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Original Article

# 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, 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 by 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 semiarid 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, epitasis, 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

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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. Khazayi 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 ($O_2^-$), singlet oxygen ($O_2^{-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl ions ($^{\cdot}OH$) [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 $H_2O_2$ several antioxidative enzymes act in synchrony. SOD, POX, GPX catalysis superoxide to hydrogen peroxide: $2O_2^- + 2H^+ = H_2O_2 + O_2$. 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 champers 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 μmols $m^{-2}$ $s^{-1}$. 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 $8^{th}$ 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 imbibitions 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

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