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RESEARCH ARTICLE

# Trehalose Expression in Hexaploid Wheat (*Triticum aestivum* L.) Germplasm under Drought Stress

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**ABSTRACT**

Plant productivity is greatly influenced by environmental stresses. Under stressful condition, plants develop a plethora of biochemical and molecular mechanisms to cope with new situations that causes expression of several genes by signal transduction, associated with stress tolerance. Plant osmotic stress responses are associated with changes in gene expression due to environmental stresses like drought, salinity and low temperature. Trehalose is a non-reducing disaccharide that occurs in a large range of organisms, such as bacteria, fungi, nematodes and crustaceans. Plants that produce trehalose are highly tolerant to desiccation stress. Present study was focused on biochemical estimation of trehalose levels in different wheat genotypes viz Local Genotype, Mapping Population, Synthetic and NIBGE varieties, by HPLC method. Drought was induced by the application of polyethylene glycol (PEG 8000). Root/Shoot ratio was enhanced in the drought affected genotypes. Trehalose promoted a positive effect in wheat germplasm under the drought stress that has lead to the conclusion that it might be utilized to enhance the growth of wheat under the stress situation.

## INTRODUCTION

Plant stresses like oxidative, chemical toxicity, drought, salinity, extreme temperatures along with the attack of insects, pests and plant pathogens result in significant crop losses which are serious threat to agriculture (Jamil et al., 2005). Drought significantly damages plant that further limits crop productivity. Many of the countries are facing this big disaster including Pakistan, and affect almost every aspect of plant growth.

Wheat (*Triticum aestivum* L.) occupies an important place as staple food and the yield improvement of the wheat germplasm under different stresses and agro-climatic situations is mandatory (Zhu et al., 2000). About 35% of the human population consumes wheat as food, covering 29% of caloric intake. Wheat shares largest cereal market due to its global production at more than 651.4 million metric tones per annum (FAO, 2012). Water shortage definitely limits plant growth and productivity even more than any other environmental factor (Boyer, 1982). Suitable environmental conditions are necessary for optimum growth of crop and in field the crop yield also depends upon the row spacing between plants (Tahir et al., 2012). Elevated levels of stress results in the accumulation of osmolytes like proline, glycine-betaine (Ashraf and Foolad, 2007) manitol, and soluble sugars like trehalose which lower the osmotic potential of the cell sap and thus prevent the movement of water out of the cell (Taiz and Zeiger, 2003).

The conventional breeding techniques have been unsuccessful in transferring the drought-tolerance trait to the target species (Jamil et al., 2005). The basic biotechnology tools can be employed to manage stress tolerance and hence improving yield stability. Our main focus was on biochemical estimation of trehalose levels within genotype groups of wheat (*Triticum aestivum* L.) germplasm of different groups like Local genotype, Mapping population, Synthetic and NIBGE varieties. Root-shoot ratio was the basic determinant of crop growth parameters which was affected under drought situation. Trehalose levels were found to be increased in the plants under the stress. Based on this study strategies may be devised to increase crop productivity under drought conditions.

## MATERIALS AND METHODS

The study was conducted on four different genotypes of wheat viz Local genotype (LG), Mapping population (MP), Synthetic (Syn) and NIBGE varieties. The wheat samples were collected from Plant Genomic Molecular Biology lab at NIBGE. Estimation of trehalose was done at Molecular Biochemistry Lab., and Central Hi-Tech Lab., University of Agriculture, Faisalabad.

**Cultivation of plants and culture conditions**

Healthy seeds of sixty six (66) wheat cultivars were screened out and geminated in sand filled plastic pots. Hoagland solution was applied per pot first time. Later on, polyethylene glycol (PEG 8000) was applied after every second day to induce drought to the experimental pots up to 25-30 mL each pot. Control seedlings were fertilized with full strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). After 9$^{th}$ day, thinning was done to select healthy plants for ongoing trail. Selection of the wheat germplasm was made on the basis of Local Genotype (LG: 20), Mapping Population (MP: 20), Synthetic (Syn: 20) and NIBGE (06) varieties. Root/Shoot ratio was also recorded according to standard procedure.

**Trehalose Estimation**

After five-week experiment, the plants were collected in polyethylene bags and placed in liquid nitrogen for some time and stored in -80ºC freezer till further analysis. Plant samples were accurately weighed to 0.25 g and chopped with the help of pestle and mortar. Extract obtained was preserved at -20°C till further use for analysis on HPLC. Trehalose was analyzed by HPLC (SCL-10A, Shimadzu) using column (Rezex RCM-monosccharide $Ca^{+2}$, Phenomenex) for separation of the sugars and refractive index detector for the detection. Quantitative determination of Trehalose was achieved by comparing peak surface areas with those obtained with pure Trehalose standard solutions in the range 0-5 mM. The mobile phase was DD-$H_2O$ with 1 mL/min as flow rate at 80°C.

## RESULTS AND DISCUSSION

The study was conducted on selected wheat cultivars from four genotype groups with or without PEG application up to 41 days in a controlled growth chamber. The results were recorded as under:

**Root/Shoot Ratio**

Root/shoot ratio is an important parameter while studying drought stress in different crops. The root/shoot ratios of the selected wheat (*Triticum aestivum* L.) genotypes are reflected in Table 1. Considerable variations in root to shoot ratios were observed among the genotypes.

Among the 20 Local genotypes, all the roots showed increase in length as compared to their respective control plants under PEG application. In the drought situation, plant roots expanded more to qualify for water and nutrients as compared to the shoot length. Maximum increase in the ratio of root/shoot for drought samples was found to be 1.88 while control had shown 0.48. Minimum ratio of root/shoot for drought sample was found to be 0.71 as compared to 0.59 for the control sample of the Local Genotype. The changes in root and shoot lengths after drought stress have shown that the increase in root-shoot ratio in all the genotypes reflected the indication for drought induction. Maximum root/shoot ratio of the synthetic genotypes from the parent cross of *Triticum durum* X *Aegilops squarrosa* i.e., Synthetic 257 x OPATA present in each genotype was 2.63 for drought and 0.46 for control sample. The minimum range was 0.71 for drought and 0.44 for its respective control sample. The root/shoot ratio of the mapping population genotypes was found to be 2.54 for drought and 0.56 for the control sample. The minimum range was 0.70 for drought and 0.43 for its respective control. The root/shoot ratios of the NIBGE genotypes were 1.16 (maximum) for drought and 0.55 for control sample. The minimum range was 0.50 for drought and 0.59 for its respective control.

**Table 1: Root/shoot ratio for all genotypes of wheat under drought and control conditions**

| Genotypes | Drought Mean ±SD | Control Mean ±SD |
|---|---|---|
| NIBGE | 0.81a ± 0.28 | 0.86a ±0.42 |
| Mapping population | 1.81a ±0.56 | 0.50b±0.12 |
| Local genotype | 1.44a ±0.41 | 0.62b ±0.13 |
| Synthetic | 1.61a ±0.51 | 0.46b ±0.08 |

Abiotic stresses which cause depletion of cellular water are responsible for greatest agricultural losses. Upon exposure to these prevalent stresses, the accumulation of osmoprotectants in sufficient quantity to facilitate osmotic adjustment. The increase in cellular osmolarity due to these compatible solutes is accompanied by the influx of water into the cells providing the turgor necessary for cell expansion (Hare et al., 1998). Water deficit develops slowly enough to allow changes in developmental processes as water stress has several adverse effects on plant growth. In this situation, compatible solutes like proline, glycine-betaine and trehalose produces to counter the unfavorable cellular conditions. The osmotic potential fluctuation of soil solution creating a water stress in plants ultimately leads to plant death due to growth arrest and molecular damage. Osmotic adjustment of cells helps to maintain plant water balance to establish internal milieu (Taiz and Zeiger, 2003).

Increase in root growth in different plants under drought stress was also shown by Tahir et al. (2002) and Jaleel et al. (2008). Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy and perform function accordingly. Root length is a better measure than the surface area of the absorbing ability of roots. Water moves slowly in soil so that a small root is almost as effective as a larger one in absorbing water and nutrients. According to Perry (1982), the large proportions of shoot production are characteristic of vegetation in early successional phases, while high proportions of root production are characteristic of climax vegetational phases. Except for injury to the roots, a reduction in the root-shoot ratio is almost always in response to more favorable growing conditions. An increase in the root-shoot ratio would indicate that a plant was probably growing under less favorable conditions.

Production of ramified root system under drought is important to above ground dry mass and the plant species or varieties of a species have shown great differences in the production of roots (e.g. rice). The importance of root systems in acquiring water has long been recognized to support accelerated plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation in legumes (Johansen et al., 1992). The development of root system increases the water uptake and maintains requisite osmotic pressure through higher trehalose and proline levels in *Phoenix dactylifera* (Djibril et al., 2005). An increased root growth due to water stress was reported in sunflower (Tahir et al., 2002) and *Catharanthus roseus* (Jaleel et al., 2008). The root dry weight was decreased under mild and severe water stress in *Populus* species (Wullschleger et al., 2005). An increase in root to shoot ratio under drought conditions was related to ABA content of roots and shoots (Sharp and LeNoble, 2002; Manivannan et al., 2007). The root growth was not significantly reduced under water deficits in maize and wheat (Sacks et al., 1997).

**Trehalose estimation**

Trehalose increased the biomass production in shoots and roots in all wheat cultivars under water stressed conditions as an osmoprotectant under adverse environmental conditions. The trehalose was separated and detected by HPLC; conditions were optimized with retention time of the osmolyte at 5.13 min (Fig. 1). The samples from each genotype of wheat were analyzed by HPLC in order to compare the production of trehalose among all other varieties. The results of HPLC indicated that these varieties produced trehalose in varying amounts to adjust the osmoticum under drought stress (Table 2).

The results indicated that the varieties produced trehalose in varying amounts to adjust the osmoticum under drought stress.

**Table 2: Level of trehalose (µg/g) in different wheat genotypes**

| Germplasm | Drought Mean ±SD | Control Mean ±SD |
|---|---|---|
| NIBGE | 2392.0a ± 32.71 | 476.67b ± 151.22 |
| Mapping population | 2406a ±109.98 | 502.40b ±71.80 |
| Local genotype | 2346.20a ±113.43 | 453.0b ±44.38 |
| Synthetic | 2509.75a ± 228.95 | 522.0b ±68.02 |

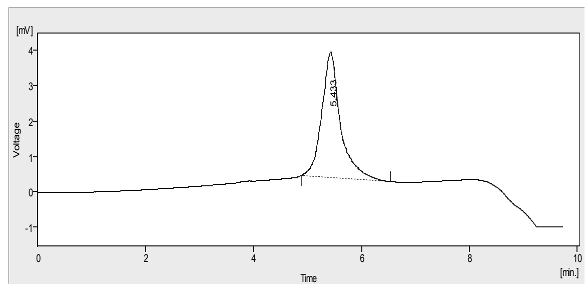


**Fig 1: Trehalose standard chromatogram for estimation of the osmolyte in wheat genotypes indicating retention time of trehalose as 5.13 min.**

The values of all genotypes showed higher levels of trehalose in drought situation as compared to their respective controls (Fig. 2A-D) indicating the high need of this osmolyte rise in case of oxidative stress for plant emergency management. Genotypes LG 64 reflecting higher levels of trehalose with lower levels of control sample i.e. 2566 µg/g for drought and 488 µg/g lower levels (Fig. 2A). Genotypes MP 37 reflecting higher levels of trehalose with considerable levels of control sample i.e. 2576 µg/g for drought and 462 µg/g with control levels (Fig. 2B). Genotypes Synthetic 7-7D reflecting higher levels of trehalose with lower levels of control sample i.e. 3486 µg/g for drought and 545 µg/g with control levels (Fig. 2C). Genotypes NIBGE 2 had 2477 µg/g for drought and 491 µg/g in control samples (Fig. 2D).

Overall, mean standard deviation of the presented genotypes in case of trehalose has shown that there was a marked difference between drought and control samples. The trehalose levels in all the genotypes had higher values under drought stress as compared to their respective control samples (Table 2). Higher levels of drought samples in all genotypes have shown indication of stress induced during the experiment in plants. On the other hand, control samples showed normal range of trehalose released under Hoagland solution medium. Statistical analysis for one-way ANOVA (Steel and Torrie, 1990) for trehalose has also shown that there

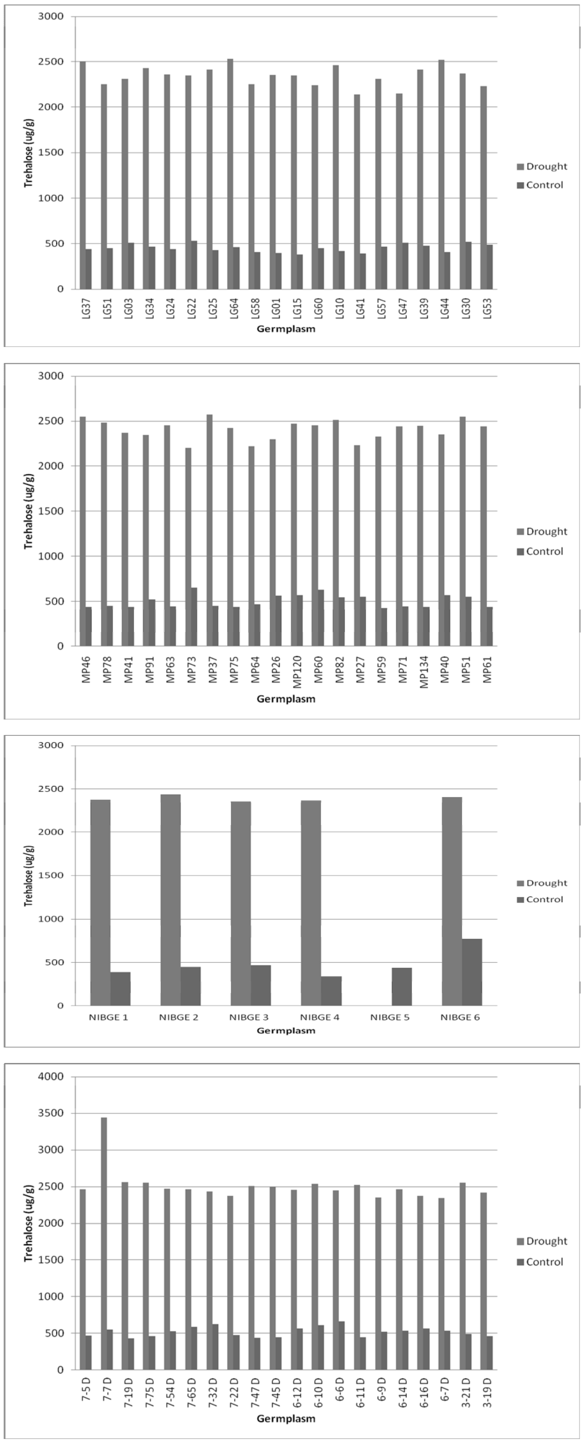


**Fig 2: Trehalose concentration in wheat samples of (A) Local genotype, (B) Mapping population, (C) Synthetic genotype, (D) NIBGE genotype**

**Table 3: One way ANOVA for Trehalose versus Treatment group**

| S.O.V | DF | Sum of Squares | Mean Square | F-Ratio | P-Value |
|---|---|---|---|---|---|
| Btw genotype | 3 | 283692 | 94564 | 3.96 | 0.012* |
| Error | 62 | 1480078 | 23872 | | |
| Total | 65 | 1763770 | | | |

*Level of significance P<0.05

was significant difference between various genotypes under drought stress (Table 3).

Trehalose is a vital soluble sugar osmolyte frequently used by cells to accommodate osmotic pressure within the affected cells to avoid cellular injury due to oxidation phenomenon. According to Paul et al. (2008), sugar signaling mechanism plays a vital role in accelerating the photosynthetic performance of plants to its maximum rate in association with trehalose metabolism. These positive effects of trehalose on gas exchange parameters are due to its role in osmoregulation which may affect the stomatal opening (Hare et al., 1998). It can be concluded from our study that improvement in growth in wheat cultivars under water stressed condition with trehalose application may have been due to the role of trehalose in osmotic adjustment.

## Conclusion

PEG induced drought stress in the selected wheat germplasm enhanced the root/shoot ratio in the drought affected germplasm. Trehalose expression was enhanced significantly under drought stress as determined by HPLC and it promoted a positive effect in the wheat germplasm under the stress. Induction of trehalose, therefore, may be exploited for enhancing the yield of wheat under drought stress.

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