Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars

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ABSTRACT

In order to investigate the effects of drought stress on germination components of barley cultivars, a laboratory experiment was conducted in a factorial randomized complete design with four replications. The controlled experiment included ten of Egyptian barley cultivars namely; (Giza 123, 124, 125, 126, 127, 129, 130, 134, 135 and 2000) as first factor. The second factor included 4 levels of drought stress inducer by applying 0, 5, 10 and 20% of polyethylene glycol-6000 (PEG) which is equivalent to four osmotic potential levels including – 0.001, – 0.27, – 0.54 and – 1.09 MPa, respectively. The results showed that, the highest reduction was related to the drought level of 20% PEG among the barley cultivars. The best cultivars in terms of germination traits were Giza 134, Giza 127, and Giza 126 this indicate their tolerance to drought stress and Giza 130, 135, 2000 cultivars was moderately tolerance and remaining is less tolerance. The protein band 27 kDa and 78 kDa showed high intensity after stress in almost all cultivars. Those two protein bands their exciting was very clear in treated barley leaf tissue. It could be related to dehydrine and oxygen evolving enhancer protein 2 (OEE2) which involved in drought stress tolerance response. Cultivars Giza 127, 130 and 134 showed highest tolerance response under drought stress. The antioxidant enzymes PAGE pattern of Peroxidase (POX), Sodium dismutase (SOD) and Ascorbate peroxidase (APX) for Barley cultivars under drought stress revealed a high activities for Giza 126, 127, 130, 134, 136 and 2000 under – 0.5 MPa osmotic stress by PEG in most of their isoforms. Based on similarity coefficient values the highest values were 1.0 with 100% similarly between tolerant cultivars Giza 130 and Giza 127. Similarly between the susceptible cultivars 125 and Giza 129 was 60%. These data confirmed the growth parameters which we ranked as tolerant to drought stress.

1. Introduction

Water is one of the major limiting factors for the agricultural production in arid and semi-arid areas. Drought is the main environmental constraint, which often having devastating effects on crop productivity. Hence, improved tolerance to drought has been an important goal in crop improvement programs [35]. Drought tolerance is a complex trait affected with many genes and mostly conditioned by many component responses, which may interact and may be different with respect to types, intensity and duration of water deficit. Moreover, most agronomic traits are expressed differently in normal and stress conditions and are known to be affected by environmental factors. Therefore, selection based on the phenotype would be difficult for such traits Hittalmani et al. [24].

Stress tolerance in plants is a complex trait and direct selection for grain yield under stress conditions has been hampered by low heritability, polygenic control, epistasis, and high genotype by environment interactions. Determination of the molecular basis of drought tolerance would allow and facilitate the targeted breeding of cultivars adapted to stress [7].

Barley (Hordeum vulgare L.) is a grain cereal in dry land farming systems of semi-arid areas. In these areas water deficit and unsuitable distribution of rainfall decrease the germination and
establishment of barley. Barley is one of the most important cereal crops grown in many developing countries, where it is often subject to extreme drought stress that significantly affects production [12]. Barley is grown over a broader environmental range than any other cereals where unfavorable climates prevail. In such conditions the barley encounters with drought stress during seed germination and early growth stages. These stages are the most vulnerable to drought stress and presenting a challenge in barley production [30].

Duman [16] reported that dryness stress decreases seed germination percentage and the length of radicle and plumule. The creation and maintenance of a pure water potential in the environment of soil is almost a difficult job. So in this regard, establishing conditions of dryness stress using different osmotic materials to create the osmotic potential is considered as one of the best methods to study the effects of dryness stress on germination. Among these substances, due to the simulation of natural environmental conditions, polyethylene glycol has many applications and is widely used in vitro [25]. Because this compound has a high molecular weight, it cannot pass through the cell wall and therefore it is used to regulate water potential in germination tests. Khashayi et al. [31] found that negative potentials between 0.4 and −0.8 MPa are the best condition for studying germination features of different genotypes of plants under drought stress. El-Kholy et al. [18] emphasized that the highest N was recorded for barley cultivars (Giza123, 124, 125, 126, 129 130 and 2000) grown under normal conditions. Plant nutrients such as, P, K, and Na in all cultivars decreased under water stress condition. Taha et al. [46] evaluated three barley cultivars (Giza 123, Giza 124 and Giza 125) for its ability to release root exudates under iron deficient condition. Barley cultivars Giza 123 proved better dry matter, and exudates contents over Giza 124 and Giza 125. Abdel-Moneam et al. [1] indicated that drought susceptibility index (DSI) over both conditions indicated that line-2, line-7, Giza 130 and Giza 131 were tolerant for most traits, indicating the importance of these parents in this regard. So, these genotypes should be involved in breeding programs for developing new tolerant varieties to water stress [19].

El-Denary and El-Shawy [17] studied the water stress induced by PEG application on three barley (Hordeum vulgare L.) genotypes. Results showed that germination percentage, shoot length, root length and total dry mater were the most effective traits between sensitive and tolerant genotypes. Giza 126 and California Marriott were tolerant and stable under different stress levels, while the sensitive variety Giza 129 showed sharp decrease in germination percentage, shoot length and total dry mater.

In addition to other biochemical and molecular changes that follow when plants are under stress, it is very well established that the effects of various environmental stresses, including drought stress, are mediated, at least partly, by enhanced generation of reactive oxygen species (ROS) like superoxide radical (O2·−), singlet oxygen (O2(1∆g)), hydrogen peroxide (H2O2) and hydroxyl ions (•OH) [34,37]. Chloroplasts, mitochondria and peroxisomes are important intracellular generators of activated oxygen species [42,13]. The increased production of toxic oxygen derivative is a common feature of stress conditions. Plants have evolved a wide range of mechanisms to contend this problem. The capacity and activity of antioxidant defense system are important in limiting the oxidative damage and in destroying the active oxygen species that are produced in excess of those normally required for metabolism. The plant cells have evolved antioxidant defense mechanism to prevent the danger posed by these reactive oxygen species. This mechanism includes scavenging free radicals by natural antioxidants such as glutathione, ascorbate [45]. For the destruction of H2O2 several antioxidant enzymes act in synchrony. SOD, POX, GPX catalysis superoxide to hydrogen peroxide: 2O2 · + 2H+ = H2O2 + O2. Hydrogen peroxide is broken down to water by catalase [2].

Molecular markers reveal many polymorphisms at the DNA level have been shown to be a very powerful tool for cultivar characterization and found gene(s) related to specific traits. Among these, simple sequence repeats (SSR) or microsatellite were showed to be high potential for identification and estimation and found gene(s) related to specific traits of barley genotypes. More than 775 microsatellites have been used by Varshney et al. [3]. The genetic maps based on microsatellites for all seven barley chromosomes were conducted [4].

This study was conducted elucidate the effects of various polyethylene glycol (PEG) osmotic solutions on molecular and biochemical parameters and early seedling growth of Egyptian barley cultivars.

2. Material and methods

The research was conducted in the growth chambers of the Plant biotechnology department, National Research Centre, Dokki, Egypt in 2016. Growth chambers conditions are: Light intensities at mid-canopy were maintained at approximately 400 μmol m−2 s−1. A photoperiod of 16 h light and 8 h 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%.

The experiment was carried out as factorial in the form of randomized complete design with four replications. The first factor contained ten barley cultivars (Giza 123, 124, 125, 126, 127, 129, 130, 134, 135 and 2000). Barley (Hordeum vulgare L.) cultivars seeds were kindly provided by Barley Department, Agricultural Research Centre, Giza, Egypt. The second factor included four levels of drought stress created by adding polyethylene glycol-6000 (PEG) at four concentrations: 0, 5, 10 and 20%. The factors were priming with polyethylene glycol (PEG 6000) at four osmotic potential levels including −0.001, −0.27, −0.54 and −1.09 MPa. PEG was used because it has a high molecular weight, it cannot pass through the cell wall and therefore it is used to regulate water potential in germination tests. Polyethylene glycol 6000 was used to evaluate resistance to drought at germination stage and to create different levels of water potential.

To assess water stress tolerance during germination, the seeds were immersed in the solution of sodium hypochlorite 1% for 5 min and were disinfected; then, washed by distilled water three times. Petri dishes and the seeds bed (Whatman paper) were all sterilized in autoclave. Ten seeds of each variety were transferred into each sterilized glass Petri dish with a diameter of 9 cm in which the filter papers were placed. Five ml of distilled water was added to each Petri dish. Then, after 24 h 10 ml of the solution related to each treatment was added to the Petri dishes. The germinated seeds were counted until full germination. The seeds whose root length is 2 mm or more are considered as the germinated ones. In the 8th day, the germinated seeds were taken out of the Petri dishes and the stem and root were separated to assess the morphological parameters. At this stage, germination component was calculated according to ISTA [27].

Germination percentages (G%) were calculated as total number of germinated seeds by total number of seed used into 100. Germination rate (GR) was calculated as the summation of newly germinated seeds on each day divided by number of days that elapsed since onset of imbibition with seed numbers adjusted to a base of 100. The Seedling vigor index (SVI) was calculated as shoot and root length into germination percentage divided by 100. Root and shoot length, root and shoot fresh weight, root and shoot dry
weight was evaluated. Root: shoot ratio calculated as root length divided by shoot length into 100. Root and shoot dry weight was obtained after drying at 70 °C for 48 h. Tissue water content calculated as shoot fresh weight minus shoot dry weight divided by shoot fresh weight percentage.

2.1. SDS–PAGE proteins profile under drought stress

Barley cultivars under study were analyzed for protein profile, total soluble protein was done as suggested by Larkindale and Huang (2004). Protein present in the supernatant was measured by a modification of the method using crystalline bovine albumin to establish a standard curve.

SDS PAGE was performed as described by Leammli et al. (1970). Changes in proteins having isoenzymic activity of the ROS scavenging enzymes were studied using PAGE under nonreduced, non-denatured conditions at 4 °C. Native PAGE analysis was performed for various enzymes involved in the ascorbate–glutathione cycle on a gel (10%) with protein load of 50 μg in each well. Specific procedures for running and staining of gels for different enzymes are given below. Staining of gels for SOD activity. Gels were soaked in NBT (2.45 mM) for 20 min followed for different enzymes having isoenzymic activity of the ROS scavenging enzymes were studied using PAGE under nonreduced, non-denatured conditions at 4 °C. Native PAGE analysis was performed for various enzymes involved in the ascorbate–glutathione cycle on a gel (10%) with protein load of 50 μg in each well. Specific procedures for running and staining of gels for different enzymes are given below. Staining of gels for SOD activity. Gels were soaked in NBT (2.45 mM) for 20 min followed by immersion in a solution containing TEMED (28 mM), riboflavin (3 μM), and potassium phosphate (50 mM, pH 7.8) for 15 min. Illumination was discontinued after maximum contrast between the achormic zones and general blue colour was achieved. Gel was pre-run for 30 min using electrode buffer containing 2 mM Ascorbate before the samples were loaded. Gel showed dark brown bands and was photographed immediately. Staining for GR isoforms was performed.

2.2. DNA extraction and PCR amplification for microsatellite markers

Genomic DNA of the ten barley cultivars under investigation was extracted from leaves using CTAB method according Doyle and Doyle [15]. DNA concentration was measured using Nane drop. Polymerase chain reaction (PCR) amplification was prepared in volume of 25 μl using 40 ng of genomic DNA, 2 μmol dNTP, 25 mM of MgCl2, 10 pmol of each primer, and a 0.5 μl of 5U of Taq polymerase. 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 stage, 45 s. at 55 °C for annealing stage and 30 s. at 72 °C for extension stage. Reaction was incubated at 72 °C for 7 min. 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 (PAleontological Statistics Version 1.94b) adapted by Hammer et al. [23]. Cluster analysis was performed to produce a dendrogram using unweighted pair-group method with arithmetical average (UPGMA).

List of SSR primers sequences used was:

| Primer name | Sequence                                      |
|-------------|----------------------------------------------|
| Bmag 603    | F- ATACCATGATACATCATCACATGCG and             |
|             | R- GGGGATGATGATCAGAATCTGTA                 |
| Ebmac 84    | F- TCTCGATGAGCTCTTATACAC and               |
| GBM1459     | F- AACAATCCATATCTCCCCAGCACACAA            |
| GBM1405     | F- TAACGCGACTGAAAAAGCGG and               |
| GBM1221     | F- ACCAGCAATCCAGTTGCTG and                |
| Bmag770     | F- AAGCTCTTTCTTGTATCTGCTG and             |

The data were statistically analyzed according to Gomez and Gomez [22]. The least significant differences (LSD) were used to compare differences among treatment means at 5% level.

3. Results and discussion

3.1. Number of germinated seeds

Number of germinated seeds of the studied barley cultivars as affected by Polyethylene glycol (PEG) presented in Table 1 and Fig. 1. Results showed that the highly germinated seed number was recorded after 5 days in cultivar Giza 127 (5.58) followed by Giza 134 (5.14), while the lowest ones were detected for Giza 123, 124, 125; 135. However, Giza 126, 130; 2000 were intermediate. Also, after 5 days, germination speed for cultivar Giza 127 and 134 was still the superior followed by Giza 125, 126; 134 and the rest of the studied cultivars were lower than the previous one. At the end of the germination test, the most studied cultivars were improved such as Giza 125, 126, 127, 130, 135; 2000 and the highest germinated seed number was registered for Giza 134 (7.75), but the Giza 124 (3.67) was the lowest one.

Drought is one of the important tensions in reducing the growth and production of plants. It can affect many aspects of plant metabolism and growth, because this tension reduces germination rate and percentage and finally delays establishment of plantlets Prisco et al. [40]. According to the effect of the PEG % on the germinated speed, resulted data revealed that increasing PEG % associated with decrease in germination rate with decreasing percentage 24%, 49%, 81%; 25%, 46%, 77% and 19%, 42%, 45% at PEG % 5%, 10%; 20% after 3, 5; 7 days as compared with untreated ones, respectively. So it is clear that PEG% had a negative effect on the germinated seeds in early stage while this negative effect was minimized at the end of germination test.

3.2. Root and shoot length

Root and shoot length measured at the end of the germination test. Data in Table 1 noticed that the highest values were recorded at Giza 126, 127, 130; 134 followed by Giza 125, 2000 for root length. Similar trend was observed in case of the shoot length at the end of the germination test, except Giza 126, 134 cultivars which were the best followed by Giza 125, 127, 130; 2000. Whereas, the lowest values in both characters were Giza 123, 124, 129, 135 (root) and Giza 124 (shoot).

Root/ shoot ratio as affected by PEG % indicated that to similar trend for the highest value (2.03, Giza 134) followed by Giza 123 (1.93), while the lowest values was recorded for Giza 123 (1.55). This finding could be explained on the base of the cultivars tolerant to osmotic pressure that gained from PEG %. Also, data noticed that there were highly significant correlation between roots and shoot length with germination studied periods. The obtained r values of the correlations increased with increase the germination periods for both root and shoot length and the values were 0.820**, 0.829*, 0.868**, and 0.871**, 0.919**, 0.968** for the germination periods 3, 5 and 7 days, respectively.

3.3. Germination percentage and rate

Germination is one of the most critical periods in the life cycle of plants. Under water stress, low water potential is a determining factor inhibiting seed germination [47]. Table 1 and Figs. 2 and 3 illustrated the effect of the investigated barley cultivars on the germination percentage. Resulted data revealed that there were three groups could be recognized. The first one...
Table 1
Effect of PEG on germination and plant length of barley.

| Barley cultivars | PEG levels (%) | Number of germinated seeds at 3 day | Plant length (cm/plant) | Root:Shoot ratio | Germination Rate (%) |
|------------------|----------------|-------------------------------------|-------------------------|------------------|---------------------|
|                  |                | 5 day                                | 7 day                   | Root:Shoot       |                     |
| Giza 123         | 0              | 4.33                                | 5.67                    | 6.33             | 48.33               | 4.83 |
|                  | 5              | 3.33                                | 3.67                    | 5.00             | 2.83               | 56.67 |
|                  | 10             | 2.33                                | 3.33                    | 4.67             | 2.83               | 22.20 |
|                  | 20             | 0.00                                | 0.33                    | 0.67             | 0.50               | 25.00 |
| Giza 124         | 0              | 6.00                                | 8.00                    | 9.00             | 5.50               | 70.10 |
|                  | 5              | 3.00                                | 3.67                    | 4.67             | 2.83               | 43.50 |
|                  | 10             | 0.67                                | 0.67                    | 1.00             | 0.67               | 22.20 |
|                  | 20             | 0.00                                | 0.00                    | 0.00             | 0.00               | 0.00 |
| Giza 125         | 0              | 5.67                                | 8.00                    | 9.00             | 4.67               | 55.40 |
|                  | 5              | 3.67                                | 5.33                    | 7.00             | 3.00               | 47.65 |
|                  | 10             | 2.67                                | 4.00                    | 5.33             | 2.17               | 49.69 |
|                  | 20             | 2.00                                | 3.33                    | 3.67             | 2.17               | 59.52 |
| Giza 126         | 0              | 7.00                                | 9.00                    | 9.67             | 4.17               | 47.49 |
|                  | 5              | 4.33                                | 6.00                    | 7.67             | 3.67               | 60.23 |
|                  | 10             | 3.00                                | 4.00                    | 5.00             | 3.17               | 52.54 |
|                  | 20             | 2.00                                | 2.67                    | 3.67             | 2.67               | 66.87 |
| Giza 127         | 0              | 8.67                                | 10.00                   | 10.00            | 5.17               | 53.42 |
|                  | 5              | 7.67                                | 9.33                    | 9.33             | 4.50               | 56.97 |
|                  | 10             | 6.00                                | 8.00                    | 8.33             | 3.50               | 58.61 |
|                  | 20             | 0.00                                | 0.00                    | 0.00             | 0.00               | 0.00 |
| Giza 129         | 0              | 3.67                                | 5.33                    | 7.00             | 4.67               | 50.87 |
|                  | 5              | 3.33                                | 4.00                    | 5.33             | 2.33               | 58.86 |
|                  | 10             | 2.67                                | 3.67                    | 4.00             | 1.83               | 63.10 |
|                  | 20             | 0.00                                | 0.00                    | 0.00             | 0.00               | 0.00 |
| Giza 130         | 0              | 5.33                                | 6.33                    | 8.33             | 5.33               | 67.09 |
|                  | 5              | 4.67                                | 5.00                    | 7.33             | 3.33               | 40.75 |
|                  | 10             | 3.67                                | 4.33                    | 5.67             | 2.83               | 57.07 |
|                  | 20             | 2.67                                | 3.67                    | 4.67             | 2.17               | 65.08 |
| Giza 134         | 0              | 7.33                                | 8.00                    | 10.00            | 4.17               | 50.24 |
|                  | 5              | 6.67                                | 7.67                    | 9.00             | 3.50               | 49.83 |
|                  | 10             | 4.33                                | 5.33                    | 7.67             | 3.00               | 45.38 |
|                  | 20             | 2.33                                | 3.67                    | 4.33             | 2.17               | 55.29 |
| Giza 135         | 0              | 4.67                                | 6.00                    | 7.67             | 4.17               | 49.84 |
|                  | 5              | 2.67                                | 5.00                    | 6.00             | 3.17               | 86.60 |
|                  | 10             | 2.33                                | 3.67                    | 4.67             | 1.83               | 64.29 |
|                  | 20             | 0.33                                | 0.33                    | 0.67             | 1.33               | 50.00 |
| Giza 2000        | 0              | 6.33                                | 7.67                    | 8.67             | 4.50               | 47.10 |
|                  | 5              | 5.33                                | 6.00                    | 7.67             | 3.17               | 51.50 |
|                  | 10             | 2.33                                | 3.33                    | 3.33             | 2.33               | 45.37 |
|                  | 20             | 2.00                                | 2.67                    | 3.33             | 2.17               | 67.14 |

| LSD (0.05) Barley varieties (V) | 0.77 | 1.12 | 0.91 | 0.47 | 1.28 | 9.35 | 0.37 | 6.18 |
| LSD (0.05) PEG levels (T)       | 0.39 | 0.45 | 0.41 | 0.33 | 0.56 | 11.79| 0.88 | 4.93 |
| LSD (0.05) (V*T)                | 1.19 | 1.34 | 1.36 | 0.90 | 2.52 | 41.19| 1.15 | 12.71 |

Fig. 1. Germination speed of barley cultivars as affected by PEG levels.

include cultivars (Giza 134, 127) that scored germination percentage more than 69% and the second one include and Giza 125, 126; 130 that germination percentage ranged between 60% and 69% and the third group (Giza 135; 2000) that ranged between 47% and 60%, while the rest of the studied cultivars were less than 47%. This finding represented the strength of the cultivars and expresses their storage from carbohydrates. Also, it is clear to observe the narrow range between the first two groups, where the highest one was relative to the genetic features of the cultivars.
Regarding to the effect of the osmotic pressure resulted from PEG rates on the germination percentage, data on hand showed that the increase in PEG dramatically decreased germination percentage, where the increase in PEG by 5% decreased germination percentage by 19% relative to the untreated one. But increasing PEG from 5% to 10% decreased germination percentage by 9%. Whereas, increased PEG from 10% to 20% decreased germination percentage by 30%. The results affected directly on the total germination percentage. Salehi [43] reported the reduction of germination percentage and the increase of osmotic potential produced by polyethylene glycol. Kafi et al. [29] stated that as the water potential decreased, germination percentage, germination rate, root length, stem length, root dry weight, and stem dry weight decreased. The interaction effect of the PEG rates on the examined barley cultivars mentioned that PEG at 20% had a harmful effect on the germination percentage especially for barley cultivars Giza 124, 127 and Giza 123, 135 recorded the lowest germination percentage. Also, Fig. 2 showed highly correlation between germination percentage and rate except for the cultivar Giza 127.

It is worthy to mention that cultivars Giza 127, 134 were superior since the gained the highest germination percentage, followed by Giza 126 (96%), 124(90%); Giza 125 (90%) under control treatments. The most tolerant cultivars were 134 (77%) and Giza 127 (83%) and the following cultivars comes intermediate (Giza 125/53%, Giza 126/50%; Giza 123/47%) relative to the PEG 10%. Also, it could rank the reduction in germination percentage as a result of the PEG addition as follows 20% (57.7) >10% (25.0) >5% (9.5%) relative to the untreated control treatment. Germination is one of sensitive step to drought stress; trait modification related to germination is important purposes in regions which plant establishment is failed due to drought. Bayoumi et al. [10]. Germination process is controlled by environmental and hormonal factors. Light, oxygen, temperature degree and water availability plays important role among other factors [20].

3.4. Fresh and dry weight

Regarding to the fresh and dry weight of shoot and root barley cultivars as affected by PEG rates. Data presented in Table 2 showed that with each cultivars, increasing PEG led to progressively decreased in both fresh weight and dry weight of root, but it is clearly mentioned that highly fresh weight of root were recorded at Giza 124, Giza 125 were superior due to their values that exceeds 125 mg/plant, while Giza 123, Giza 134, Giza 2000 were more than 50 mg/plant. Another trend was attained in case of the fresh weight of shoot where Giza 125 was the best one and scored 146.2 mg/plant. Also, there were two cultivars (Giza 124 > Giza 2000) have got the second highest values (more than 100 mg/plant) as well as Giza 127, 123, 134 recorded fresh weight values 86.6, 72.3; 70.0 mg/plant, respectively. Similarly, Baalbaki et al. [9] reported that root and shoot weights of all wheat cultivars declined when osmotic potential was decreased, but the extent of reduction in root growth was less than that for shoots. Regardless of barley cultivars, Table 2 revealed the effect of PEG rates on the fresh weight of root and shoot. Data on hand pointed out that increasing PEG% associated with reduction in fresh weight of both root and shoot and the reduction values were 69.3, 80.9, 90.6 and 57.6, 75.2 and 85.2 at PEG % 5, 10; 205 for root and shoot fresh weight relative to the control, respectively. Also, data indicated that increase PEG level led to reduction by 7; 11% and 58, 18% for PEG at 5% and 10% relative to the untreated treatment, respectively. Jamshidi [28] investigated safflower genotypes under water stress and reported that at lower potential levels the seedlings had thinner and longer roots than the control treatment and as the stress increased up to about 1.2 MPa, the root length reduced more. Oskooei [38] reported that as the drought stress increased, the stem growth decreased (Table 3).

According to the fresh weight of root and shoot as affected by different studied barley cultivars, data in Table 2 showed that barley cultivars Giza 124, 125 were superior ones for fresh weight of root and gained the highest values as 40.6, 45.2 mg/plant,
respectively. While the lowest values recorded with barley culti-
vars Giza 129–14.0). In case of fresh weight of shoot still Giza
125 the best variety (58.3 mg/plant) followed by Giza 2000 (49.1
mg/plant) and Giza 127 (47.5 mg/plant). Whereas, the variety Giza
124, 126, 130, 134 were intermediate values and the rest examined
cultivars were the lowest, especially Giza 135 (18.5 mg/plant).

The maximum and minimum dry weight values were attained
at control treatment (distilled water) and at PEG at 20%, respec-
tively. Barley cultivar Giza 124 was superior in both root and shoot
dry weight. Regarding to the root and shoot dry weight, data in
Table 2 illustrated that within barley cultivars, PEG rate had a neg-
ative effect on the dry weight of both root and shoot. This negative

### Table 2
Effect of PEG on root and shoot weight and seedling vigor index.

| Barley cultivars | PEG levels (%) | Fresh weight (mg/plant) | Dry weight (mg/plant) | Tissue water content (%) | Seedling vigor index |
|------------------|----------------|-------------------------|-----------------------|--------------------------|----------------------|
|                  | Root Shoot Root Shoot Root Shoot | | | | |
| Giza 123         | 0 84.4 72.3 3.67 6.58 95.7 90.9 6.80 | | | | |
|                  | 5 20.4 28.7 1.07 2.21 94.7 92.3 4.55 | | | | |
|                  | 10 12.0 4.6 0.71 0.51 94.1 88.9 3.30 | | | | |
|                  | 20 0.6 1.1 0.04 0.09 31.1 30.6 0.08 | | | | |
| Giza 124         | 0 126.1 116.1 6.01 8.93 95.2 92.3 12.00 | | | | |
|                  | 5 23.6 28.7 0.98 2.61 95.8 90.9 4.36 | | | | |
|                  | 10 12.7 8.1 0.70 0.81 31.5 30.0 0.17 | | | | |
|                  | 20 0.0 0.0 0.00 0.00 0.0 0.0 0.00 | | | | |
| Giza 125         | 0 125.2 146.2 7.83 16.25 93.8 89.9 12.00 | | | | |
|                  | 5 22.2 34.8 1.23 2.48 94.4 92.9 6.53 | | | | |
|                  | 10 17.8 28.1 0.94 3.51 94.7 87.5 3.56 | | | | |
|                  | 20 16.9 24.3 1.05 2.21 93.8 90.9 2.14 | | | | |
| Giza 126         | 0 24.4 47.8 1.06 4.34 95.7 90.9 12.57 | | | | |
|                  | 5 15.6 38.9 0.78 2.99 95.0 92.3 7.67 | | | | |
|                  | 10 8.9 24.6 0.49 2.73 94.4 88.9 4.58 | | | | |
|                  | 20 8.0 22.2 0.47 1.85 94.1 91.7 2.44 | | | | |
| Giza 127         | 0 45.6 86.7 2.40 6.67 94.7 92.3 14.83 | | | | |
|                  | 5 21.8 57.3 1.36 5.21 93.8 90.9 11.67 | | | | |
|                  | 10 17.8 31.7 1.19 3.17 93.3 90.0 7.92 | | | | |
|                  | 20 12.2 14.4 0.58 1.81 31.7 29.2 0.00 | | | | |
| Giza 129         | 0 30.4 65.9 1.32 7.32 95.7 88.9 9.68 | | | | |
|                  | 5 13.3 15.0 0.70 1.07 94.7 92.9 3.38 | | | | |
|                  | 10 12.2 12.2 0.72 1.53 94.1 87.5 1.93 | | | | |
|                  | 20 0.0 0.0 0.00 0.00 0.0 0.0 0.00 | | | | |
| Giza 130         | 0 45.6 53.3 2.17 4.85 95.2 90.9 11.25 | | | | |
|                  | 5 18.9 40.9 0.79 3.08 95.8 92.3 6.60 | | | | |
|                  | 10 6.9 21.1 0.38 2.35 94.4 88.9 4.44 | | | | |
|                  | 20 4.3 8.9 0.31 0.74 61.9 61.1 2.57 | | | | |
| Giza 134         | 0 24.0 70.0 1.50 5.38 93.8 92.3 12.50 | | | | |
|                  | 5 18.2 33.4 1.01 3.04 94.4 90.9 9.45 | | | | |
|                  | 10 14.0 31.1 0.74 3.11 94.7 90.0 7.41 | | | | |
|                  | 20 6.0 11.9 0.38 1.49 93.8 87.5 2.67 | | | | |
| Giza 135         | 0 55.6 50.2 2.42 5.58 95.7 88.9 9.58 | | | | |
|                  | 5 18.5 40.0 0.79 3.08 95.8 92.3 6.60 | | | | |
|                  | 10 6.9 21.1 0.38 2.35 94.4 88.9 4.44 | | | | |
|                  | 20 4.3 8.9 0.31 0.74 61.9 61.1 2.57 | | | | |
| Giza 2000        | 0 56.7 101.1 2.98 7.78 94.7 92.3 12.28 | | | | |
|                  | 5 30.0 54.4 1.88 3.89 93.8 92.9 7.16 | | | | |
|                  | 10 11.7 28.7 0.78 2.87 62.2 60.0 1.94 | | | | |
|                  | 20 9.4 12.2 0.45 1.22 95.2 90.0 1.83 | | | | |

LSD (0.05)
Barley cultivars (V) 16.06 16.86 1.47 0.85 17.15 17.83 1.99
PEG Levels (T) 10.77 12.14 0.97 0.60 10.43 10.84 2.06
(V*T) 35.17 42.46 3.29 1.94 35.15 37.41 3.11

### Table 3
Similarity coefficient values among ten barley cultivars.

| Giza 134 | Giza 130 | Giza 127 | Giza 2000 | Giza 126 | Giza 124 | Giza 123 | Giza 135 | Giza 125 |
|----------|----------|----------|-----------|----------|----------|----------|----------|----------|
| 0.25     | 0.25     | 0.29     | 0.29      | 0.33     | 0.20     | 0.40     | 0.60     |
| 0.25     | 0.43     | 0.29     | 0.29      | 0.60     | 0.50     | 0.17     |
| 0.13     | 0.13     | 0.14     | 0.14      | 0.17     | 0.00     |
| 0.00     | 0.00     | 0.00     | 0.00      | 0.00     |
| 0.25     | 0.43     | 0.13     | 0.29      |          |
| 0.57     | 0.22     | 0.67     | 0.07      | 0.00     |
| 0.38     | 0.38     | 1.00     | 0.00      | 1.00     |
| 0.33     | 1.00     | 0.00     | 0.00      | 1.00     |
| 0.33     | 0.00     | 0.00     | 0.00      | 0.00     |
effect was observed in descending order, the following barley cultivars Giza 124 > Giza 129 > Giza 135 > Giza 125 relative to their values at PEG at 10% and 20%. Total dry matter of root and shoot, germination percentage, shoot length, root length were the most effective traits between sensitive and tolerant barley cultivars [17].

Regarding to the effect of investigated barley cultivars on dry weight of root and shoot, data in Table 2 represented that Giza 125 gained the highest value, which is doubled of the following cultivars Giza 127, Giza 2000, Giza 134 and Giza 124. Whereas, under shoot dry weight same trend observed. With respect to the effect of PEG rates on the dry weight of root and shoot, data found that PEG had a negative effect where a progressively decrease was noticed. The highest and the lowest values of dry weight of root and shoot were recorded at distilled water (control) and PEG 20%, respectively. The rate of reduction in dry weight of root and shoot were highly after 5% than other rates of PEG and the values were 67.8, 78.1, 89.4; 62.7, 70.2, 87.1 at PEG 5%, 10%, 20% for root and shoot dry weight, respectively. Akhondi [6] reported that as the stress increased, morphological traits such as root length decreased. Shahriari and Hassan [44] reported that as the levels of stress increased the length of root decreased. The decrease of elongation of stem and root (stem and root) due to drought stress could be associated with the fact that meristem cells of the root and stem are affected and the cell division and elongation process is disrupted. Water deficit conditions affect the water absorption by the cells and thus the necessary turgescence pressure for the cells enlargement decreases which accelerates the growth stopping or slowing.

3.5. Tissue water content

From Table 2 results showed that same trend was obtained regarding to the effect of interaction between barley cultivars and PEG rates on the tissue water content (root and shoot). The barley cultivars (Giza 124; Giza 129) had affected by high rate of PEG at 20%. Also, the highest values of tissue water content were recorded at distilled water followed by 5% PEG. In addition, results noticed that variability Giza 125 and Giza 2000 were the superior ones. In case of the cultivars effect on the tissue water content (root and shoot), Table 2 indicated that Giza 124, 126, 134 scored the highest values followed by Giza 130, 200, Giza 123 and Giza 127, in sequences. Regarding to the germination rates effect on the tissue water content (root and shoot), Table 2 recorded that the highest and lowest values were attained in distilled water (untreated) and PEG at 20% in both root and shoot. The rate of change resulted from PEG rates relative to the control were 0.3%, 10.7%, 43.9% and –1.4%, 12.0%, 43.7% for root and shoot, respectively. Water stress therefore appears to reduce the absorption and utilization of water to such an extent that the tolerance mechanisms employed by these plants in a drought are insufficient to maintain normal growth. Depending on decrease in shoot growth, tissue water content (TWC) gradually declined with the increasing of concentration of PEG. Relative water content (RWC) was used as a measure of drought. This index may be useful for determining the plant leaf water status. Drought induced with PEG decreased shoot water status in the present study. But seedlings of large seeds had higher relative water content than that of medium and small seeds. This result indicated that large seeds having longer root lengths had more water uptake abilities resulting in higher TWC of shoot [14].

3.6. Seedling vigor index

With respect to the seedling vigor index (SVI) as affected by both barley cultivars and PEG %, results in Table 2 and Figs. 4 and 5 showed that the highest SVI were recorded for barley cultivars Giza 2000 (12.26), Giza 134 (12.50), Giza 127 (14.83), Giza 126 (12.57), Giza 124, 123 (12.0), while the lowest values of seedling vigor index were recorded in Giza 124, 129 (0.0) and Giza 123, 135 gained the lowest values. Dryness stress decreases seed germination percentage and the length of radicle and plumule [16]. Regarding to the effect of barley cultivars on the seedling vigor index, resulted data pointed out that cultivars Giza 127, 134 scored the highest values (8.60, 8.01, respectively. While cultivars Giza 123, 129 recorded the lowest values (3.68, 3.75). In addition Giza 125, 126, 130 were in between (about 6) or (6.06, 6.82).

The rate of change in seedling vigor index relative to the increase PEG% was highly. Meanwhile, the increase in PEG combined with decrease in seedling vigor index. The rate of decrease was 41.8%, 43.1% and 68.1% at PEG 5%, 10%; 20% as compared with control. Also, it is clear to mention that the rate of the decrease relative to PEG unit (5%) were 41.8% and 2.3% for 5% and 10% PEG comparing with untreated. While increased PEG by doubled (from 10% to 20% PEG) led to decrease seedling vigor index by 25%. PEG solutions caused a growth reduction in shoots of triticale seedling; however, the root dry matter increased with rising osmotic stress (−0.45 MPa) [8]. The presence of increased concentrations of PEG during the growth of seedling inhibits the developmental traits and survival of barley seedling. Shoot length, root length, germination percentage and dry weight were always decreased by exposure to all the stress levels tested. It was clear that as the stress level increases, the seedling vigor index decrease. A similar observation was reported by Radhouane [41]. Nemat et al. [36] showed that Giza 126 was better than the other genotypes under the stress levels tested. The tested genotypes varied significantly in their reaction to PEG. However, the reduction in shoot and root length may be due to an impediment of cell division and elongation leading to kind of tuberization. This tuberization and lignification of the root system allows the plant to enter a slowed-down state, while waiting for the conditions to become favorable again [21].

3.7. SDS- PAGE profile of germinated barley cultivars under PEG treatment

The SDS–PAGE profile revealed that the soluble protein accumulation increased after 20% PEG treatment for all nine barley genotypes (Giza 123, 125, 126, 127, 130, 134 and 2000), except Giza 124 and 135 which showed decreasing of total soluble protein under PEG treatment (photo 1). It is very obvious that the upper band with molecular weight 78 KDa existed in all PEG treated samples and it was very hard to detect it in normal samples. Also there is another drought induced band with molecular weight 14 KDa at the end of the SDS–PAGE gel. The overall profile of the nine barley
genotypes is highly accumulation of soluble protein in leaf tissue after 10 days of 20% PEG.

The study the effects of drought stress on barley may change their gene expression and protein accumulation during a biotic stress. In an attempt to understand the molecular basis of barley drought tolerance, a SDS–PAGE method was used to screen proteins involved in drought stress response. It is widely known that there are numerous transient responses to environmental shock and that many of these genes are common to several types of stresses, such as cold, salinity, heat and drought stress. Some proteins were found to express at a lower level in drought stress plants. This could be due to the inhibitory effects of drought stress on transcriptional process. Another protein may induce after stress like dehydrin proteins. It seems that the initial increase in total soluble proteins during drought stress was due to the expression of new stress proteins, but the decrease was due to a severe decrease in photosynthesis. Photosynthesis is decreased in drought stress and materials for protein synthesis weren’t provided; therefore, protein synthesis dramatically reduced or even stopped. Those two bands were present in the stress and control (normal) condition which indicates that some inducible protein like dehydrin proteins was produced either in stressed or normal condition. This finding is in accordance to the research reported previously that a similar size of dehydrin protein (approximately 78 kDa) was expressed in both irrigated leaf tissues and drought-stressed tissues in P. bulbosa.

However, based on the band thickness showed by all cultivars, it was observed that dehydrin protein produced by plants under stress condition thicker than the one in the normal condition. Dehydrins are a group of plant proteins which respond to any type of stress that causes dehydration at the cellular level, such as cold and drought stress. Previously, three dehydrins of 65, 60, and 14 kDa were identified as the predominant proteins present in cold acclimated blue berry and in response to drought. Biochemical markers also are key tools in the evaluation of genetic variability in both natural populations and germplasm accessions. As example, storage protein (Hordein and glutenin) has a great intergenotypic variation, and has been used as marker in cultivar identification, genetic diversity studies, determination of phylogenic origins [11] and in covered and hulless barley [32].

3.8. ROS scavenging system of barley germinated cultivars under PEG

In gel antioxidants enzymes pattern activities of barley seedlings cultivars under drought condition. Screening of electrophoretic profile of detoxification enzymes (POX, SOD and APX) ware already studded in 10 barley cultivars shoots under two levels of osmotic stress using PEG (0 and 10%) on seedlings stage (Fig. 3). The POX activity in barley cultivars was so high under –0.5 MPa of 10% PEG specially in the lower group of peroxidase isozyme II and III comparing to the basal level of activity profile in normal conditions. G135 and G136 was showing highest activity rather than G126 and G127 for the isozyme no II. All barley seedlings of the Pox gel pattern showed low regulation of almost all POX isoforms in normal condition and up regulation under osmotic pressure specially isozymoform no III in all cultivars. The specific SOD isozyme activities were also tested in barley shoot seedlings (Fig. 3). The base activity level of Mn SOD and Cu, Zn SOD isoforms were very low in all barley cultivars. Under –0.5 MPa the Cu, Zn SOD isoform were up regulated and their bands shows high activities comparing to Mn SOD was dramatically decreased G125, G126 and G127 revealed a heights activity under 10% PEG germination treatment. The Fe SOD isoform could not be detected in barley shoot during all course of PEG experiment. Only G 2000 and 136 showed another third band of Cu SOD in osmotic stress conditions. The APX activity of Barley shoot under osmotic stress showed interesting activity response. All the three isoforms were upregulated under drought stress for all barley cultivars (G 126, 127, 136 and 20,000) specially isozymoform I and III. The activity of isozyme No. II did not change after increase the osmotic pressure (Fig. 3). All the isozyme assay of three detoxification enzymes (POX, SOD and APX) by PAGE technique confirmed the previous data.
3.9 DNA extraction and PCR amplification for microsatellite markers

Out of six used primer pairs, three primers showed monomorphic fragment profiles (GBM 1459, GBM1405 and GBM 1221) which were discarded from analysis. The outstanding three primer pairs (Bmac603, Ebmac 84, and Bmag 770) generated clear fragment patterns with high polymorphism (100%) (photo 4).

Based on used SSR Primers (Bmag 603, Ebmac 84, GBM1459, GBM1405, GBM1221 and Bmag770) results gave bands ranged between (110 to 220 bp) in Ebmac 84 and Bmag770, respectively with two alleles using Bmag 770 to three alleles using Bmag 84. Some of these bands found only in same tolerant cultivars using specific primers like 770. Band with size 220 found only on Giza 124, 126 and Giza 134. Similarly some of these SSR markers were used by Mariedy et al. [33] and Khatab and Mariedy [5] for salt stress for Egyptian barley genotypes. Among different types of molecular markers available for barley, microsatellite or simple sequence repeats (SSRs) have proven to be the markers of choice for marker-assisted selection (MAS) in breeding and genetic diversity studies. The value of microsatellite markers for both genetic diversity studies and for barley breeding was demonstrated [26].

Using Polygenetic tree in Fig. 6 there were found main clusters, cluster one include only cultivar Giza 135. However, cluster 2 and 3 include most of tolerant cultivars (Giza 126, 134, 2000, 130, 127) which classified as tolerant cultivars and last cluster consist susceptible cultivar (Fig. 7).

Based on similarity coefficient values the highest values were 1.0 with 100% similarly between tolerant cultivars Giza 130 and Giza 127. Similarly between the susceptible cultivars 125 and Giza 129 was 60%. Contrary, between tolerant and susceptible cultivars should low similarity between Giza 123 and all tolerant cultivars Giza 134, 2000 and 126 showed 0%.In the other hand, molecular markers have been used as a valuable tool in the characterization and evaluation of genetic diversity within and between species and population. The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as RAPD, simple sequence repeats (SSR), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP) and inter-simple sequence repeat polymorphic DNA (ISSR), and so on. These molecular markers had been used in genotype identification, genetic mapping and in genes differentially expressed [39].

4. Conclusion

Drought stress had a significant effect on all the measured traits at all PEG levels used. In this experiment, germination speed was affected by drought stress more than germination percentage. Most cultivars had acceptable germination in drought stress of about 0.54 MPa (10% PEG) which indicates the characteristics of barley in tolerating drought stress and its suitability for being cultivated in arid and semiarid areas. Treatment with 0.54 MPa can be the germination sensitivity threshold for the studied barley cultivars. In summary, due to its better growth responses to drought stress, Giza 134, 127 and 126 cultivars was the superior one and Giza 129 and 124 was the lowest.

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