolerance as shown in Fig. (4). Contrary primer (Bmac 0040, 6H) was a negative marker which has a specific fragment with molecular size 216 bp found only in sensitive cultivars (Giza 124, Giza 129, Giza 132 and Giza 130) but not found in tolerance cultivars as shown in Fig. (5). Regarding primer (Bmac 0032, 1H) as shown in Fig. (6), no any fragments were generated to be related to salt tolerant but could be used for study genetic diversity in barley.

As shown in Table (1), the highly polymorphism information content (PIC) values were 0.54, 0.67 and 0.79 for primer (Bmac0040, 6H), (Bmac0032, 1H) and (Bmag0770, 1H), respectively. The primers with high value of PIC were sufficient to differentiate all of the studied cultivars. These PIC values were comparable to values obtained by Pandey *et al*. (2006). The genetic relationships among the tested 15 barley cultivars based on phylogenic trees using UPGMA method. Dendrogram (Fig. 7) showed that all cultivars clearly grouped into two major groups. The first group divided in two cluster, first cluster had only one cultivar Giza 125 and the second cluster include most of the tolerant cultivars which divided into two sub clusters, the first one consisted of the tolerant cultivars (Giza 123, Giza131 and Giza 136) and second sub-cluster includes Giza 127 and Giza 2000 which could be and show moderate tolerance. While the second group divided in two cluster the first one consisted of all the sensitive cultivars (Giza 124, Giza 130 and Giza 132) and the other cluster was divided in two sub cluster the first sub-cluster included the moderate salt tolerance cultivars in one group (Giza 128, Giza 126, Giza 134) and the other sub include the moderate sensitive (Giza 133, Giza 135 and Giza 129). The highest genetic similarity in this group as shown in Table (4) was between Giza 135 and Giza 133 with value (100%) as well as between Giza 128 and Giza 134. However, the lowest one is observed between Giza 124 and Giza 126 was (20%). Based on the results of UPGMA

cluster analysis the results was in covenant with Agro-physiological estimation, which indicated that tolerant were closely related to each other and in general, this is revealed from their response to salt stress. Our results were in good harmony with Ben Naceur *et al.* (2012), Mariey *et al.* (2013), Khatab and Mariey (2013) and El-Akhader *et al.* (2016). The polymorphic microsatellites recognized from this study could be potentially applied to identify barley microsatellite loci linked to salt stress in barley selection and breeding programme.

In conclusion, physiological traits in barley cultivars were strongly influenced under salt stress by adaptive changes. The scavenging system in salt tolerance genotypes exhibited higher proline content, CAT and POX activities, than in the salt sensitive genotypes. Thus, the salt tolerant barley cultivars Giza 123, Giza 131 and Giza 136 seems to be linked to the activities of proline content and the antioxidant enzymes. Besides, the identified polymorphic fragment could be considered as potential markers to identify salt tolerant cultivars for marker-assisted selection (MAS) in barley breeding programs.

SUMMARY

Salinity is a major abiotic stress which affecting all crops in Egypt especially in the northern part of Nile Delta. More than 30% of the total cultivated areas are irrigated by mixed or saline water. This study was amid to evaluate and clarify the adaptive response in agro-physiological and molecular aspects of 15 Egyptian available barley cultivars. The experiment was conducted during two seasons 2012/2013 and 2013/2014 in randomized complete block design with three replications under both saline and normal conditions. The results showed that Giza 123, Giza 131 and Giza 136 had the highest number of grains spike^{-1}, grain yield, flag leaf area and chlorophyll content under both normal and saline, which were considered as tolerant cultivars. Moreover, proline content, catalase and peroxidase activities were higher in these cultivars than activities in the sensitive cultivars under salt stress. Based on molecular analysis using informative SSR markers, the data represents in total 13 fragments with high polymorphism (100%) ranged from one to four fragments per locus with fragment sizes ranged from (120 to 290 bp). Bmag0770 primer amplified specific fragment in most tolerant cultivars, which was absent in susceptible cultivars with higher PIC value (0.79%). Dendrogram based on SSR marker successfully discriminated the barley cultivars for salt stress.

REFERENCES

Abdel-Hamid, A. (2014). Physiological and molecular markers for salt tolerance in four barley cultivars. Eur. Sci. J., 10: 252-272.

Amirjani, M. R. (2010). Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. American

Journal of Plant Physiology, 5: 350-360.

Anderson, J. A., G. A. Churchill, J. E. Autrique, S. D. Tanksley and M. E. Sorrells (1993). Optimizing parental selection for genetic linkage maps. Genome, 36: 181-186.

Ashraf, M. and M. R. Foolad (2007). Roles of glycinebetaine and proline in improving plant to abiotic stress tolerance. Environ. Exp. Bot., 59: 206-216.

Araus, J. L., M. P. Salfer, C. Royo and M. D. Serett (2008). Breeding for yield potential and stress adaptation in cereals. Critical Rev. Plant Sci., 27: 377-412.

Bates, I. S., R. P. Waldrn and I. D. Teare (1973). Rapid Determination of Free Proline for Water Stress. Plant Soil, 39: 205-207.

Bayoumi, T. Y., M. H. Eid and E. M. Metwali (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr. J. Biotech., 7: 2341-2352.

Bchini, R. Chaabane, M. Mosbahi, M. Ben Naceur and R. Sayar (2012). Application of salt tolerance indices for screening barley (*Hordeum Vulgare* L.) cultivars. International Journal of Current Research, 3: 8-13.

Ben Naceur, A., R. Chaabane, M. El-Faleh, Ch. Abdelly, D. Ramla, A. Nada, M. Sakr, M. Ben Naceur (2012). Genetic diversity analysis of North Africa's barley using SSR markers. Journal of Genetic Engineering and Biotechnology, 10: 13-21.

Chaves, M. M., J. S. Pereira, J. Maroco, M. L. Rodrigues, C. P. Ricardo, M. L. Osorio, J. Carvalho, T. Faria and C. Pinheiro (2002). How Plants Cope with Water Stress in the Field Photosynthesis and growth. Annals of Botany, 89: 907-916.

Clark, R. B. and R. R. Duncan (1993). Selection of plants to tolerate soil salinity, acidity and mineral deficiencies. Int. Crop Sci., 1: 371-379.

Dai, Q, C. Chen, B. Feng, T. Liu, X. Tian, Y. Gong, Y. Sun, J. Wang and S. Du (2009). Effects of different NaCl concentration on the antioxidant enzymes in oilseed rape (*Brassica napus* L.) seedlings. Plant Growth Regulation, 59: 273-278.

Davidson, D. J. and P. M. Chevalier (1987). Influence of polyethylene Glycol-induced water deficits on tiller production in spring wheat. Crop Sci., 27: 1185-1187.

Doyle, J. J. and J. L. Doyle (1990). A rapid DNA isolation procedure for

small quantities of fresh leaf tissue. Focus, 12: 13-15.

El-Akhader, A., M. Abd El-Sattar, K. Amer and T. Kumamaru (2016). Genetic diversity and association analysis among Egyptian barley (*Hordeum vulgare* L.) genotypes with different adaptations to saline conditions analyzed by SSR markers. AJCS, 10: 637-645.

Fotovat, R., M. Valizadeh and M. Toorehi (2007). Association between water-use-efficiency components and total chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.) under well-watered and drought stress conditions. J. Food. Agric. Environ., 5: 225-227.

Garland, T. Jr., P. E. Midford and A. R. Ives (1999). An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. American Zoologist, 39: 374-388.

Hammer, Ø., D. A. T. Harper and P. D. Ryan (2001). Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4: 1-9.

Khatab, I. A. and Mariey A. Samah (2013). Development of agronomical and molecular genetic markers associated with salt stress tolerance in some barley genotypes. Current Research Journal of Biological Sciences, 5: 198-204.

Khosravinejad, F., R. Heydari and T. Ferboodnia (2008). Antioxidant responses of two barley varieties to saline stress. Pakistan Journal of Biological Sciences, 11: 905-909.

Lobarzewski, J., M. Brzyska and A. Wojcik (1990). The influence of metal ions on the soluble and immobilized cytoplasmic cabbage peroxidase activity and its kinetics. J. Mol. Catal., 59: 373-383.

Mariey, A. Samah (2013). Molecular markers for salinity tolerance in some barley genotypes. PhD thesis Tanta University, Egypt.

Mariey, S. A., M. N. Mohamed, I. A. Khatab, A. N. EL-Banna, A. F. Abdel Khalek and M. E. Al-Dinary (2013). Genetic diversity analysis of some barley genotypes for salt tolerance using ssr markers. Journal of Agricultural Science, 5: 12-28.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.

Mohammad, B., V. Mostafa and M. V. Mohammad (2015). Catalase and peroxidase antioxidant enzyme activities in barley cultivars seedling under salt stress. Bull. Env. Pharmacol. Life Sci., 4: 29-35.

Muller, J. (1991). Determining leaf surface area by mean of linear measurements in wheat and triticale (brief report). Archiv Fuchtungs Frschung, 21: 121-123.

Pandey, M., C. Wagner, W. Friedt and F. Ordon (2006). Genetic relatedness and population differentiation of Himalayan hulless barley (*Hordeum vulgare* L.) landraces inferred with SSRs. Theor Appl Genet., 113: 715-729.

Pirasteh-Anosheh, H., G. Ranjbar, Y. Emam and M. Ashraf (2014). Salicylic acid–induced recovery ability in salt-stressed *Hordeum vulgare* plants. Turkish Journal of Botany, 38: 112-121.

Ramsay, L., M. Macaulay, S. Degli, K. MacLean, L. Cardle, J. Fuller, K. Edwards, S. Tuvesson, M. Morgante, A. Massari, E. Maestri, N. Marmiroli, T. Sjakste, M. Ganal, W. Powell and R. Waugh (2000). A simple sequence repeat-based linkage map of barley. Genetics, 156: 1997-2005.

Russel, D. (1996). MSTAT, Director. Crop and soil science department, Michigan state university, USA.

Sadasivam, S. and A. Manickam (1996). Biochemical Methods, New Age International Publishers (P) Ltd., New Delhi, India.

Saghai-Maroof, M., R. Biyashev, G. Yang, Q. Zhang and R. Allard (1994). Extraordinarily polymorphic microsatellite DNA in barley: species diversity, hromosomal locations, and population dynamics. Paper presented at the 91st Proceedings of the National Academy of Sciences of the United States of America, 7: 5466-5470.

Sayar, R., H. Bchini, M. Mosbahi and H. Khemira (2010). Response of durum wheat (*Triticum Durum* Desf.) growth to salt and drought stresses. Czech J. Genet. Plant Breed, 46: 54-63.

Schlemmer, M. R., D. D. Francis, J. F. Shanahan and J. S. Schepers (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agron. J., 97: 106-112.

Shao, H. B., Z. S. Liang and M. A. Shao (2007). Osmotic regulation of 10 wheat (*Triticum aestivum* L.) genotypes at soil water deficits. Colloids Surf., 47: 32-139.

Unal T. B., L. Y. Aktas and A. Guven (2014). Effects of salinity on antioxidant enzymes and proline in leaves of barley seedlings in different growth stages. Bulg. J. Agric. Sci., 20: 883-887.

Varshney, R., T. Marcel, L. Ramsay, J. Russell, M. Röder, N. Stein, R.

Waugh, P. Langridge, R. Niks and A. Graner (2007). A high density barley microsatellite consensus map with 775 SSR loci. Theor. Appl. Genet., 6: 1091-1103.

Walia, H., C. Wilson, A. Wahid, P. Condamine, X. Cui and T. J. Close (2006). Expression analysis of barley (*Hordeum vulgare* L.) during salinity stress. Functional and integrative Genomics, 6: 143-156.

Yildiz, M. and H. Terzi (2013). Effect of NaCl stress on chlorophyll biosynthesis, proline, lipid peroxidation and antioxidative enzymes in leaves of salt-tolerant and salt-sensitive barley cultivars. J. Agri. Sci., 19: 79-88.

Table (1): List of multiplexing sets of the used SSR primers, motifs, Location on chromosome, no. of alleles and polymorphism information contents (PIC).

Primer name	Sequence	motifs	Location on chromosome	No. of alleles	PIC	Polymorphism %
Bmac0032	F- CCATCAAAGTCCGGCTAG R- GTCGGGCCTCATACTGAC	(AC)7(CA)13 (AT)19	1H	3	0.67	100
Bmac0040	F- AGCCCGATCAGATTTACG R- TTCTCCCTTTGGTCCTTG	(AC)20	6H	4	0.54	100
EBmac0788	F-TAACTTACTTTATATCCATGGCA R- ATGATGAGAACTCTTCACCC	(TG)23	4H	2	0.45	100
EBmac0755	F- AGCCTTGTGTATCAGGACA R- CTGCTGGTGTTCTCTAAAAGT	(AC)16	7H	2	0.43	100
GBM1459	F- AACACATCCATACTTCCCCG R- AGCTGAATAAATGCCCATGC	(AC)7	2H	1	0,00	0
GBM1405	F- TACACGCACTGAAAAGACGG R- CTCGCTGCTGAGTTTGTCTG	(CGCA)5	3H	1	0.00	0
GBM1221	F-ACCAGCAATCCAAGTTACGG R-TGCCTTGGTCTTGGTGTGTA	(AC)10	4H	1	0.00	0
GBM1464	F- ATAGCCGTGCTCTTGCTCAT R- CAAGACCACCATTTGCATTG	(CAG)8· ·(CAG)5	7H	0	0.00	0
Bmag0770	F- AAGCTCTTTCTTGTATTCGTG R-GTCCATACTCTTTAACATCCG	(GT)13 (AG)19	1H	4	0.79	100

Table (2): Combined mean performance of the 15 barley cultivars for four traits under control and saline stress conditions in the two seasons.

Barley cultivars	No. grains spike[1]		Grain yield (g/plant^{-1})		Total chlorophyll content		Flag Leaf Area (cm^2)	
	Control	Saline	Control	Saline	Control	Saline	Control	Saline
Giza 123	72	66	1450	950	49.37	44.73	11.00	9.60
Giza 124	66	58	1066	870	46.77	46.67	9.48	7.20
Giza 125	64	48	1000	830	49.37	44.93	8.23	6.79
Giza 126	68	56	880	640	50.37	43.70	6.37	5.33
Giza 127	28	22	1055	750	44.47	39.33	5.80	4.60
Giza 128	24	22	833	520	46.83	43.47	6.72	5.05
Giza 129	66	54	750	580	43.33	35.50	6.35	5.42
Giza 130	66	54	1055	480	47.43	44.27	10.55	5.27
Giza 131	72	62	1300	930	53.97	50.03	12.34	9.91
Giza 132	62	48	650	480	46.93	37.50	8.84	7.40
Giza 133	68	54	800	580	44.30	42.40	6.46	3.80
Giza 134	66	62	820	840	47.70	43.80	8.55	3.12
Giza 135	62	60	740	520	45.57	44.30	10.68	6.85
Giza 136	72	66	1410	930	51.73	47.13	11.43	9.33
Giza 2000	72	60	1150	690	46.40	35.60	6.30	3.30
Average	66.40	55.13	0.99	0.67	48.15	43.12	9.24	6.19
C.V%	10.53	6.02	6.02	8.35	2.77	2.77	5.31	7.93
L.S.D	11.69	5.62	96.32	95.7	2.22	1.98	0.81	0.81

Table (3): Analysis of variance of four traits for 15 tested barley cultivars grown under salinity stress.

Traits	Cultivars	Salinity	season	Season x Cultivar	Location x Cultivar
No. grains spike-1	**	**	**	ns	ns
Grain yield (g/ plant^{-1})	**	**	**	*	**
Total chlorophyll content	**	**	**	**	*
Flag Leaf Area (cm^2)	**	**	**	**	**

*and **; indicate significance at 0.05 and 0.01 levels, respectively

Table (4): Similarity coefficient values among 15 barley cultivars based on band polymorphisms generated by SSRs primers.

Cultivars	G.133	G.135	G.136	G.134	G.2000	G.126	G.131	G.130	G.129	G.128	G.127	G.132	G.125	G.124
G.135	1.00													
G.136	0.20	0.20												
G.134	0.57	0.57	0.38											
G.2000	0.30	0.30	0.86	0.33										
G.126	0.50	0.50	0.50	0.83	0.44									
G.131	0.20	0.20	1.00	0.38	0.86	0.50								
G.130	0.30	0.30	0.30	0.33	0.40	0.30	0.30							
G.129	0.86	0.86	0.30	0.71	0.40	0.63	0.30	0.40						
G.128	0.57	0.57	0.38	1.00	0.33	0.83	0.38	0.33	0.71					
G.127	0.40	0.40	0.75	0.44	0.88	0.56	0.75	0.50	0.50	0.44				
G.132	0.63	0.63	0.08	0.33	0.17	0.30	0.08	0.56	0.56	0.33	0.25			
G.125	0.27	0.27	0.56	0.44	0.50	0.56	0.56	0.25	0.36	0.44	0.60	0.15		
G.124	0.50	0.50	0.09	0.22	0.18	0.20	0.09	0.44	0.44	0.22	0.17	0.86	0.08	
G.123	0.20	0.20	0.71	0.38	0.63	0.50	0.71	0.44	0.30	0.38	0.75	0.18	0.56	0.09

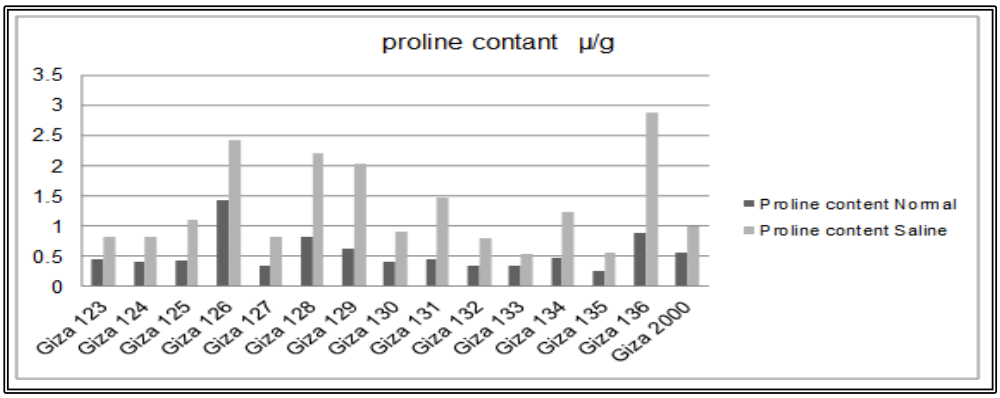

Fig. (1): Effect of salt stress on proline content for the 15 tested barley cultivars.

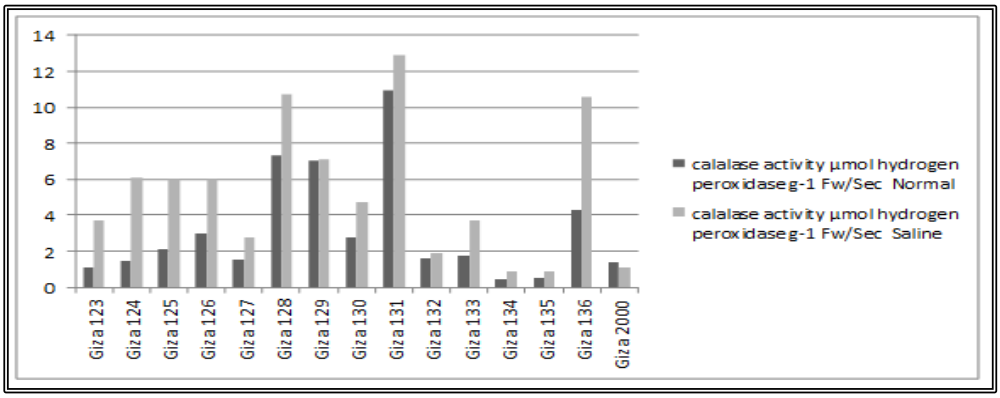

Fig. (2): Effect of salinity stress on catalase activity of 15 tested barley cultivars.

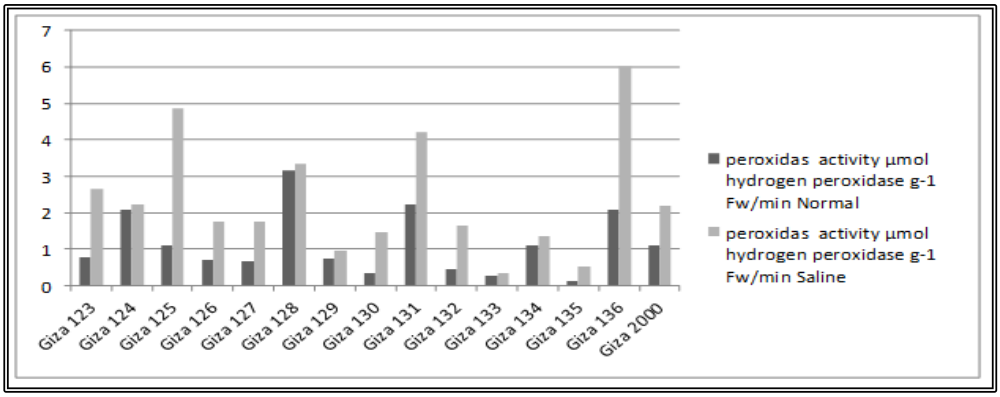

Fig. (3): Effect of salinity stress on peroxidase activity for 15 tested barley cultivars.

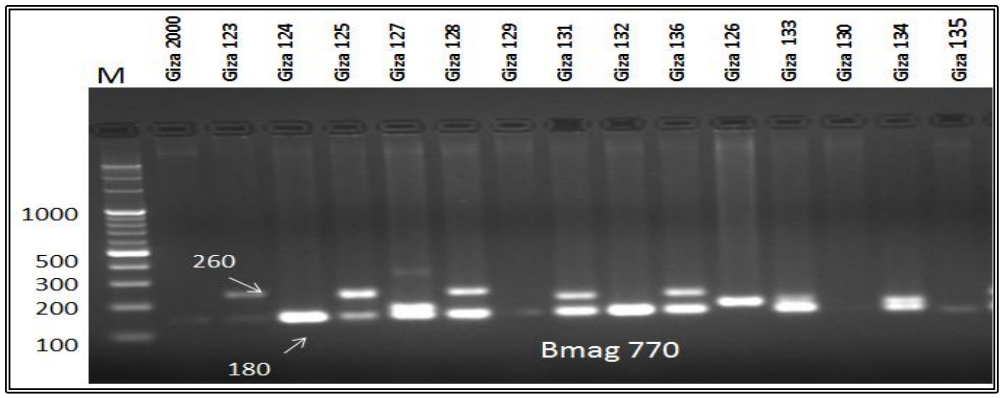

Fig. (4): Banding pattern using Bmag0770 SSR primer for 15 barley cultivars, M: Marker.

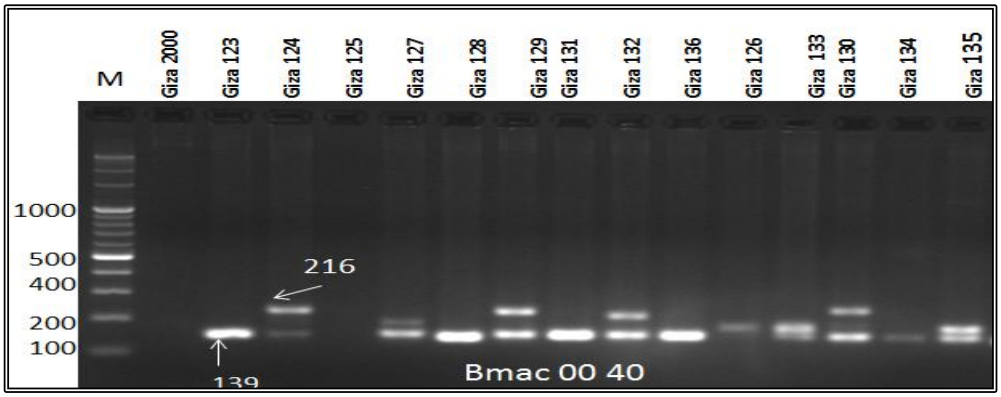

Fig. (5): Banding pattern using Bmac0040 SSR primer for 15 barley cultivars, M: Marker.

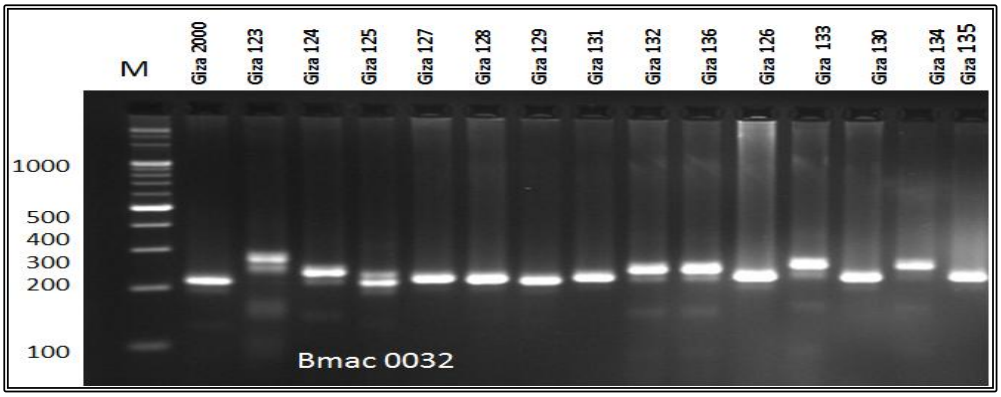

Fig. (6): Banding pattern using Bmac0032 SSR primer for 15 barley cultivars, M: Marker.

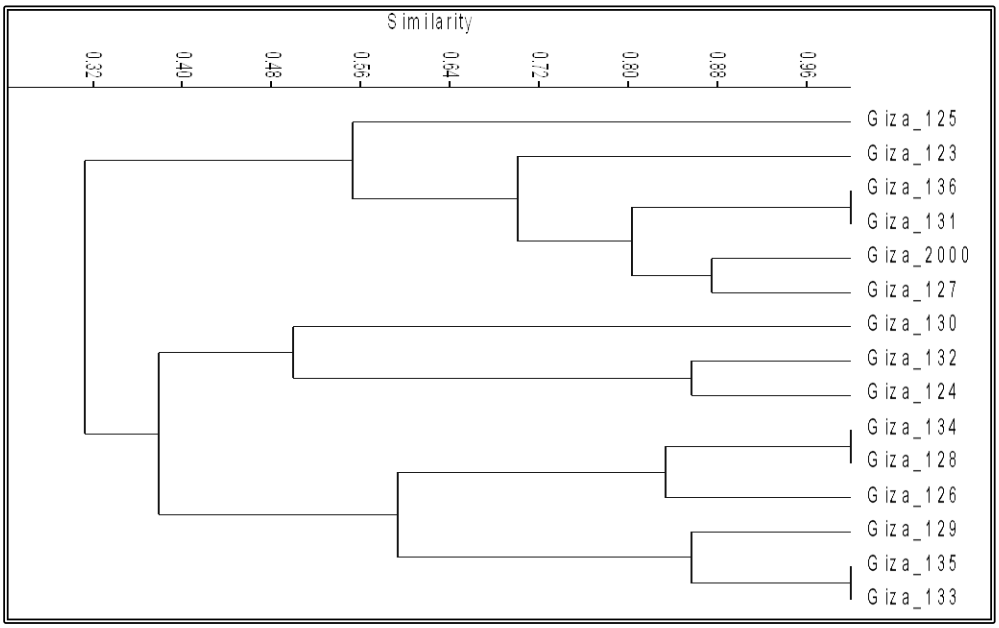

Fig. (7): Similarity dendrogram of fifteen cultivars based on band polymorphisms generated by SSR primers.