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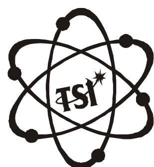

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Effect of water deficit stress on protein, proline, lipid peroxidation, chlorophyll and carbohydrates of maize leaves

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ABSTRACT

The effects of water deficit stress on accumulation of proline, as well as contents of chlorophyll, carbohydrates, malondialdehyde, and lignin were studied in leaves of *Zea mays* L. cv. 704. The experiments were conducted using six replications in a completely randomize design treatment including control and water deficit treatments before and after flowering. The results showed that under water deficit stress the proline, malondialdehyde, chlorophyll b, lignin and glucose contents of leaves were significantly higher than those of control plants, while protein, chlorophyll a (as well as total chlorophyll) and xylose contents were decreased by water deficit stress. The results suggested that water deficit stress affects adversely on normal plant metabolism through increasing of reactive oxygen species that resulted in peroxidation of membrane lipids in turn.
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KEYWORDS

Carbohydrates;
Maiz;
Malondialdehyde;
Proline;
Protein;
Water deficit.

INTRODUCTION

One of the most crucial functions of plant cells is their ability to respond to fluctuations in their environment. Understanding the connections between a plant's initial response and the downstream events that constitute successful adjustment to its altered environment is one of the next grand challenges of plant biology. Adverse environmental conditions such as drought are among the major factors limiting the growth and productivity of land plants. Water stress results in stomatal closure and reduction of transpiration rates, decrease of water potential of plant tissues, decrease of photosynthesis and inhibition of growth, Accumulation of abscisic acid (ABA), proline, mannitol and sorbitol, and in some cases, increasing of radical scavenging compounds (e.g., ascorbate, glutathione, and α-tocopherol) are among other symptoms usually observed in plants under water deficit conditions[1].

The increase of proline content in response to water deficit is a well-documented fact[2] and a large body of data indicates a positive correlation between proline accumulation and enhanced tolerance to drought and salt stress[3]. Other experimental evidences suggest that the accumulation of proline is a symptom of stress injury rather than an indicator of stress tolerance[4]. Nevertheless, proline accumulation seems to be a useful index of drought stress in plants[5].

Chlorophyll content also can be changed by stress condition and can be considered as a parameter showing salt tolerance in crop plants[6]. Chlorophyll fluorescence has been regarded as a tool for determining the photoinhibition of photosynthesis and an indicator of oxidative stresses[7]. Rapid estimates of photosynthetic potential can be important for studies on gas exchange and the C cycle. Reactive oxygen species (ROS) e.g., superoxide radical ($O2^{•}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{•}$) which are naturally produced by plants are increased and accumulated during salinity, water stress, and some other stresses[8]. Accumulation of ROS damages the membranes and essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids[9].

The objective of this work was to investigate the effect of drought stress on protein content, proline, chlorophyll, carbohydrates, MDA and Lignin in leaves of maize.

MATERIALS AND METHODS

Seeds of maize (*Zea mays* L. cv. 704) were sown in 25 Kg pots containing silty loam soil. The experiment was conducted in a completely randomize design with three treatments i.e., control conditions (S0), water deficit before (S1) and after flowering (S2), with six replications each. The plants were irrigated at field capacity level until the treatment being applied. Water potential (Ψ(was measured between 11:00 AM and 01:00 PM , twice, 24h after irrigation (initiation of stress and at the end of stress). Water potential was about -0.5 bar at the initiation of stress and was -7.5 bars at the end of the treatment.

Sampling

2 hours before ending of water stress period the middle leaves of each plant were harvested and frozen by liquid N_2 and kept at -80°C until used for biochemical analysis.

Preparation of extracts

Leaf samples (0.2 g) were homogenized in a mortar and pestle with 3 ml ice-cold Na-Pi buffer 25 mM, (pH 7.8). The homogenate was centrifuged at 18,000 g for 30 min at 4°C, and then the supernatant was filtered through filter paper. The supernatant was used as a crude extract for the assay of enzymes and protein content. All experiments were carried out at 4°C.

Protein assay

Total protein content was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford (1976)[10].

Proline assay

Samples of leaves (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v), and then the homogenate was centrifuged at 18,000 g for 15 min. Two milliliters of the supernatant were then put into a test tube into which 2 ml of glacial acetic acid and 2 ml of freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid) were added. Tubes were incubated in a water bath for 1 h at 100°C, and then allowed to cool to room temperature. Four milliliters of toluene were added and mixed on a vortex mixer for 20 seconds. The test tubes were allowed to stand for at least 10 min to allow the separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube, and its absorbance was measured at 520 nm by spectrophotometer (GBC, Cintra 6, Australia). The concentration of proline was calculated from a proline standard curve and was expressed as mmol per gram of fresh weight[11].

Chlorophyll assay

Chlorophyll content was determined by extraction with 80% acetone followed by reading absornbances at 645 and 663 nm[12]. The concentration of chlorophyll was expressed as mg/g FW.

Lipid peroxidation rate

The level of membrane damage was determined by measuring MDA as the end product of peroxidation of membrane lipids[13]. In brief, samples were homogenized in an aqueous solution of trichloroacetic acid (10% w/v), and aliquots of the filtrates were heated in 0.25% thiobarbituric acid. The amount of MDA was determined from the absorbance at 532 nm, followed by correction for the non-specific absorbance at 600 nm. The concentration of MDA was determined using the extinction coefficient of malondialdehyde(ε=155µM

cm^{-1}).

Determination of lignin content

Cell wall preparations were obtained by homogenization of frozen samples in water with a mortar and pestle followed by centrifugation at 1000 g and sequential washing of the pellet with EtOH, $CHCL_3$-MEOH (2:1) and acetone and then drying in air. Lignin content of wall preparations was measured via a modified acetyl bromide procedure[14]. The lignin content was determined by measuring of absorbance at 280 nm using specific absorption coefficient value 20.0 g^{-1} L cm.

Carbohydrates assay

Samples (0.2g) were homogenized with 3 ml of distilled water and homogenates were filtered by filter paper. Then 0.5ml of aquatic phenol solution (5%) and 2.5 ml of high concentrated sulfuric acid (98%) were added to 50µl of homogenates and incubated at 25°C for 15 minutes. The absorbance was recorded at 480 (xylose), 485 (glucose) and 490 (mannose) nm[15].

Statistical analysis

Each experiment was carried out with six replications and data were analyzed using SAS software[16]. When analysis of variance (ANOVA) showed significant treatment effects, Duncan's multiple range test was applied to compare the means at P<0.05.

RESULTS

Water stress resulted in remarkable reduction of protein content of maize leaves before flowering, compared to that of control plants which had the most protein content (Figure 1).

Water stress increased proline content of maize leaves along with the treatments, so that proline content of the plants at the flowering stage was higher than that of those stressed plants before flowering and of the latter was higher than that of the control ones (Figure 2).

The content of chlorophyll a was decreased by water deficit and although it was, at least in part, compensated with the increase of chlorophyll b in treated plants, the total chlorophyll content of treated plants was lower that of non treated plants (Figures 3-5).

The rate of peroxidation of membrane lipids was also affected by water stress and increasd along with

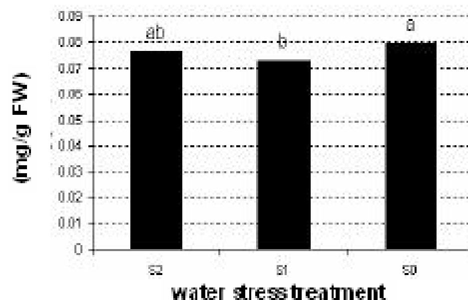

Figure 1: Protein content of maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

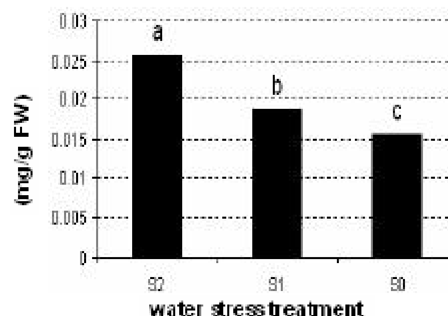

Figure 2: Proline content of maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

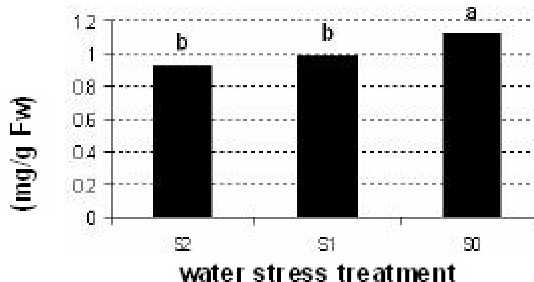

Figure 3: Chlorophyll a obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

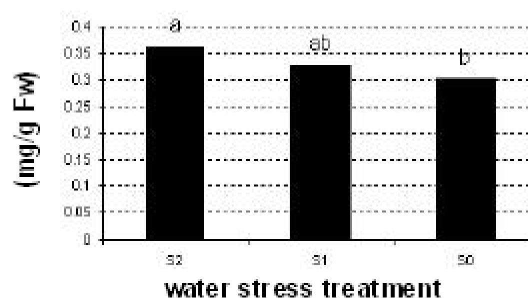

Figure 4: Chlorophyll b obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

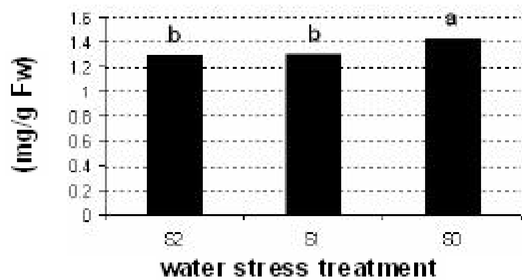

Figure 5: Total chlorophyll obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

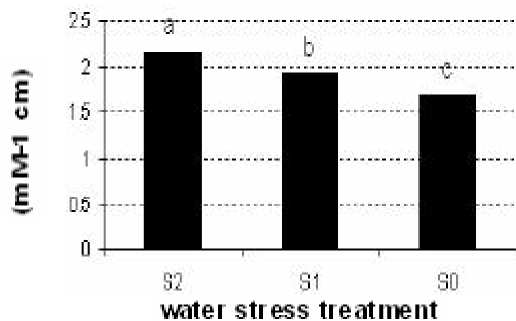

Figure 6: MDA obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

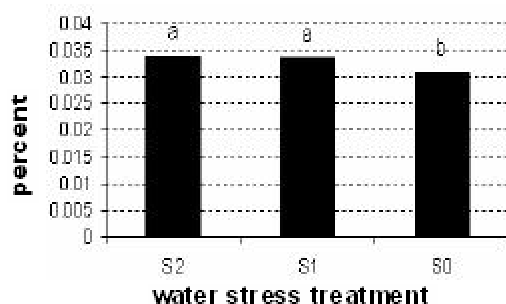

Figure 7: Lignin percent obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

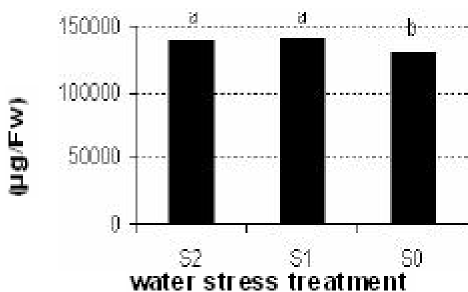

Figure 8: Glucose obtained from maize leaves in control conditions (S0), and water deficit stress before (S2) and after flowering (S1), (p≤0.05)

the treatments. The most level of MDA was again seen in those plants under water deficit conditions after flowering stage of their development (Figure 6). Like wise, the level of MDA of the stressed plants before flowering stage was higher than that of the non-stressed plants (Figure 6).

The lignin content of wall cells of water deficit stress-plants was higher than that of non-stressed plants. There was not however, significant difference between lignin content of, plants before and after flowering stages (Figure 7).

The content of soluble carbohydrates of leaves was affected by water stress. Glucose content of water stressed-plants before and after flowering was both higher than that of the control plants (Figure 8). Xylose content of plants however, decreased under water deficit stress after flowering and xylose content of water stressed-plants before flowering was identical to that of the control ones (Figure 9) Mannose content of stressed- and non-stressed plants were identical (Figure 10).

DISCUSSION

It is well accepted that stress conditions, e.g., drought, salt stress, extreme temperatures, nutrient deprivation, UV-B radiation, and air pollutants can cause changes in cell metabolites.

Reduction of protein content results from a variety of environmental stresses such as water stress has been reported by other studies[17]. Water stress may inhibit protein synthesis via decrease in the number of polysomes[18], or accelerate degeneration of proteins through accumulation of reactive oxygen species (ROS)[19]. Lipid peroxidation is often used as an indicator of increased oxidative damage[20]. Increased rate of proxi dation of membrane lipids of water deficit stressed-maize in the present study and reduced levels of proteins of them imply the hypothesis that one aspect of adverse effects of water deficit on plants growth is conducted via production of ROS.

Accumulation of proline in leaves of water stressed-maize in the present study is in accordance with other reports[21]. The beneficial role of proline in plant stress tolerance as suggested by early correlative studies was recently confirmed by genetic as well as transgenic stud-

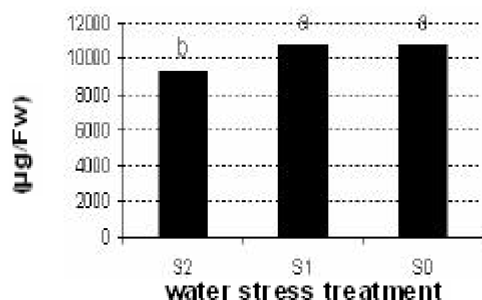

Figure 9: Xylose obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), ($p \leq 0.05$)

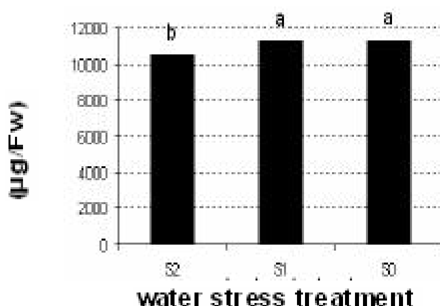

Figure 10: Mannose obtained from maize leaves in control conditions (S0) and water deficit stress before (S2) and after flowering (S1), ($p \leq 0.05$)

ies, which demonstrated that proline can increase the tolerance of plants to abiotic stress[22]. This is probably due to, among others, the ability of osmolytes to scavenge reactive oxygen species, although the underlying mechanism is presently unclear[22].

Water deficit stress by increase of ROS can play important role in production of lignin. Hydrogen peroxide, a dangerous species of oxygen and an electron acceptor for wall bound peroxides, plays a major role in polymerization of phenolic monomers in the synthesis of lignin and establishment of covalent bonds between lignin and carbohydrate in cell walls[23]. Increase of lignin in cell walls of plants under water deficit conditions in the present research, again implies that similar to other abiotic stresses, adverse effect of water deficit may also be mediated by increasing ROS .

In the present study in water stressed-maize photosynthetic pigments (chlorophyll a, and total chlorophyll) as chief components of the photosynthetic system governing the dry matter participation, decreased. Chlorophyll content is fundamental to understand a plant's response to the environment in which it resides[6]. Chlorophyll fluorescence has been regarded as a tool for determining the photoinhibition of photosynthesis and an indicator of oxidative stresses[9]. Dela-Rosa and Maiti[24] found an inhibition in chlorophyll biosynthesis in sorghum plants because of salt stress[24]. Chlorophyll content has also been reported as one of the parameters of salt tolerance in crop plants[6]. Higher chlorophyll degradation was observed in sodium chloride sensitive pea cultivar as compared to tolerant one[25].

Result showed increasing in glucose and decreasing in xylose and no change in mannose. Water deficit stress as a stressful condition leads to mobilization of stored carbohydrates to supply for energy and carbon skeletons to synthesize stress molecules. Water deficit stress causes stomata closure, limits transpiration, and increases leaf temperature as a consequence. Both stomata closure and heat stress decrease photosynthesis yield[26]. Many tissues of stressed plants are likely to have an increased demand for rapidly metabolizable carbohydrate in order to initiate the responses that would guarantee stress tolerance. This must be satisfied despite a likely decrease in carbon fixation and may lead to the mobilization of carbon from storage. The mobilization of stored carbohydrates could increase the glucose content a consequence of sucrose catabolism. Glucose itself may have a role as osmolyte[27]. Stored carbohydrates could also be mobilized in order to synthesize proline to cope with drought stress. The pattern of glucose accumulation in sugar beet roots recorded in this work resembles that of proline: the level of glucose increased in the last stage of the crop. Environmental stresses like drought, cold and salinity lead to major alterations in carbohydrate metabolism[28], and up regulation of many genes corresponding to carbohydrate metabolism[29].

CONCLUSIONS

In conclusion, this study has shown that water deficit stress can decreased protein and chlorophyll and increased proline and lipid peroxidation for plants under water stress conditions. These indexes can be used for determine resistance to water deficit stress. Partial inhibition of water stress-induced increased in lipid peroxidation, and an increased damage to plant cell.

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