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# Influence of salt stress on molecular and biochemical changes of barley at early seedling stage

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Barley is an important food source in many parts of the world. It considered the most drought and salinity tolerant among cereals. This experiment was conducted to study the effect of salt stress on germination and seedling growth of ten barley cultivars (*Hordeum vulgare* L.). The studied barley cultivars were treated with different levels of salt (0, 25, 50, 100 and 150 mM NaCl) in sandy soil under growth chamber condition. Results indicated that increased salinity caused a significant reduction in percentage of germination and seed germination rates. Increasing salt levels caused a significant reduction in root and shoot length at the seedling stage. In this study Giza 126, 130,135, 2000 have got higher germination growth component indicating their tolerance to salinity stress in sandy soil. The intensity of the 78 kDa protein band in leaf was very low in treated samples and the 30 and 27 kDa protein bands in barley leaf were more abundant in control seedlings than in the salt-treated plants.Except the cultivar Giza 130 shows higher intensity of the 30 kDa and 27 kDa and 6 kDa protein bands.The genetic variation and relationships among different barley cultivars with different responses to salt stress were also studied by SSR markers. Detected SSR markers were polymorphic and generate cultivars- specific, some of these markers distinguished cultivars for salt and found related alleles. These markers could be verified as being genetic markers associated with salt tolerance in the studied barley cultivars and help in marker-assisted selection breeding program.

**Keywords: Barley, percentage of germination, salt stress, SDS PAGE, SSR**

## INTRODUCTION

Barley (*Hordeumvulgare* L.) is the fourth cereal crop worldwide with application in brewing and as a fodder crop for animal feeding. Barley considered as a one of the most cereals salt tolerant. Among the abiotic stresses, salinity limits barleyproduction and it is one of the major a biotic stresses (Pareek et al. 2010). Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu, 2001). Salinity is one of the most important environmental parameter that determines the success or failure of plants establishment. Consequently, the study of salt tolerance during germination and growth stages of plants is important for determining saline limits at each developmental phase (Zapata et al. 2004).

Barley is considered highly salt tolerant and can grow in the areas with elevated salt contents. (Munns and Tester 2008) stated that existence of

differences in salt tolerance not only amongst different species, but also within certain species offers an opportunity for identifying and developing salt-tolerant genotypes. (Abdel-Hamid, 2014) concluded that cultivar Giza 124 was higher in germination percentages than those for other cultivars (Giza 125, Giza 126 and Rihane 3 under salt stress condition.

Simple sequence repeat (SSR) markers are very useful for plant breeding and genetic diversity studies for several reasons. Using small amounts of sample DNA, are easy to amplify by polymerase chain reaction (PCR), are amenable to high-throughput analysis, are largely co-dominantly inherited, multi-allelic, highly informative, and abundant in plant genomes (Powell et al. 1996). Mariey et al. (2013) and Samah et al. (2013) generated clear patterns with high polymorphism using SSR primers (Bmac 0209, Bmac 0316, Scssr 03907, Bmag770, HVM67 and HVHOTRI), to evaluate the genetic diversity of salt tolerance in the some barley genotypes. Based on phylogenic trees the data from the dendrogram constructed with SSR markers showed four clusters. All the salt tolerant genotypes and some moderately salt tolerant genotypes were found in two closely related clusters, while all the sensitive genotypes and moderate ones were closely related in the other two clusters. The aim of this study was to evaluate the NaCl stress on germination, growth and biochemical of barley cultivars. And try to find any molecular markers liked to salt stress based on SSR markers.

## MATERIALS AND METHODS

This research was conducted to examine the influence of NaCl salinity on germination and growth of barley cultivars. Seeds were sown in sandy soil in germination cups (5 cm length x 10 cm diameter) wetted with different concentration of NaCl. Ten barley cultivars (Giza 123,124,125,126,127,129,130,134,135 and 2000) were used. Seeds of these cultivars were provided from barley Dept, ARC, Egypt. This experiment was carried out using a factorial completely randomized design with four replicates and five NaCl concentrations (0, 25, 50, 100 and 150 mM).

Firstly, surface of the seeds were sterilized using 5% sodium hypochlorite solution for 10 min and then the seeds were rinsed with sterile distilled water, several times. Four replicates of 10 seeds were arranged on a blotter to which 10 mlof the test solution was added. Seeds were germinated in sandy soil under growth chamber condition. Light intensities at mid-canopy were maintained at approximately 400 µmols $m^{-2}$ $s^{-1}$. A photoperiod of 16 hours light and 8 hours dark was maintained using a combination of fluorescent lights and incandescent lights. Temperatures were maintained at 23°C daytime and 18°C nighttime and were monitored using chart recorders. Relative humidity was maintained at approximately 50%.When coleoptiles and root length of a seed reached to 2 mm, the seed was scored as a germinated seed. At the end of $8^{th}$ day, the germinated seeds were collected and the stem and root of each seedling were separated to assess the morphological parameters. At this stage, germination percentage, germination rate and seed vigor index were calculated according to **ISTA** (2008).The extraction for total soluble protein and SDS PAGE was performed as described by Larkindale and Huang (2004).

### PCR Amplification for Microsatellite Markers

Genomic DNA of the ten barley cultivars under investigation was extracted from leaves using CTAB method according to Laemmli (1970) and Doyle and Doyle (1990).DNA quantity and quality was measured using Nano-drop Spectrophotometer. PCR amplification was prepared in volume of 20 µl using ~ 40- 100 ng of

**List of SSR primers sequences under salt stress**

| Primer name | Sequence |
|---|---|
| Bmag 603 | F- ATACCATGATACATCACATCG, R- GGGGGTATGTACGACTAACTA |
| Ebmac 84 | F- TTCCGTTGAGCTTTCATACAC, R- ATTGAATCCCAACAGACACAA |
| GBM 1459 | F- AACACATCCATACTTCCCCG, R- AGCTGAATAAATGCCCATGC |
| GBM 1405 | F- TACACGCACTGAAAAGACGG, R- CTCGCTGCTGAGTTTGTCTG |
| GBM 1221 | F-ACCAGCAATCCAAGTTACGG, R-TGCCTTGGTCTTGGTGTGTA |
| Bmag 770 | F- AAGCTCTTTCTTGTATTCGTG, R-GTCCATACTCTTTAACATCCG |
| Bmag 0032 | F-CCATCAAAGTCCGGCTAG, R- GGGGGTATGTACGACTAACTA |
| Bmag 649 | F-CGTCCGTCCTAGCAAAAG, R- GGGTGTACGGTAGCACTAATA |

isolated DNA, 2 µmoldNTP., 25 mM of $MgCl_2$, 5 pmol of each primer, and a 0.5 µl of *Taq* polymerase and 10 µl of 10X PCR buffer. Amplification of SSR were compared with each other and DNA bands were scored as present (1) or absent (0), using Jacared coefficient using PAST program (Pale ontological 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 data were statistically analyzed according to Gomez and Gomez (1984). The least significant differences (LSD) were used to compare differences among treatment means at 5% level.

## RESULTS AND DISCUSSION

### Germination rate and percentage

Percentage of germination was decreased in all barley cultivars (Fig. 1&2). The examined barley cultivars and NaCl concentrations on the germination rate and percentage is often mentioned to the highest and lowest numbers were got at 0 or 25 and 150 mM NaCl, respectively. Also, there is a markedly decrease with increasing NaCl concentrations under all studied barley cultivars where Giza 2000 (9.17-25 mM NaCl) and 100% (Giza 126, Giza 2000 at 100 mM NaCl) established the highest values of germinated rate and percentage, respectively.

The obtained results revealed that Giza 126, 125, 135,and 2000 scored the highest values where they ranked in descending order and the values were 6.8, 6.6, 6.2, 6.1 for germination rate and same trend was observed in case of germination percentage. The present findings clearly indicate that the salinity significantly reduced either germination rate or percentage, by causing delay in germination and by lowering the final population of germinated seeds. (Kocacaliskan, 1990) also found that the seed germination rate decrease as salinity increases and the seed germination failed at high NaCl concentration in maize. Salt stress and seeds are desiccation sensitive, which caused physiological injuries in seeds and finally leads to reduction in seed germinability. It is clear to mention that control as well as 25 mM NaCl gained the closest values and highest too. Where, 150 mM NaCl scored the lowest values in both. The percentage reduction was estimated and the numbers were 1.8, 15.9, 15.8, 66.4% and 6.0, 17.2, 8.0, 55.2 % for 25, 50, 100; 150 mM NaCl, respectively as compared with control treatment. In general this study indicates that salinity led to a significant decrease of germination rate and percentage in all studied barley cultivars. The result also pointed out clearly that the salinity led to decrease of growth parameter in both root and shoot length

### Root and shoot weight

In order to evaluate salt tolerance of barley cultivars at early seedling stage, dry weight of shoot and root were tested under the effect of NaCl salinity. Salinity stress significantly reduced shoot and root dry weight in both barley cultivars (Fig. 3). Root and shoot fresh weight was negatively affected by NaCl concentrations. Meanwhile, 0 and 150 mM NaCl gained the minimum and maximum values of fresh weight of root and shoot, respectively under all examined barley cultivars. The highest and lowest values were recorded at Giza 134 (245.7 mg/plant) for root and Giza 130 under control while, the lowest ones were observed at Giza 130 (912.2 mg/plant) for shoot. It is clear that the differences of root weight between NaCl concentrations were more than of shoot. The highest and lowest values of the dry weight of root and shoot took same trend of the fresh weight. Emam (2016) evaluated six barley cultivars (Giza 128, 127, 130, 129, 126, and 123) under hydroponic conditions for green fodder production and quality. Results concluded that barley green sprout production by using Giza 127 barely cultivar while with respect the animal nutrition needs Giza 123 and 130 considered the best option because Giza 123 and 130 produced the highest dry matter, protein contents and fiber content. Regarding the effect of investigated barley cultivars on the fresh and dry weight of root and shoot. Barley cultivars Giza 126, 129, 123 and 135 scored the highest values and Giza 123, 134, 126 and134 recorded the lowest ones, respectively. Biomass differences among plant cultivars under saline conditions are important in determining tolerance (Bagci et al. 2003). But in case of NaCl concentrations effect, data on hand revealed that increasing NaCl concentrations faced by reduction in the studied parameters, where the large and small numbers were attained at control and 150 mM NaCl, respectively. In addition, the differences between NaCl concentrations in fresh weight were more than the dry weight. Shoot dry weight of both cultivars was affected more than root dry weight by salinity and this led to higher root/shoot ratio. It is suggested that the root/shoot dry weight ratio could be correlated to differences in salt tolerance of barley cultivars.

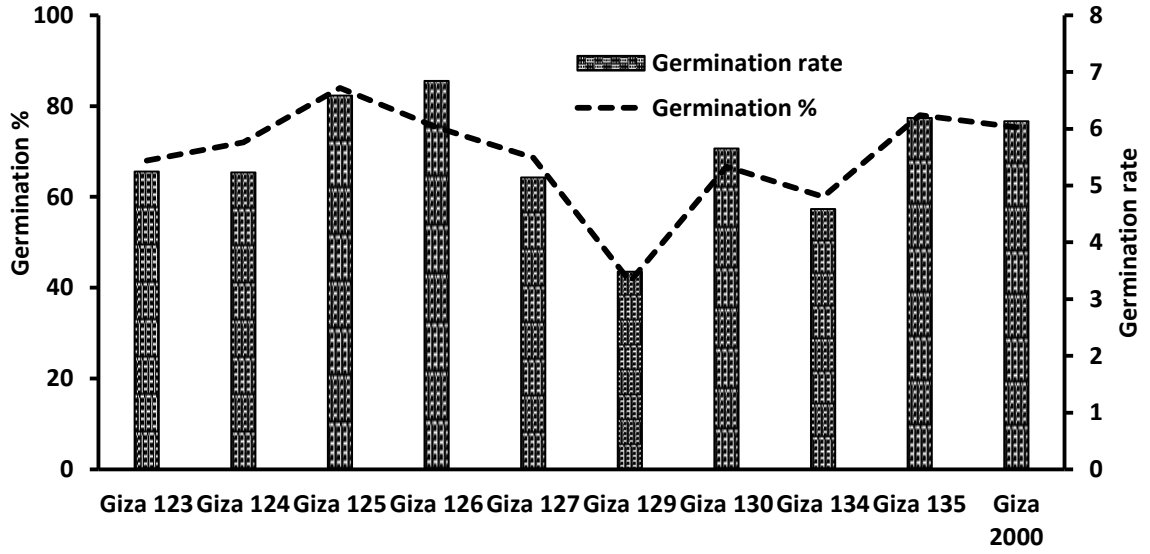


**Figure (1): Barley cultivar differences on germiation percentage and rate**

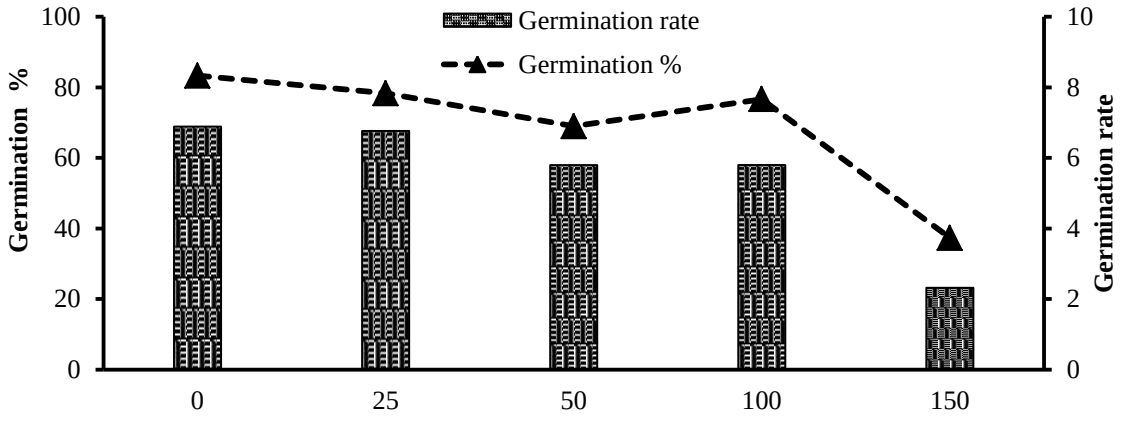


**Figure (2): Effect of NaCl concentration (mM) on germination percentage**

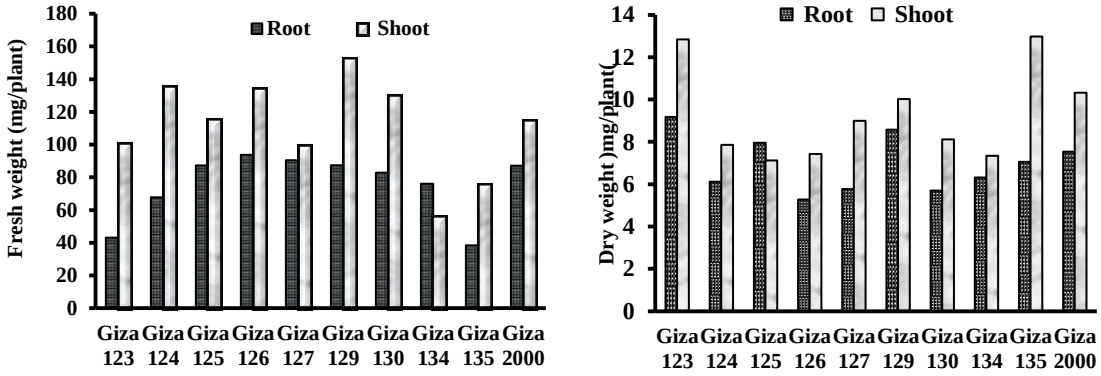


**Fig. (3): Barley cultivar differences in fresh and dry weight per plant**

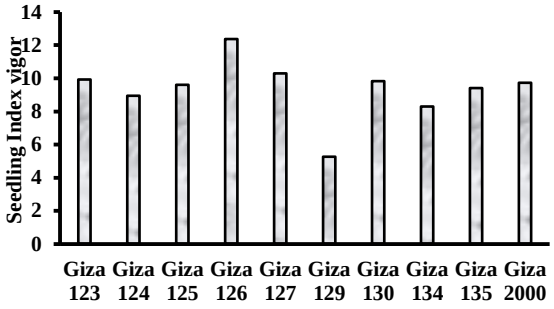


**Fig. (4): Barley cultivar differences in SVI**

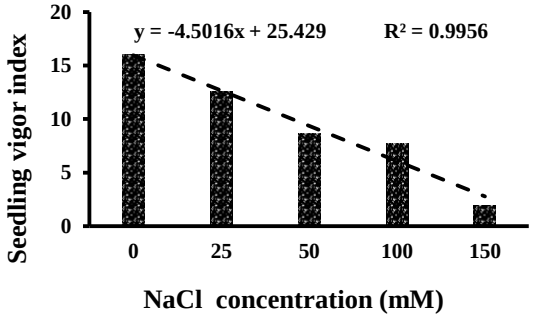


**Fig. (5): Relation between NaCl and SVI**

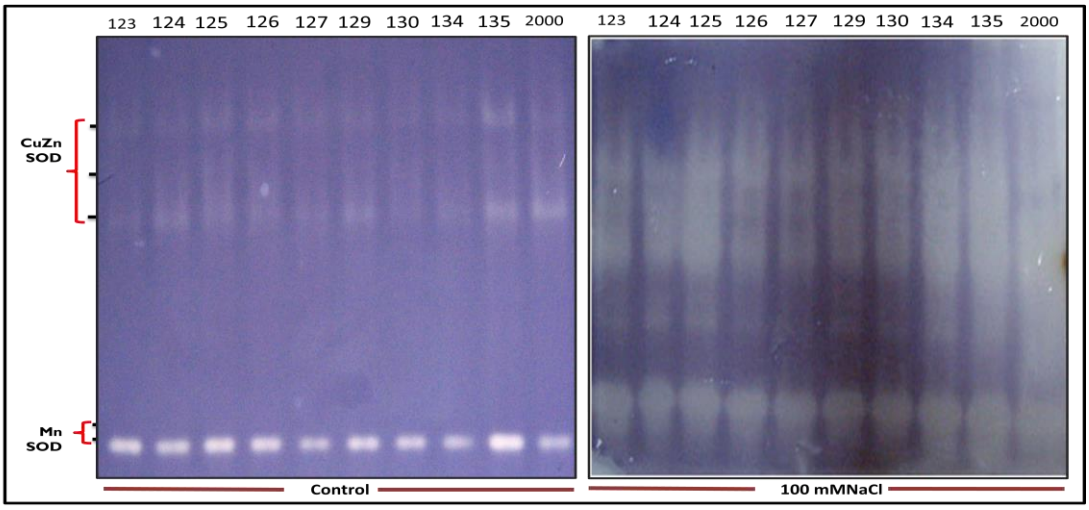


**Figure (6): Isozyme banding patterns and relative activity of SOD in leaf tissue of barley seedling cultivars, after 10 days germination under normal (Right figure) and 100 Mm NaCl (left figure).**

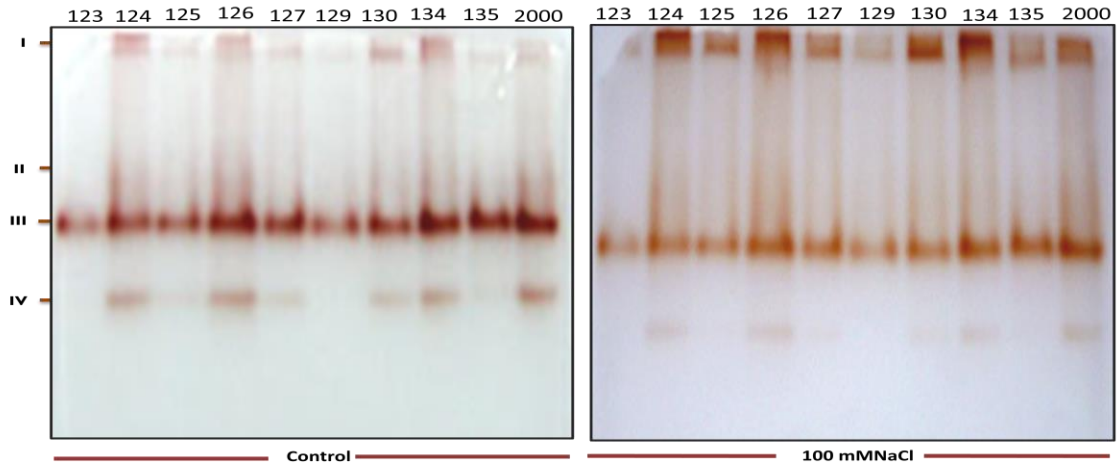


**Figure 7: Isozyme banding patterns and relative activity of POX in leaf tissue of barley seedling cultivars, after 10 days of germination under normal (Right figure) and 100 Mm NaCl (left figure).**

**Seedling vigor index (SVI)**

The effect of NaCl treatments, established highly significant effect on the seedling vigor index (SVI) under all examined barley cultivars (Fig. 4 and 5). It was observed that the highest and lowest SVI values were obtained at 25 mM NaCl under all investigated barley cultivars, where Giza 123, 126; 127 were the superior ones and they got the highest barley cultivars values and the opposite was true with Giza 127, 134; 126. Regardless NaCl effect, barley cultivars were greatly affected on SVI where the Giza 126 got the highest value (9.3) with difference more than the nearest value (Giza 127) by about 34 % and 173 % with the smallest value (Giza 129–3.4). It is clear that NaCl concentrations inhibited the SVI especially at more than 25 mM and the decrease percentage comparing control with 150 mM NaCl was 88%. In other way increase NaCl concentrations led to decrease SVI by about 17, 45, 51, 88 % comparing its treatments with control.

**Biochemical changes of barley seedlings under salt stress**

The isozyme pattern of SOD in seedling barley cultivars under salt stress showing high activity of almost all the cultivars specially the CuZn SOD isozyme comparing to control conditions. Giza 135 and Giza 2000 showed the highest activity under 100 mM NaCl treatment (Fig. 6). Giza 127 and Giza 129 their SOD activity was lowest activity comparing to the other cultivars. The Mn SOD isozyme base level was higher than CuZn SOD isozyme in normal condition then under 100 mM NaCl the CuZn SOD overrides it. It is obvious that superoxide dismutase (SOD) activity is sensitive to salt stress in barley specially in seeding stage since the stress response of plant are varied based on the developmental stage. Four peroxidase (POX) isozyme were detected in barley seedlings cultivars under NaCl treatment and POX I and POX III were major one. Isozyme pattern data did not show significant difference between barley cultivars stress response against salt stress in seedling stage. The POX I activity was higher in Giza 124, Giza 126, Giza 130, Giza 134 in leaf tissue under 100 mM NaCl while the POX III have very much same response for all barley cultivars in normal and stress condition. The isozyme pattern of POX IV and II showed the lowest activity. Still it reveals difference in stress response between barely cultivars. Giza124, Giza 126, Giza 134 and Giza 2000 were having higher activities in POX IV. The in-gel isozyme profile of POX II was not detected under NaCl treatment, but in control condition was present in cultivars Giza124, Giza 126, Giza 134 and Giza 2000 (Fig. 7).DAB stain data showed the presence and distribution of hydrogen peroxide $H_2O_2$ in leaf tissue of seedling barley cultivars under 100 mM NaCl treatment comparing to control condition. After 10 day of germination under salt stress barley cultivars have response differently to NaCl treatment based on the accumulation of $H_2O_2$ in leaf tissue in (Fig. 8).

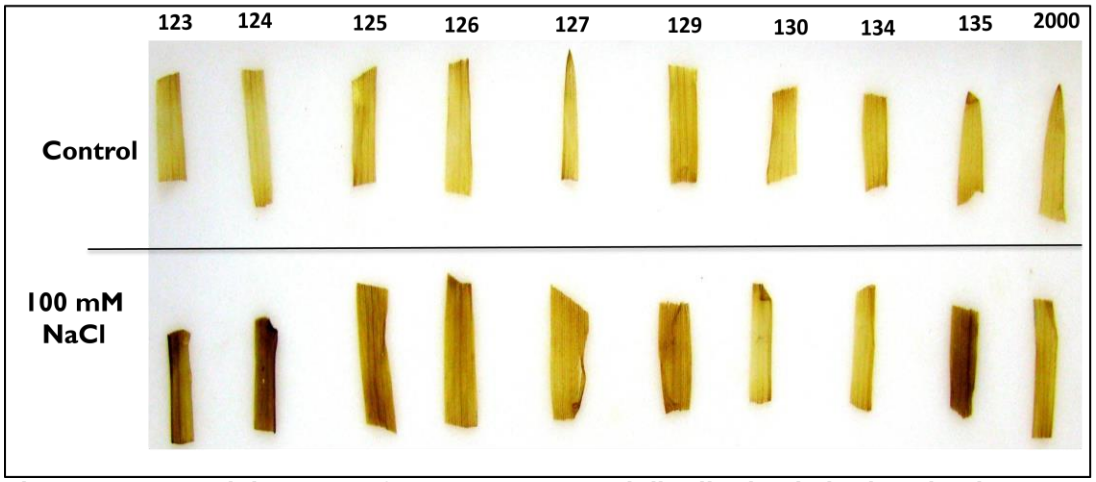


**Figure 8: DAB staining assay for $H_2O_2$present and distribution in barley plantlets Egyptian seedlings cultivars under 100 mM NaCl**

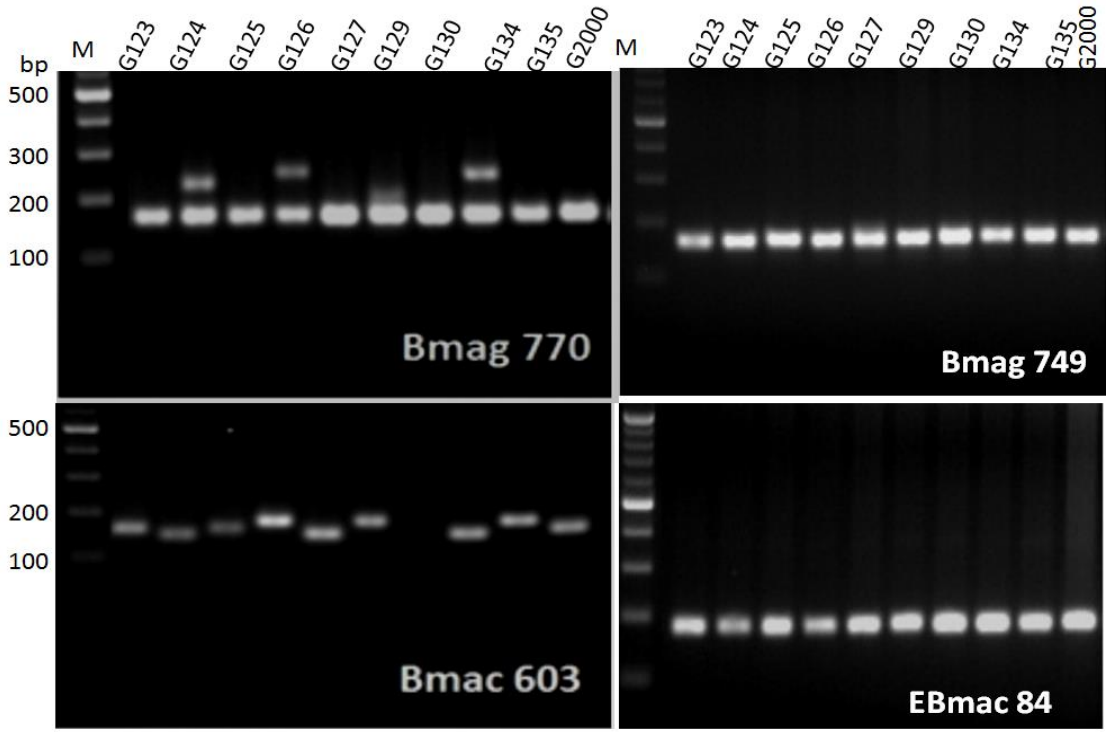


**Figure 9. Banding pattern using SSR primers for ten barley cultivars**

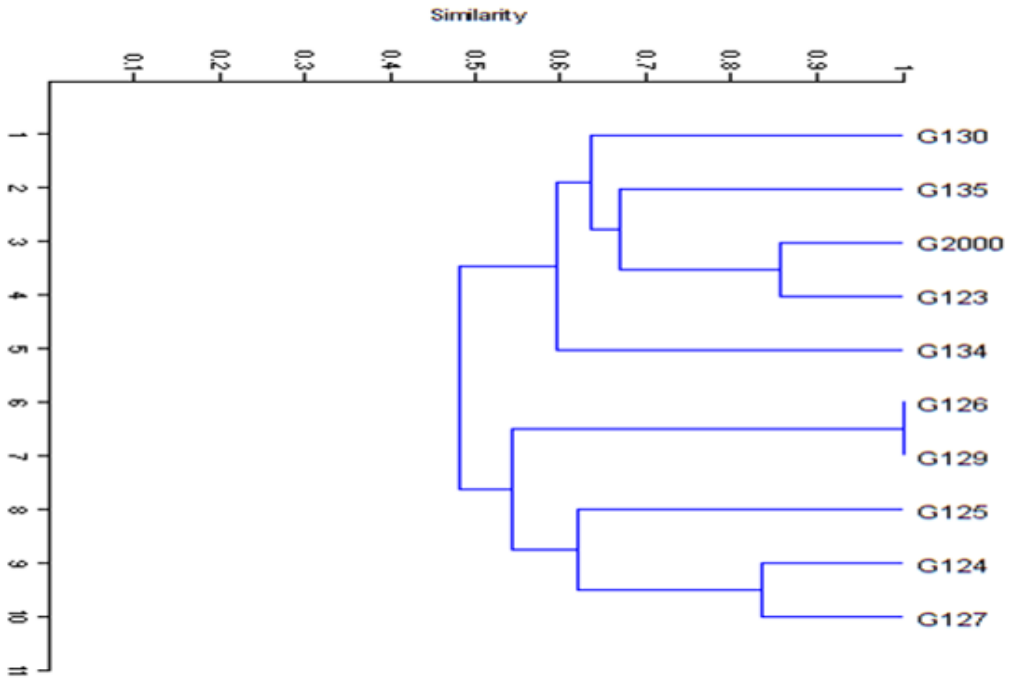


**Figure10. UPMGA polygenetic tree using Dice similarity reporting SSR-based genetic relationships among 10 barley cultivars**

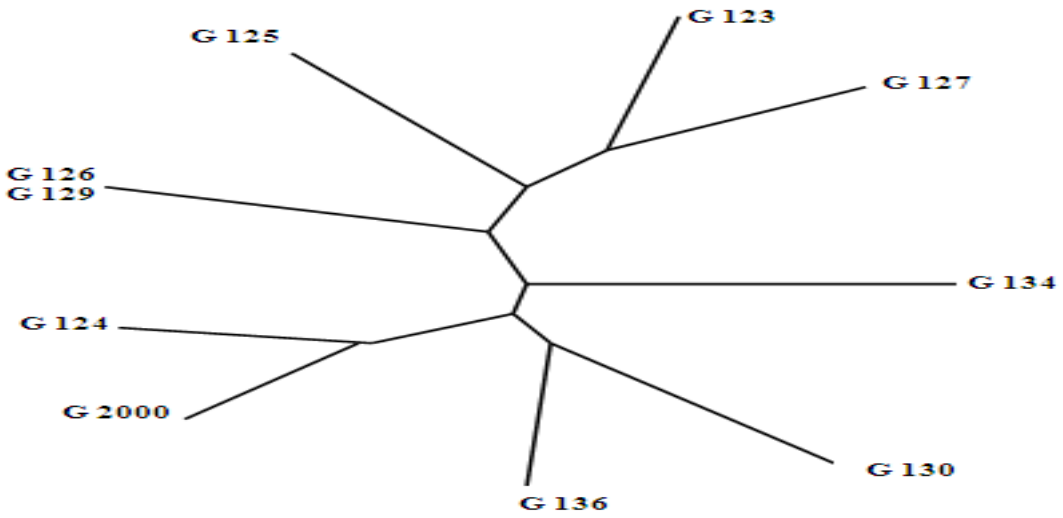


**Figure (11): Unrooted polygenetic tree reporting SSR-based genetic relationships among barley cultivars**

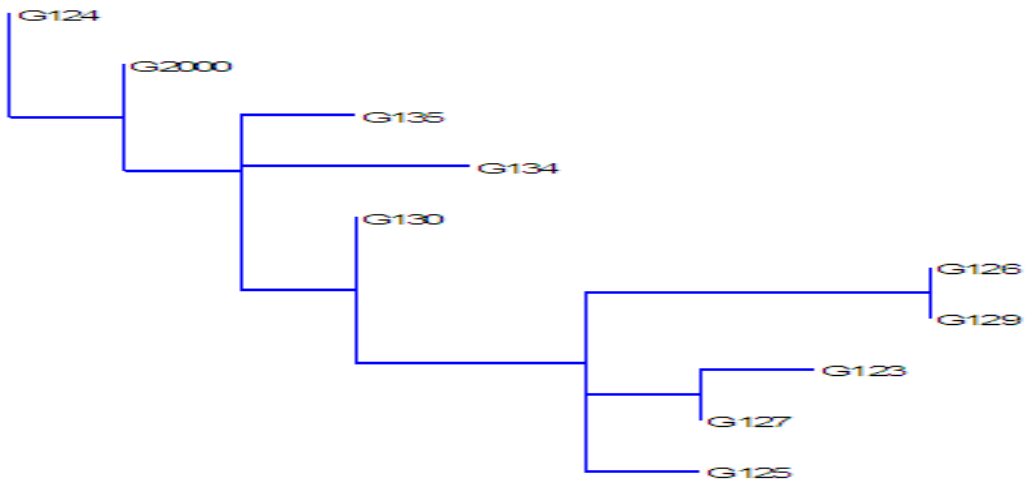


**Figure (12): Parsimony polygenetic tree reporting SSR-based genetic relationships among barley cultivars**

The cultivar Giza 123, 124, 125 and135 showed high accumulation of $H_2O_2$ and dark brown of DAB stain, which indicated the sensitive of those barley cultivars under salt stress in seedling developmental stage. In the other hand, the cultivars Giza 127, 130 and Giza 134 were having a very faint brown stain because of the low accumulation level of hydrogen peroxide.

**PCR Amplification for Microsatellite Markers**

Out of eight used primer pairs, some primers showed monomorphic fragment profiles (GBM 1459, GBM1405, GBM 1221and Bmag 749) which were discarded from analysis, also GBM1464 has no amplification. The outstanding other SSR primers (Bmac 603, EBmac 84 and Bmag 770) generated clear fragment patterns with high polymorphism (100%). N total 10 bands were generated using tested primers. Using Polygenetic tree in Fig (9) using UPMGA polygenetic tree using Dice similarity reporting SSR-based genetic relationships among 10 barley cultivars and showed by Unrooted polygenetic tree (Fig 10) were found two main clusters, cluster one include tolerant cultivar However, other cluster include most of sensitive cultivars.

And this compared with Parsimony polygenetic tree reporting SSR-based genetic relationships among 10 barley cultivars (Fig 11 and 12) all could classify the genotypes into two major groups the most tolerant based on SSR markers were G 123, G125 and G127. These more confirmed by unrooted and Parsimony trees. Moreover, in fig Plant growth responses to salinity vary with plant life cycle; critical stages sensitive to salinity are germination, seedling establishment and flowering (Ashraf and Waheed 1990; Flowers 2004). Criteria for evaluating and screening salinity tolerance in crop plants vary depending on the level and duration of salt stress and the plant developmental stage (Shannon 1985; Neumann 1997). That is why some known tolerant barley cultivars were tolerant in adult stage from previous studies and showed sensitive or visa versa (Samah et al. 2016)

## CONCLUSION

The imposed salinity led to a significant decrease in the percentage of germination and seed germination rate in all studied barley cultivars. The result also pointed out clearly that salinity led to a decrease in the growth parameter as root and shoot length. Also, our results showed that Giza 126, 130, 2000 and 135 cultivars have the highest root and shoot weight indicating their tolerance under the same condition of salt stress. Based on phylogenetic tree the cultivars under study could be classified into major groups.

## CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

## ACKNOWLEGEMENT

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## AUTHOR CONTRIBUTIONS

FH, SE and MA designed and performed the experiments and also wrote the manuscript. HE and AE performed the biochemical analysis and reviewed the manuscript. IK performed the molecular analysis, and data analysis. All authors read and approved the final version.



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