

Full Length Research Paper

# Controlling water deficit by osmolytes and enzymes: Enhancement of carbohydrate mobilization to overcome osmotic stress in wheat subjected to water deficit conditions

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The present study demonstrates the effect of polyethylene glycol-6000 (PEG) induced water-deficit stress on growth, physiological and biochemical responses in six wheat (*Triticum aestivum* L.) cultivars viz. C 306, C 273, PBW 175 (drought tolerant), PBW 534, PBW 343 and PBW 550 (drought susceptible) at seedling stage for six days. Stress caused short stature in shoot and elongated roots in all cultivars. Significant reduction in chlorophyll content and increase in membrane injury index and lipid peroxide content were observed in all the cultivars. Water stress accentuated sucrose synthase/phosphate synthase activities while sucrolytic enzymes namely; acid, neutral and sucrose synthase (cleavage) got repressed. Higher build up of sucrose and lower activity of amylase in root and shoot further decreased reducing sugar (glucose, fructose) content in stressed seedlings. Though the sugar supply (sucrose) under stress was not a limiting factor for germination but the poorer 'metabolic conversion efficiency' via sucrolytic enzymes in root and shoot resulted in their favoured utilization in amino acid, proline and glycine betaine. It may therefore be concluded that certain traits namely: membrane stability parameters, sugars, enzymes and osmolytes might help the cultivars to tolerate water deficit conditions.

**Key words:** Carbohydrates, chlorophyll, sucrose metabolism, membrane stability parameters, proline, protein, starch, *Triticum aestivum*, water-deficit.

## INTRODUCTION

Water deficit is one of the most common environmental limitations affecting growth and productivity of plants. Exposure of plants to water deficit conditions induces several physiological and biochemical changes which

enable plants to adapt to limited environmental conditions (Basu et al., 2010). Reduction of photosynthetic activity, changes in carbohydrate metabolism, accumulation of organic acids and osmolytes are typical responses

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affected by stress (Valliyodan and Nguyen, 2006; Saeedipour and Moradi, 2011). Genotypic variation exists for water deficit tolerance and identification of key metabolic steps will enhance our understanding and fill our knowledge gaps.

One of the earliest responses against water stress is stomatal closure that limits CO<sub>2</sub> diffusion towards chloroplast, which in turn alters carbohydrate metabolism. Another group of compounds which may be affected are amino acids and proline that are often increased in water stressed-leaves (Pinheiro et al., 2004).

Proline, an osmoprotectant, plays an important role in the protection of subcellular structures and scavenging of free radicals. Whereas, glycine betaine is effective in the protection of membranes and the quaternary structures of complex proteins and enzymes. Accumulation of protective solutes like soluble sugars acts as osmoprotectant during stress.

Biochemical conversion of starch to sucrose in the endosperm during germination and seedling growth is a fast process and the sucrose formed is transported to roots and shoots, where it is utilized by invertase and sucrose synthase to provide hexoses for growth and sugar nucleotides for cell wall synthesis (Rosa et al., 2009). Sucrose cleavage catalysed by sucrose synthase is an easily reversible reaction. Apparently, hydrolysis of sucrose by invertase is an irreversible reaction, producing twice as many hexoses. A key function of invertase is a regulation of turgor and the control of sugar balance (Bogdan and Zagdanska, 2009).

The functional significance of sucrose synthase is related to the carbon direction toward both biosynthesis of polysaccharide and respiration (Weschke et al., 2003). Under water deficit, the conversion of starch to sucrose is inhibited and sucrose transport to growing tissues is also decreased, leading to increase in sucrose content in residual endosperm (Regier et al., 2009). In addition, water stress inhibits photosynthesis causing changes in chlorophyll content and thereby damage to photosynthetic apparatus (Nikolaeva et al., 2010). Measurement of such processes in response to drought stress may provide valuable information on the various strategies of the plant that intends to remove or to reduce the harmful effects of water-deficit in soil or plant tissues.

The prerequisite for a successful breeding program for drought tolerance is the availability of tolerant cultivars (Kosturkova et al., 2008). Therefore, the present investigation was aimed to characterize the tall traditional cultivars C 306 and C 273 suitable for rainfed agriculture and is known for drought tolerance but is low yielding cultivars (Gupta et al., 2010). Other cultivated varieties PBW 343, PBW 534 and PBW 550 are widely grown with high yield but not water stress tolerant.

The obtained results would complement the ongoing efforts at our institute for creating drought tolerant varieties derived from crosses of tall traditional varieties with modern semi-dwarf wheat (C 306/PBW 534//PBW 534 and C 273/ PBW 343// PBW 343). This will pave the way

for tagging of drought resistance genes.

The present study was examined for differential response to water deficit in six wheat cultivars C 306, C 273, PBW 175 (drought tolerant), PBW 534, PBW 343 and PBW 550 (drought susceptible) based on growth, physiological and biochemical parameters.

## MATERIALS AND METHODS

### Plant materials and treatment

Wheat (*Triticum aestivum* L.) cultivars, namely: C 306, C 273 (pre dwarfing era varieties and suitable for rainfed agriculture), PBW 343 (widely grown wheat variety for irrigated conditions), PBW 534 (an advanced breeding line for irrigated conditions), PBW 550 (recently released variety for irrigated conditions), PBW 175 (presently cultivated under rainfed conditions) were evaluated for differential sensitivity to water stress during germination. The seeds of above genotypes were collected from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The seeds were sown in pots filled with field soil under two sets, that is, control and drought set, for characterization of various drought tolerance related biochemical indices. Artificial drought conditions were generated in pots using 6, 8, 10, 15% polyethylene glycol (PEG 6000) solution. The root and shoot length (cm) of seedlings under control and drought set were measured at 10th day post germination (DPG) whereas other parameters were studied at 6th day stage of germination. The plants were watered twice a week with PEG solution in drought set and with distilled water in control set.

### Relative water content

The observations on relative water content (RWC) of leaf were taken. RWC of the leaf was determined by using the formula:

$$\text{Relative water content} = (\text{Fresh weight} - \text{dry weight}) / (\text{Turgid weight} - \text{dry weight})$$

Where, turgid weight = weight after the leaf was kept immersed in distilled water for 16 h.

### Estimation of membrane injury, lipid peroxide and chlorophyll content

Membrane Injury and lipid peroxide content were estimated as described by Valentovic et al. (2006). The concentration of lipid peroxide products were determined from the thiobarbituric acid reactive substance (TBARs) contents resulting from the thiobarbituric acid reaction.

Chlorophyll content was estimated according to the method of Hiscox and Israelstam (1979).

### Estimation of soluble sugars and starch

Total soluble sugars and starch were extracted and estimated colorimetrically using the reaction with phenol as described by Rosa et al. (2009), sucrose by the method of Roe (1934) and fructose by Williard and Slattery (1945).

### Extraction and assay of enzymes

From freshly collected samples, soluble acid invertase (EC 3.2.1.26;

pH 4.8), soluble neutral invertase (EC 3.2.1.27; pH 7.5), sucrose synthase (synthesis, EC 2.4.1.13) and sucrose phosphate synthase (EC 2.4.1.14) were extracted and estimated by the procedure employed by Rosa et al. (2009). Total amylase was extracted with 50 mM sodium acetate buffer (pH 5.0) containing 1 mM  $\text{CaCl}_2$  and contents centrifuged at 10 000 *g* for 10 min. Amylase activity was determined by estimating reducing sugars as described by Rosa et al. (2009). In all enzyme assays, the condition for linear rates with respect to substrate concentration, time, optimum temperature and pH were determined in preliminary assays.

#### Estimation of amino acid protein, proline and glycinebetaine

Total free amino acids and soluble proteins were extracted and determined as described by Good and Zaplachinski (1994) and proline contents by Valentovic et al. (2006). Soluble proteins were extracted in 0.1 M NaOH and precipitated with trichloroacetic acid (TCA) and estimated by the standard procedure. For estimating proline content, fresh tissue (0.5 g) was extracted in 3% sulphosalicylic acid and the homogenates were centrifuged at 10 000 *g* for 10 min. Two ml of the supernatant was made to react with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C and the reaction terminated in an ice bath. To this 4 ml of toluene was added and mixed vigorously. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance measured at 520 nm using toluene as blank. The glycinebetaine content was determined using the method of Grieve and Grattan (1983)

#### Statistical analysis

Data was subjected to two-way analysis of variance (ANOVA) to compare genotypic variations and environmental conditions and the LSD at  $p < 0.01$ . Standard errors were computed from the values of two independent experiments with three replicates. The data was analyzed statistically according to Duncan's multiple range tests.

## RESULTS

#### Effect of water stress on growth parameters

PEG did not affect the germination percentage, but the rate of germination was markedly improved with 8% PEG than with 10 and 15% PEG in seedlings. An increase in root length and decrease in shoot length was recorded in all the cultivars under stress conditions compared to control (Figure 1A). It was observed that C 306, C 273 and PBW 175 varieties had longer root system compared to PBW 534, PBW 343 and PBW 550 ones reflecting their inherent capability to drought tolerance. Relative water content was found to decrease under water deficit conditions in all the studied cultivars.

#### Effect of water stress on membrane injury, lipid peroxide and chlorophyll content

Membrane injury index and lipid peroxide content measured in form of thiobarbituric acid reactive substances (TBARS) content increased both in root and shoot (Figure

2A and B) in the stressed seedlings, but the overall increase was more in root as compared to shoot. Roots of C 306 and C 273 had lower membrane injury and lipid peroxide content over PBW varieties both under control and stressed conditions. PBW 175 showed highest membrane injury compared to PBW 550. Total chlorophyll content decreased in shoot of stressed plants in all the cultivars (Figure 2C). Comparatively, chlorophyll a content was significantly high than chlorophyll b although both had reduced proportionally under stress. Cultivar PBW 534 had maximum chlorophyll a content (297.11  $\mu\text{g g}^{-1}$  FW) while minimum content was observed in PBW 175 (213.65  $\mu\text{g g}^{-1}$  FW) under stress. C 306 and C 273 cvs had lesser amount of total chlorophyll content over others both under control and stress conditions.

#### Effect of water stress on sucrose metabolizing enzymes

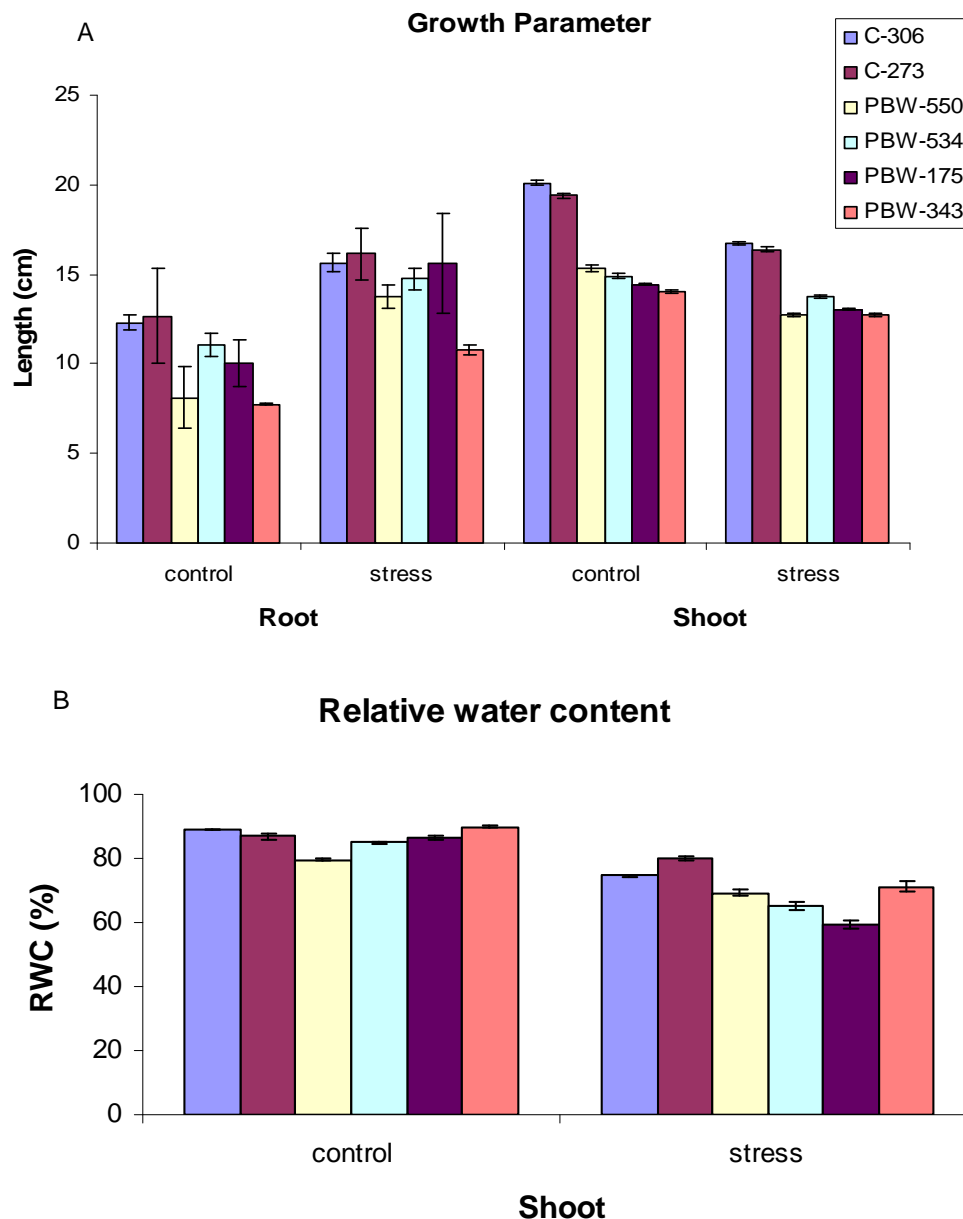
Acid invertase activity predominated over sucrose synthase (cleavage) in root and shoot (Figure 3). Water deficit caused a marked decrease in all sucrolytic activities in both root and shoot while activities of sucrose synthase and sucrose phosphate synthase were increased (Figure 3). PBW cultivars had higher invertase activities in root while sucrose synthase and sucrose phosphate synthase activities predominated in C cvs in both roots and shoot. Neutral invertase was too low in comparison with acid invertase and its interaction between cultivars and environment was statistically non significant showing little contribution towards sucrose hydrolysis.

#### Effect of water stress on starch content and amylase activity

Starch content was found to increase under stress conditions in endosperm as compared to control, in correspondence with a decrease in amylase activity (Figure 4). However, converse was found to be true in root and shoot as the content of starch decreased in root and shoot while amylase activity increased (Figure 4). Total amylase activity was significantly high in residual endosperm and was negligible in root and shoot (Figure 4) and it decreased with stress in endosperm. Maximum decrease of amylase activity was recorded in endosperm of C cultivars. Shoot and root of C cultivars maintained higher amylase activity over PBW cultivars under stress conditions showing that the hydrolysis of starch in these tissues generated higher assimilates in form of glucose as carbon source for its subsequent growth.

#### Effect of water stress on proline, glycine betaine, protein, amino acids and sugars

Enhanced accumulation of cytosolic proline, amino acid



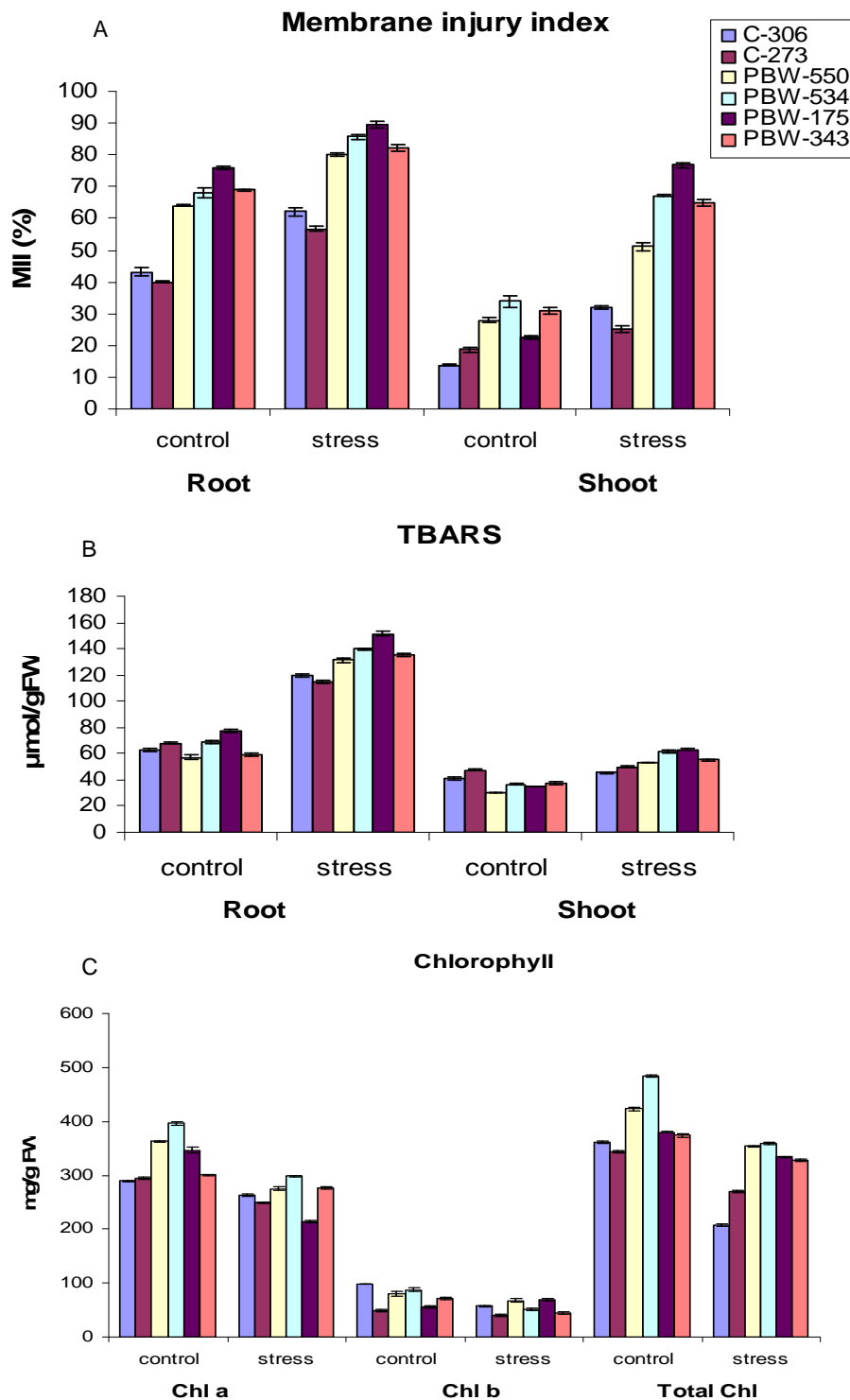
**Figure 1.** Effect of water deficit on growth parameters (A), relative water content (B) in root and shoot of germinating seedlings of six wheat genotypes. Vertical bars represents  $\pm$  SD based on three independent determinations.

and glycine betaine was observed in response to water stress in both root and shoot (Figure 5). On the other hand, protein content was decreased under water deficit conditions. Shoot maintained higher protein content over root under both control and stress conditions (Figure 5). In root, the highest increase in amino acid content was found in C 273 ( $5.13 \text{ mg g}^{-1} \text{ FW}$ ) followed by C 306 and amongst PBW cvs, it was maximum in PBW 343 ( $3.17 \text{ mg g}^{-1} \text{ FW}$ ) followed by PBW 175. Whereas in shoot; it was highest in PBW 534 followed by PBW 550. C cvs possessed lower level of amino acid than PBW ones both under control and stressed seedlings (Figure 5). Free

sugar content was higher in shoot as compared to root under control and stressed conditions (Figure 6). C cultivars maintained higher sugar content especially in roots over shoot. Sucrose was the predominant sugar present followed by glucose and fructose and its content increased both in root and shoot under stress (Figure 6).

## DISCUSSION

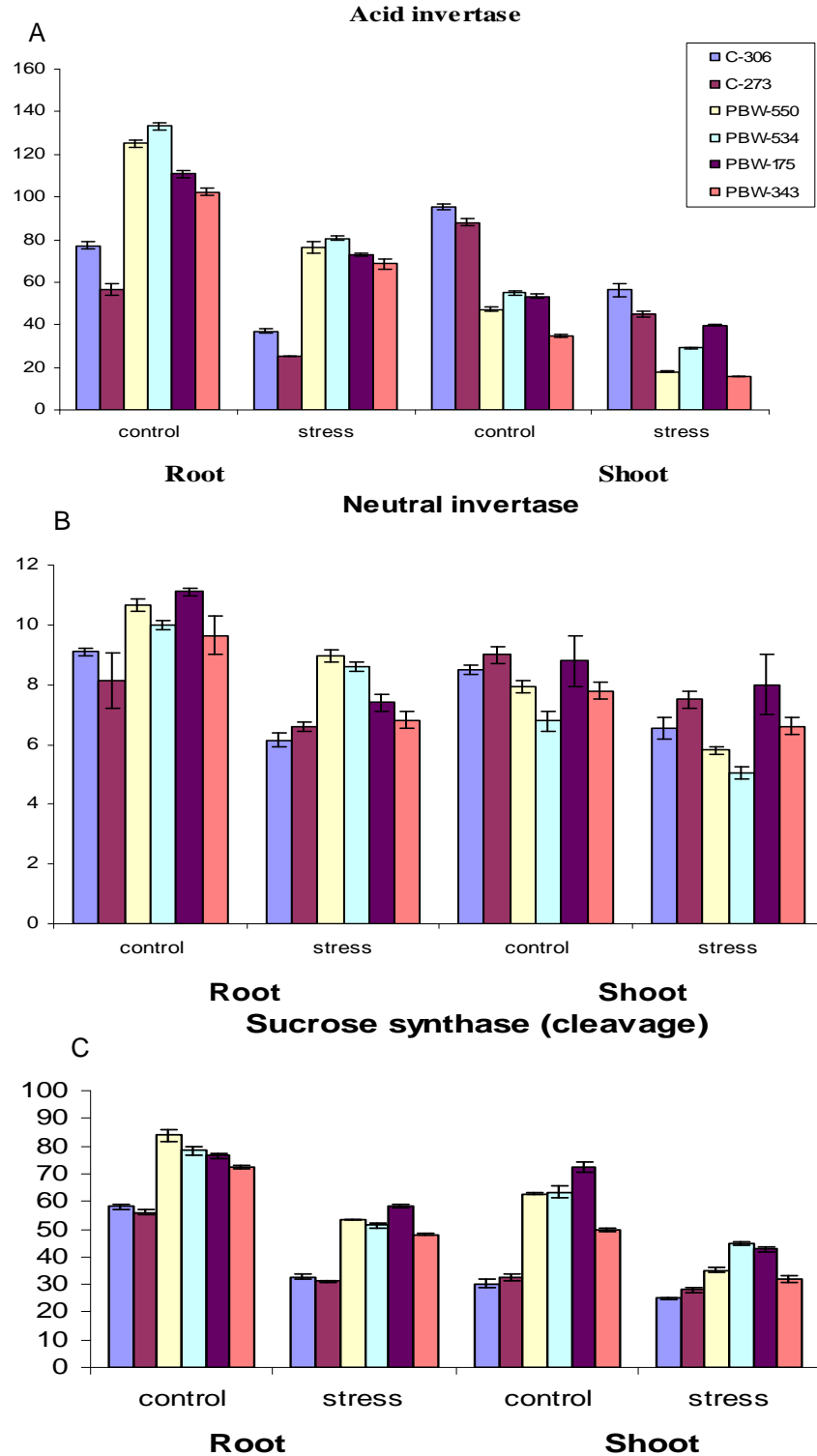
A drought/water deficit condition is the main constraint for crop production in the world and variations among plants



**Figure 2.** Effect of water deficit on membrane injury index (A), thiobarbituric acid reactive substances (TBARS, B) and chlorophyll content (C) in root and shoot of germinating seedlings of six wheat genotypes. Vertical bars represents  $\pm$  SD based on three independent determinations.

provide a valuable tool in the selection of cultivars with desirable traits. Measurement of physiological and bio-

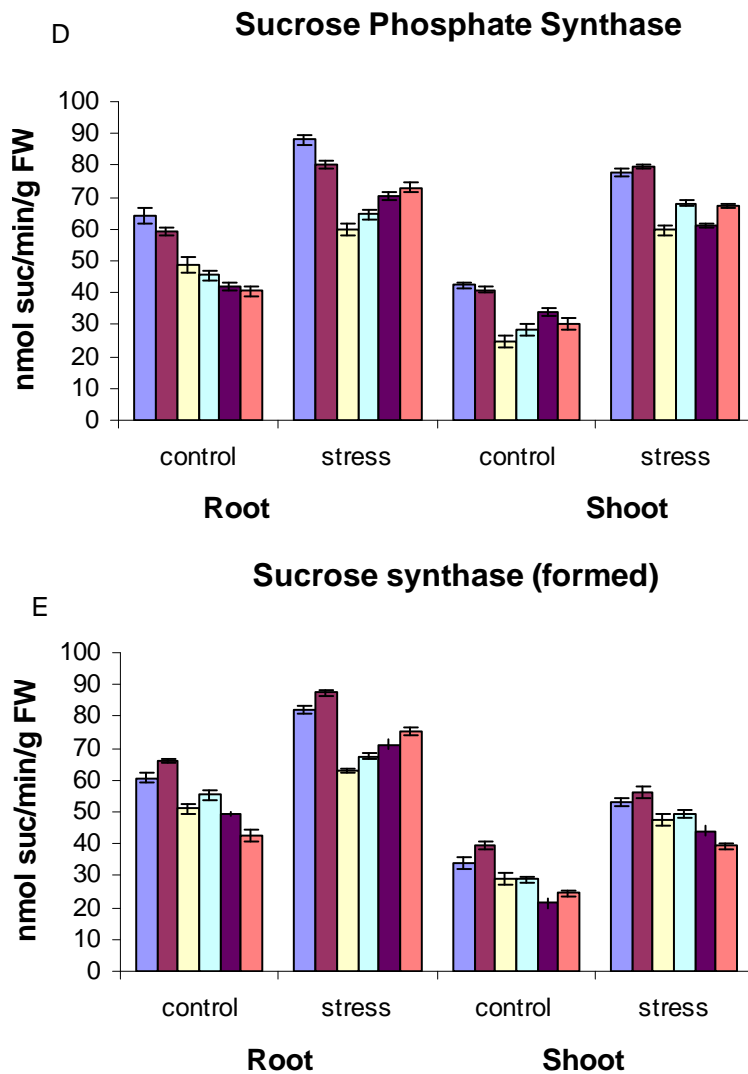
chemical responses induced by the application of PEG-6000 under laboratory condition is considered as an



**Figure 3a-c.** Effect of water deficit on sucrose metabolizing enzymes viz. acid invertase (A), neutral invertase (B), sucrose synthase (cleavage, C), sucrose phosphate synthase. Vertical bars represents  $\pm$  SD based on three independent determinations.

alternative of judging the performance of plants under field conditions (Basu et al., 2010). Although water stress

induced by PEG develops faster creating an osmotic shock, metabolic responses to such osmotic treatment by



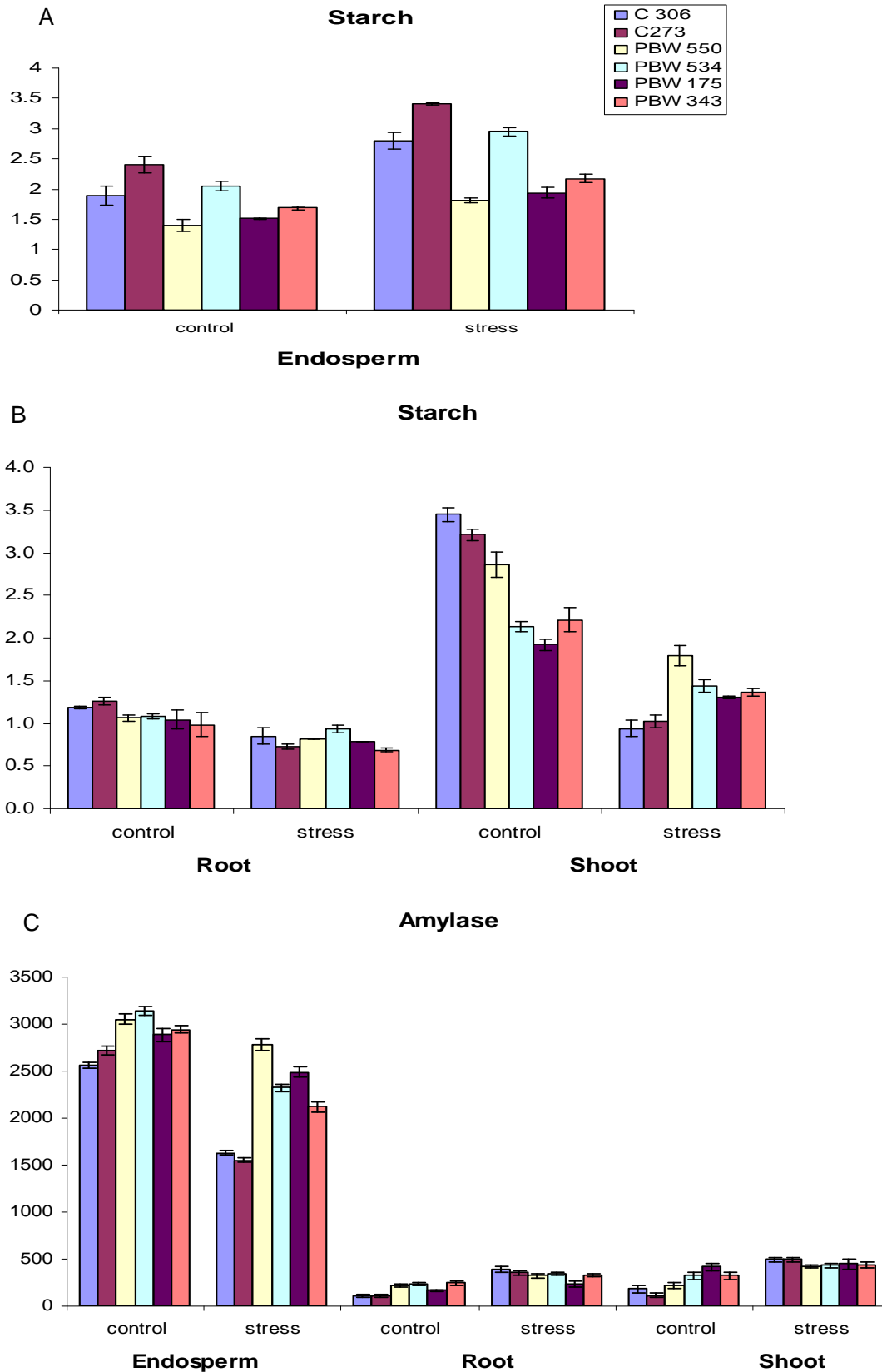
**Figure 3d-e.** Effect of water deficit on sucrose metabolizing enzymes viz. sucrose phosphate synthase (D) and sucrose synthase (synthesis, E) in root and shoot of germinating seedlings of six wheat genotypes. Vertical bars represents  $\pm$  SD based on three independent determinations.

exposing the roots at least is indicative of the relative potential of the different wheat cultivars to tolerate this stress at physiological and biochemical level.

Under water deficit conditions, the extent of increase or decrease in the rate of germination varied in a genotype-specific manner. Germination percentage was decreased under water deficit conditions but the rate of germination in form of root lengths was markedly improved especially in C 306 and C 273 cvs. C cvs had longer roots which emerged as a primary sensor of water stress by absorbing water from deeper soil layers. Cellular water status measured in form of relative water content (RWC) was used to quantify the extent of dehydration which decreased under stress conditions. Many researchers have identified the genotypes of crop species as sensitive

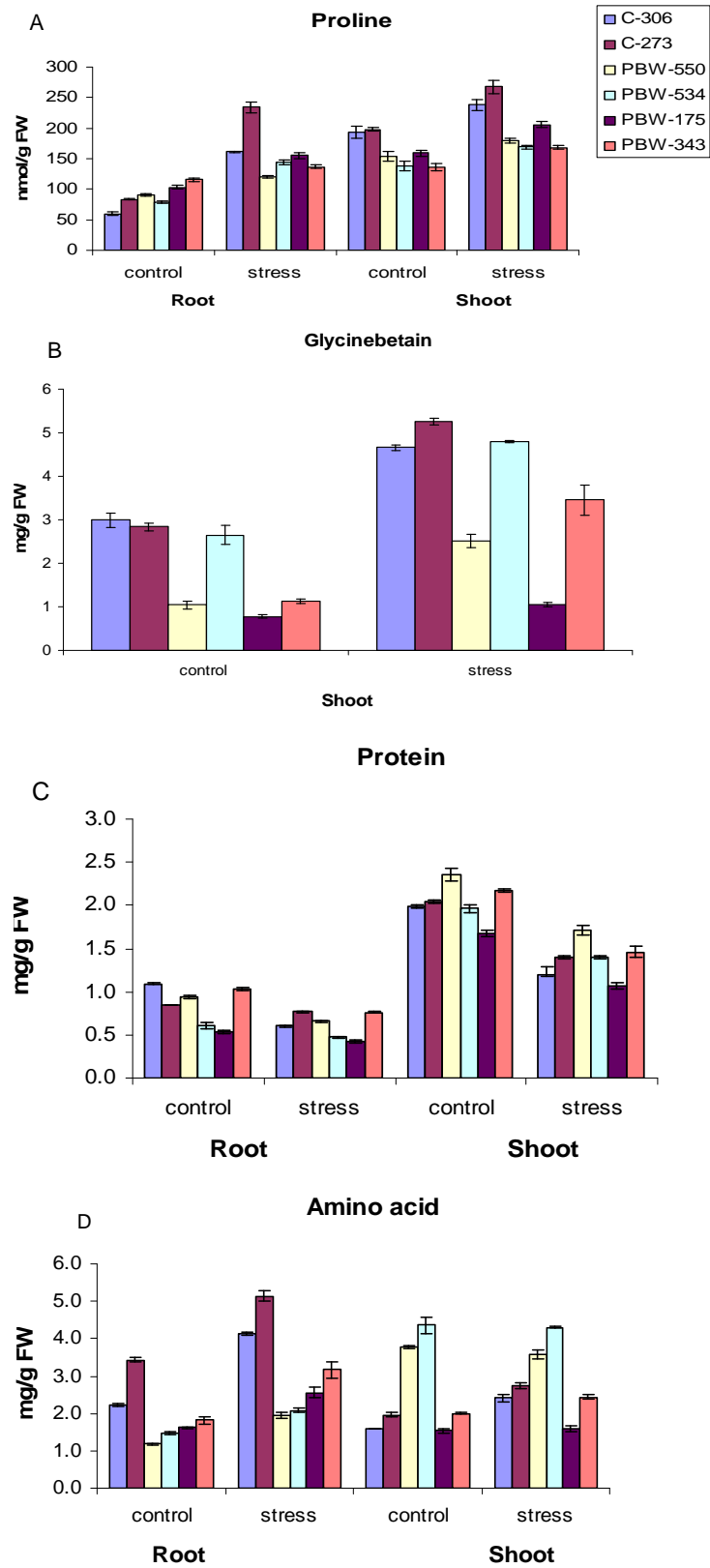
and tolerant based on RWC (Boughalleb and Hajlaoui, 2011).

Water deficit crops experiences higher membrane disruption as evident from the increased electrolyte leakage and lipid peroxide content in PBW cvs. C 306, C 273 cultivars had lower contents of lipid peroxide and membrane injury index (MII) over PBW ones suggesting a correlation between these two parameters. Chlorophyll content also decreased under stress however, less decrease was observed in PBW cultivars. Our results suggests that C 306 and C 273 cvs which are stress tolerant due to their inherent inbuilt genetic ability had more stable membranes than PBW cvs which otherwise had higher chlorophyll content. Decreased level of chlorophyll under stress might be due to peroxidative enzyme activity which

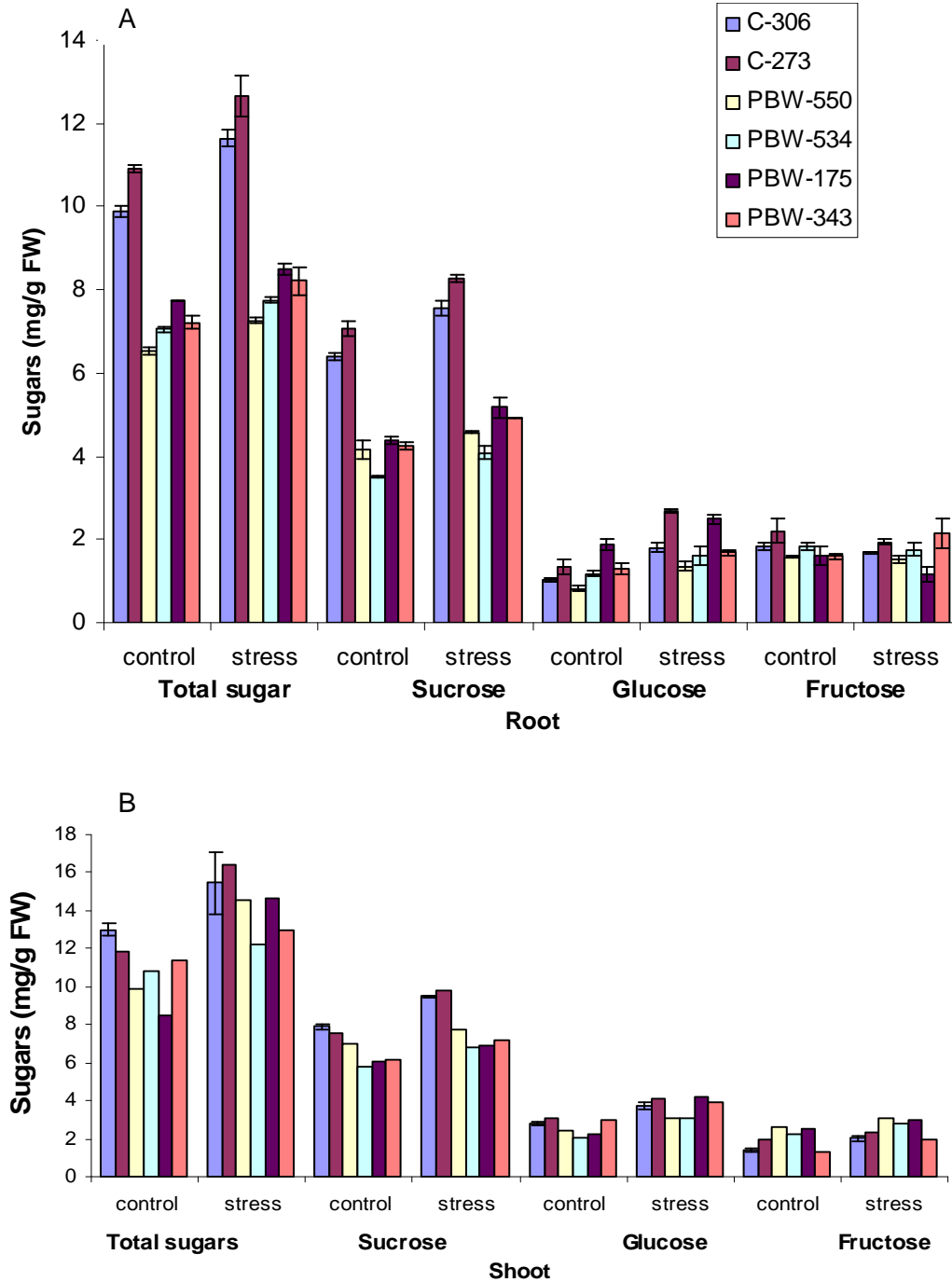


**Figure 4.** Effect of water deficit on starch content and amylase activity in endosperm, root and shoot of germinating seedlings of six wheat genotypes. Vertical bars represents  $\pm$  SD based on three independent determinations.





**Figure 5.** Effect of water deficit on proline (A), glycine betaine (B), protein (C) and amino acid (D) contents in root and shoot of germinating seedlings of six wheat genotypes. Vertical bars represent  $\pm$  SD based on three independent determinations.



**Figure 6.** Effect of water deficit on total sugars, sucrose, glucose and fructose contents in root (A) and shoot (B) of germinating seedlings of six wheat genotypes. Vertical bars represents  $\pm$  SD based on three independent determinations.

promotes thylakoid membrane damage (Rojas et al., 2004).

Water soluble sugars and starch contents also revealed differential response in cvs under water deficit conditions. For instance, total soluble sugars (sucrose, glucose and fructose) were increased in correspondence with a

decrease in starch content in root and shoot under stress conditions thus, implying a transformation of starch to sugars. However, in residual endosperm, the levels of starch increased under stress conditions. Our data showed the highest induction of sucrose level in C cultivars over PBW ones suggesting that the induced sugar

production upon stress in the tolerant varieties helped the cvs in effective osmoregulation, which appeared to be central to the development of desiccation tolerance. According to Chaves and Oliveira (2004), accumulation of sucrose promotes osmotic adjustments that act as osmolyte under stress by increasing the gradient for water flux into the cell for maintaining turgor (Kerpesi and Galiba, 2000). Consequently, water stress tolerance was enhanced by faster hydrolysis of starch in shoot and root due to stimulation of amylase activity, so as to maintain the concentration of low molecular weight carbohydrates, which helped plants to retain turgidity and protect protoplasmic constituents. A drought-induced decrease in starch contents was correlated with inhibition of starch synthase activity (Geigenberger et al., 1997). It has been suggested that under water stress, the products from starch hydrolysis could be used as substrate for sucrose biosynthesis (Lee et al., 2008).

Sucrolytic activity catalyzed by invertase and/or sucrose synthase enzyme is the first step in carbon utilization by the majority of tissues in plants, and either enzyme can predominate depending upon the tissue or developmental stage involved. Comparatively, soluble acid invertase activity was higher over other sucrolytic enzymes indicating its predominant role in sucrose catalysis. Higher activities of sucrose synthesizing (SPS) enzymes and low activity of invertases under water stress conditions corresponds to higher build up of sucrose in C varieties. Excess sucrose formed might also be utilized for the growth of seedlings of C cultivars, basically a tall variety. The sugars are taken up into the scutellum, converted into sucrose and then loaded into the phloem for transport to root and shoot. Significance of SPS in drought stress tolerance has earlier been reported by LePrince et al. (2004). Sugars that accumulate in response to stress can function as osmolyte to maintain cell turgor and have the ability to protect membranes and protein from stress damage. It is believed that the continual cycling of its degradation and synthesis is one of the common features of sucrose metabolism in many plant systems (Hill and Rees, 1995).

Proline is a low molecular weight osmoprotectant that helps to preserve structural integrity and cellular osmotic potential within different compartments of cell (Mohammadkhani and Heidari, 2008). Water stress mediated higher accumulation of proline in shoot enables it to maintain osmotic balance than root where the level decreased compared to non stress conditions. The proline level was reached, the highest level in C cvs over PBW ones. The decreased level of proline in roots of stressed wheat seedlings may be due to increased rate of proline utilization over synthesis (Marjorie and Nicholas, 2002). Total amino acid pool was increased by water deficit in all the genotypes and marginal change in protein contents reflects the mode of adjustment to water stress in wheat seedlings. Alternatively, it suggests that pathways of carbon (starch) and nitrogen (protein) biosyn-

thesis were inversely correlated under water stress. Good and Zaplachinski (1994) reported that free amino acid increased under stress which in turn stimulate protein biosynthesis for osmotic adjustment. Increase in soluble protein, proline, glycine betaine might be correlated to synthesis of osmotin-like protein or structural protein which are involved in modification of cell wall (Chaitanya et al., 2010; Zhang et al., 2011).

In summary, our results indicate that sensitivity to water stress is associated with reduced utilization of sucrose and sugars in seedling tissues and considerable differences that exist in sucrose metabolizing enzymes, amylase activity, starch, sugars, proline that could be associated with differential tolerance of cvs to water stress in pre dwarfing era and cultivated varieties. C 306 and C 273 seem to be relatively more tolerant to water deficit than cultivated ones at least partly due to its higher expression levels of sucrose metabolism and osmolytes and lower levels of TBARS content. Results from this study thus provided baseline information and a system necessary to conduct further studies related to the physiological bases of PEG-induced dehydration tolerance.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

### ACKNOWLEDGEMENT

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