Effect of Drought Stress on Proline and Relative water content in Drought Tolerant and Drought Sensitive Barley Cultivars

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ABSTRACT

Drought is a major stress that dramatically limits plant growth and productivity. Poly- ethylene glycol (PEG) has been used for osmotically induced water stress studies in plants. The present work was designed to examine the biochemical response in six barley varieties (three tolerant and three sensitive) at different osmotic potential of Polyethylene glycol -6000 (PEG- 6000). The results showed at -3.0 bars osmotic potential of PEG -6000 decreased relative water content and an increase in proline content was observed in barley leaves and roots.

Keywords: Barley, Drought stress, Proline, Relative water content (RWC)

1. INTRODUCTION

Plants are sessile in nature and, as a result, they do not have the capability to escape from the site of unfavorable environment. As per circumstances, plants often face challenges to grow under adverse environmental conditions such as water deficit or excess, high intense light, low or high temperature, salinity, heavy metals, UV rays, insects and pests attacks, etc. These stresses wield adverse effects on plant growth and development by inducing many metabolic changes, such as the occurrence of an oxidative stress [1]. Drought is one of the major threats to plants, as water deficit affects the plant–water relations at all levels from molecular, cellular and organ, to the whole plant [2]. Drought is a major stress that dramatically limits plant growth and productivity. Poly- ethylene glycol (PEG) has been used for osmotically induced water stress studies in plants [3]. Recently, many studies have focused on exploring the mechanisms of different priming effects and stress memory in the formation of drought tolerance in different plant species [4-7]. It is available in a variety of forms like whole barley, hulled barley, pearled barley as well as barley flakes. It is a rich source of minerals like zinc, copper, phosphorous, etc. as well as other nutrients like calcium and iron. Barley is considered to be the most nutritional cereal comprising the right quantity of all the vital nutrients. Fiber contains two types of nutrients, namely, soluble and insoluble fiber. The soluble fiber helps in controlling the level of cholesterol by eliminating the fatty acids, while the insoluble fiber keeps the digestive system in proper order, thereby avoiding the risk of dreadful diseases like colon cancer. Pearl barley is a rich source of protein, fiber and other nutrients, and helps in maintaining health and vitality. Barley water is known to have many medicinal properties and helps in quick healing of many diseases and ailments. The carbohydrates present in barley help in the regulation of the glucose level. Since barley has fiber levels five times more than that of the other whole grains, it helps in maintaining the sugar level as well. Barley is filled with many important nutrients like vitamin B, vitamin E, and folic acid. Another major benefit of having barley is that it helps in reducing the body weight, as it is a food appetite suppressant, making one feel filled and satisfied. Development of high yield and drought resistant cultivars has been slow. This is because of the presence of large genotype-environment interactions, causing inconsistency in yield under different environmental conditions and lack of specific methods for screening large numbers of genotypes for both yield potential and stress tolerance [8]. Stomatal regulation is one of the key mechanisms allowing plants to optimize CO2 assimilation versus evaporative water loss [9].The present study was taken up to study polyethylene glycol (PEG) induced drought stress on total proline content and relative water content in drought tolerant and drought sensitive barley varieties. Since little attention has been given by researches to improve the locally cultivated barley in this area; the present study would help to understand the responses under drought stress condition and its further improvement of present cultivar.

2. MATERIALS AND METHODS

2.1 Collection of germplasm

Six barley varieties, including three drought sensitive (K -551, K-572, K-287) and three drought tolerant (K -603, K-560, K-125), were used for the present investigation. The seeds were obtained from Rabi Cereal Section, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur.

The experiment was conducted in the Department of Biochemistry and Biochemical Engineering, SHIATS. All the seeds of different barley varieties were surface sterilized with 0.01mM $HgCl_2$ and inoculated in Petri plates with cotton beds wetted by Hoagland media. After germination, seeds were transferred into pots containing sand: soil (1:1). Seedlings were grown at 22-25^0 C and ~70% relative humidity under normal day light condition. Polyethylene glycol -6000 (PEG-6000) was used to induce drought stress. After 14 days of seedling growth, plants were divided into three groups *viz.* control, moderate (-1.5 bars osmotic potential) stress and severe (-3.0 bars osmotic potential) stress treatment. According to the method presented by some scientists, solutions with different osmotic potential were prepared for drought stress treatments. The drought stress was induced by irrigating the pot with PEG -6000 solution along with Hoagland's solution as nutrition media. Barley seedlings were subjected to drought stress for different time intervals (24, 48 and 72 hrs).

2.2 Proline Content

Proline content was determined by the method adopted by Bates *et al.*, (1973) [10]. 0.5g of fresh leaf sample was ground and homogenized in 10ml of 3% aqueous sulphosalicylic acid and the homogenate was filtered using Whatman No. 1 filter paper. 2 ml filtrate was taken in a test tube and 2 ml of acid ninhydrin (prepared by dissolving 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6M phosphoric acid) and 2 ml of glacial acetic acid was added. This was allowed to react for 1 hr. at 100^{0C} in a boiling water bath. The reaction was terminated by placing the tube in an ice box. 4 ml of toluene was added to the reaction mixture. The chromophore containing toluene was separated and absorbance was recorded at 520 nm wavelength using toluene as blank.

2.3 Relative Water Content

Relative water content was measured according to the method of Smart and Bingham (1974). Leaf segments of about 5-6 cm were sampled and immediately weighed to determine fresh weight. After fresh weight determination leaf segments were placed through two layers of filter paper and immersed in deionized water. After 5 hours when segments were totally turgid, they were gently dried with tissue paper for turgid weight measurements. Eventually, dry weights of segment were measured after incubation of the segments for 48 hours at 78^0C [11].

3. RESULTS

3.1 Proline Content

The proline content in leaves and roots, however, increased at both moderate and severe stress in all varieties of barley in response to drought (Figure 1). The increase in proline content in leaves due to drought stress was found significant. Drought tolerant barley varieties showed high proline content in comparison to drought sensitive barley varieties. Among the drought tolerant varieties, K-125 showed maximum content (137.74 µmol g-1FW) of proline whereas K-551 (drought sensitive) showed least content (117.31µmol g-1FW) of proline. Drought tolerant barley varieties showed high proline content in roots when compared to drought sensitive barley varieties. Among the drought tolerant varieties, K-125 showed maximum content (87.33µmol g-1FW) of proline whereas K-551 (drought sensitive) showed least content (68.23 µ mol g-1FW) of proline. Kabiri *et al*., 2015 showed the effect of water stress on proline content of barley. On the basis of these results, water stress caused an increase of 60% in proline content. Pireivatlou *et al.*, (2010) demonstrated that proline content was accumulated in wheat cultivars under drought stress [12-13].

3.2 Relative Water Content

Relative water content (RWC) is used to describe plant water status, and is commonly measured gravimetrically. When exposed to water stress for 24 hours, no species showed any significant difference in leaf RWC compared to their control. (Figure 2). The leaf RWC of all genotypes was significantly reduced by the drought stress prolonged, but drought sensitive varieties exhibited a larger decrease in leaf RWC under 48 and 72 hours drought stress compared with drought tolerant varieties. Among the drought tolerant varieties K125 the relative water content declined by 47.45% and 71.04% for the same time intervals, whereas in drought sensitive K-551 variety the RWC declined by 53.36 % and 77.53 % under moderate stress and severe stress of 72 hours and 31% decline at 48 hours when compared to control. The root RWC of all varieties was expressively reduced by the continued drought stress but drought sensitive varieties showed a larger decrease in root RWC fewer than 48 and 72 hours drought stresses compared with drought tolerant varieties. Among the drought tolerant varieties K-125 declined 43% and 68%, whereas drought sensitive variety (K-551) declined 52% and 72 % under moderate stress and severe stress of 72 hours as compared to control.

Salekjalali *et al.,* (2012) found that relative water content decreased 61% under drought stress in barley leaves. When compared to the control, relative water content of leaves in moderate stress and severe stress declined by 25% and 57% respectively. Others found similar results in wheat cultivars where there were significant differences between genotypes for relative water content of leaf wheat cultivars. Higher amount of relative water content was found in drought sensitive wheat cultivars than drought sensitive cultivars. Hamla *et al.,* (2014) also reported that leaf relative water content differed according to drought stress treatment. The differences were highly significant. Karmollachaab and Gharineh (2015) also found decrease in relative water content (RWC) in wheat plants under drought stress in comparison to control values (16.5%). Saeidi *et al.,* (2015) found similar results that cultivars showed significant change in relative water content. Maximum relative water content was found in drought tolerant wheat cultivars while drought sensitive cultivars had the lowest relative water content under both moderate and severe drought stress conditions [14-17].

4. CONCLUSION

Barley varieties displayed significant changes in proline and relative water content when subjected to drought stress. Drought tolerant (K-603, K-560, K-125) barley varieties exhibited higher value of proline and relative water content compared to drought sensitive (K-603, K-560, K-125) barley varieties.

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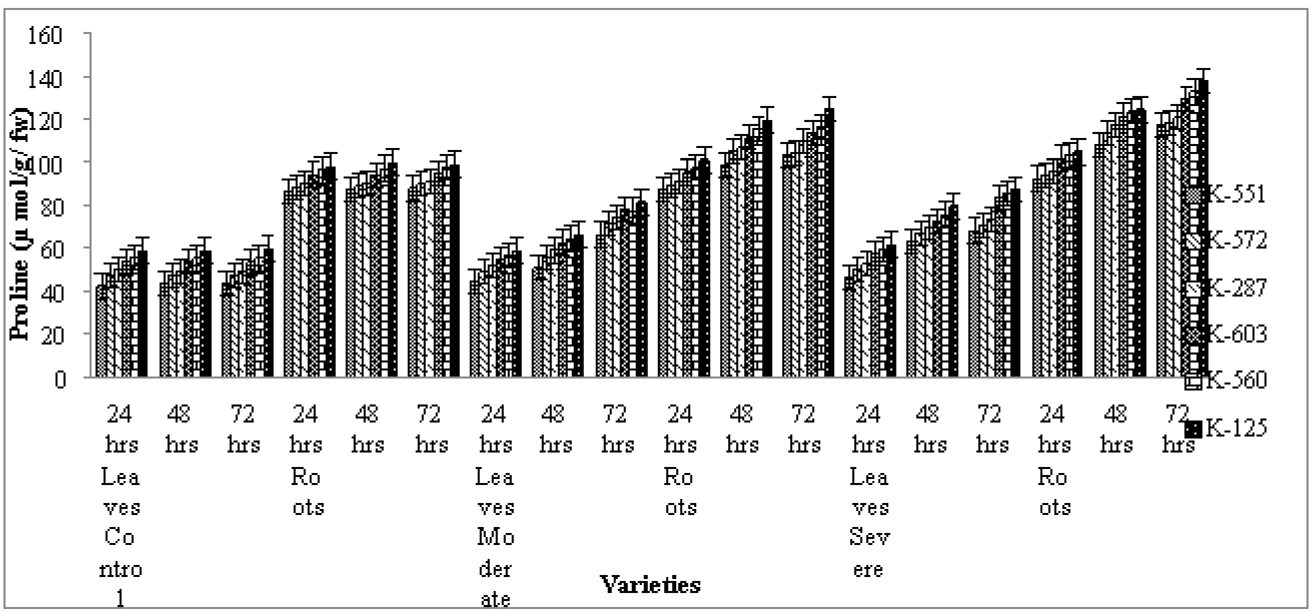

Fig. 1 Effect of drought stress on proline content (µ mol/g fw) in leaves and roots at different osmotic potential of PEG-6000 at different time intervals

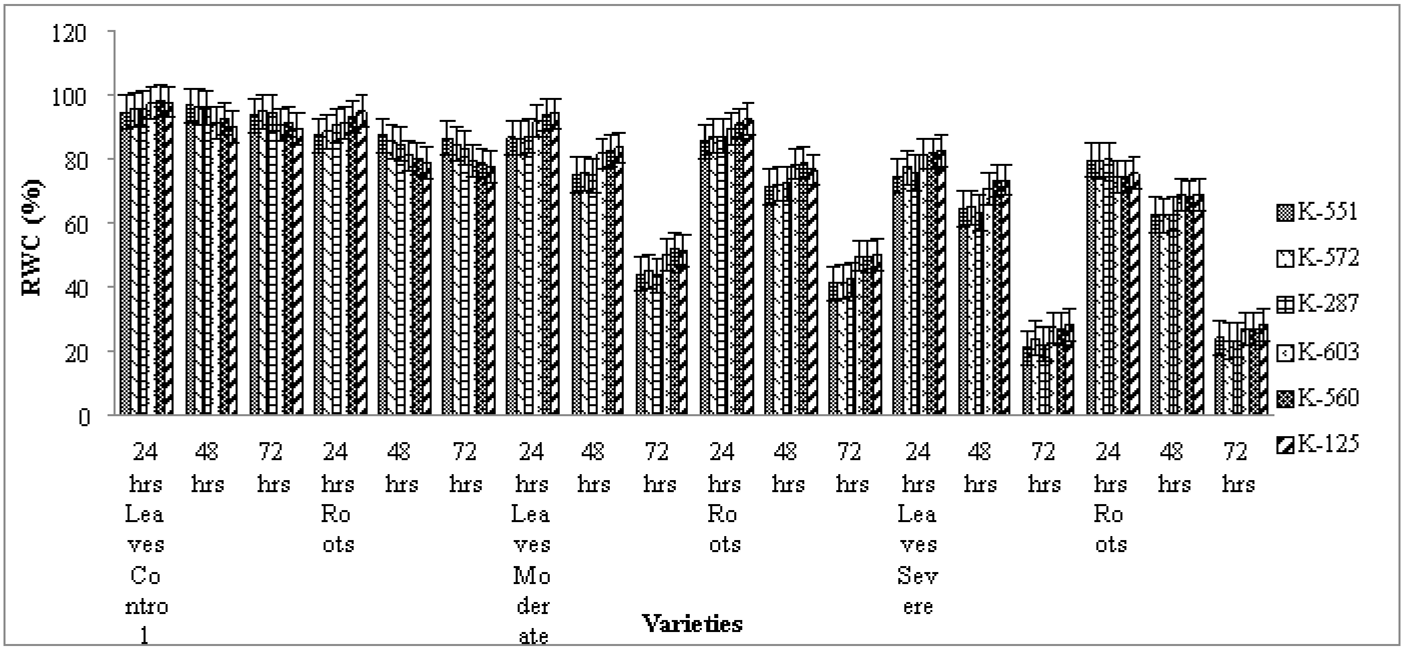

Fig. 2 Effect of drought stress on relative water content (µ mol/g fw) in leaves and roots at different osmotic potential of PEG-6000 at different time intervals