Biochemical Responses of Maize Under Drought Conditions

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ABSTRACT

This study aims to assess the biochemical changes of maize during water stress condition. Methodologically, Yellow Super Sweet Corn (YSC) and Thai Super Sweet Corn (TSC) were conducted with water stress condition in different days of interval for watering. The leaves sample were collected and determined for biochemical parameters responses to drought stress. The finding shows that increasing water stress significantly reduced the biochemical parameters as compared to control treatment which is watering everyday. The experiment show that proline accumulated highest in YSC treated with T4 (3.8 x 10^{-3} mg mL^{-1} in 200 mg of leaves) at day 65 and TSC which treated with T5 (0.08 mg mL^{-1} in 200 mg of leaves) at day 40. Protein content in YSC treated with T2 (5.6 x 10^{-4} mg mL^{-1}) was no significant differences with control treatment but showed differences in TSC which treated with same treatment (3.3 x 10^{-4} mg mL^{-1}) at day 65. There was no significant differences in chlorophyll content between maize treated with T2 and control treatment. Accumulation of MDA content was highest in YSC treated with T3 and T4 which are 0.95 \mu mol in 100 mg leaves and also in TSC treated with T3 which is 0.64 \mu mol in 100 mg leaves at the day 65. MSI was reduced in water stress treatments such as YSC treated with T3 and T4 as well as TSC treated with T2 and T3 at day 65. This study found that the three days interval of watering (T2) had the similar result as compared to everyday watering treatment (T1) and be able to produce maize cob at the day 65.

Key words: Water stress, Drought, Biochemical responses, Maize

Introduction

Water deficit is one of the most important constraints in maize production. There are two facets to maize drought resistance which are affordability of irrigation systems and increasing pressure on water resources from sectors other than agriculture (Wesley et al., 2001). Plant survival to stressful conditions such as drought is governed by the capacity for quick recognition of the stress and the rate of induction of protective mechanisms. Maize has been found to be more susceptible than sorghum to water stress. Leaf water content declined more in maize than in sorghum, attaining low values of leaf water potential (Nagy et al., 1995). In addition, the relative water content in leaves of different maize cultivars decreased significantly and with drought stress the membrane permeability of the leaf cell markedly increased (Liu and He, 1995; Chen and Dai, 1994). Furthermore, water stress decreased the relative water content in seedlings of a drought sensitive cultivar (Song et al., 1995). As a result of growth inhibition caused by stress the photosynthesize is usually diverted to storage of non structure biomass including organic compound such as proline and protein (Amthor and McCree, 1990; Setter, 1990). The accumulation of leaf soluble protein may represent a reserve of nitrogen to be used during recovery once stress has ceased (Stitt and Schulze, 1994). Free proline accumulation is one of the most frequently measured during different stress occurring in plants. Free proline contents increase when under water and osmotic stress and also subjected to salt, cold and freezing stress. Proline can also accumulate in some non-stressed plant structures that dehydrate like pollen and seeds (Hua et al., 1997). When water potential in the soil decrease, the plant increases the metabolic response known as osmotic adjustment, which accumulates cytoplasm solutes that produce a decrease in inner water potential allowing the plant to absorb water from the soil. These compatible osmolytes which are neutral organic compounds non toxic to the cell and do not inhibit enzymatic activity even at high concentration (Roger, 2001).

In general, high levels of malondialdehyde are negatively correlated with Membrane Stability Index (MSI) (Parvanova et al., 2004). Salt-tolerant genotypes have shown high MSI and low lipid peroxidation, indicating a direct relationship between stress tolerance and membrane protection. It is known that drought can affects chlorophyll content, inhibits root growth, dry matter production and severely reduces the yield and yield components (Pirdashtiet al., 2009).
The present research aimed to determine the biochemical changes in maize under drought conditions. The output of this study can be an indicator for water use efficiency in maize production.

**Materials and Methods**

Two types of maize which are Yellow Super Sweet Corn (YSC) and Thai Super Sweet Corn (TSC) were used in this study. Each variety were conducted with three replicates for each treatments. Both varieties were carried out in pot experiment. The experimental design was Completely Randomized Design (CRD). The maize grown in pot which contain peat soil for 20 days as nursery stage. After 20 days, the maize were treated with six treatments which are watering everyday (T1), watering in three days interval (T2), watering in six days interval (T3), watering in nine days interval (T4), watering in twelve days interval (T5) and watering in fifteen days interval (T6). The control treatment is T1 for both varieties. The amount of water for each watering is 500 mL.

**Free Proline Content:**

Proline accumulation in fresh leaves was determined according to the method of Bates et al. (1973).

**Protein Content:**

Protein in plant tissue of maize was isolated with protein isolation method. Fresh plant tissues in 0.1 g weight has been finely cut with a razor blade into a mortar and added with approximately 2 mL of cold extraction buffer. Cold extraction buffer contains 0.1 M potassium phosphate buffer with pH 7.8, 1 mM EDTA, 1% Triton-X-100, and 10% glycerol. The samples were grinded until smooth with a pestle until consistency of slightly watery toothpaste. The mortar and pestle together with plant tissue have to place as cold as possible during this process. Then 1 ml of slurry was transferred into a 1.5 ml microfuge tube and the tube was placed on ice until all samples are ready to next step. The samples were spinned at top speed in a microfuge for 15 minutes. After that the liquid supernatant was transferred into a new microfuge tube. Then the supernatant is ready to protein test which is Biuret method was used to determine the protein content (Gornall et al., 1949).

**Chlorophyll Content:**

Chlorophyll content was determined by using Chlorophyll Meter SPAD 502 (Japan).

**Lipid Peroxidation:**

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content using thiobarbituric acid (TBA)-reactive substances following the protocol of Sairam et al. (1998).

**Membrane Stability Index (MSI):**

The MSI was determined indirectly by measuring the electrical conductivity following the protocol of Sairam et al. (2002).

**Statistical Analysis:**

All measurement were subjected to Two-way Analysis of Variance (ANOVA) to test the differences among corresponding to water stress level. Means were compared between control and stressed treatments using Duncan test was carried out if there is significant differences among the means. In each case the number of replicates is three and the level of confidence was defined at p<0.05. All analysis were conducted using SPSS Version 17.

**Results:**

Values are the mean ± SE (n=3). Different letters on a line for each variety indicate significant differences among treatments at day 65 (p <0.05).
Fig. 1: Effect of water stress on proline content (mg mL\(^{-1}\)) for YSC and TSC at day 65.

Fig. 2: Effect of water stress on protein content (mg mL\(^{-1}\)) for YSC and TSC at day 65.

Fig. 3: Effect of water stress on chlorophyll content (SPAD unit) for TSC and TSC at day 65.

Fig. 4: Effect of water stress on MDA content (μmol / 100 mg) for YSC and TSC at day 65.
Fig. 5: Effect of water stress on Membrane Stability Index (%) for YSC and YSC at day 65.

Legend

- - YSC
- - - TSC

T1: Everyday watering
T2: 3 days interval watering
T3: 6 days interval watering
T4: 9 days interval watering
T5: 12 days interval watering
T6: 15 days interval watering

Discussion:

Free Proline Content:

In many plant species, free proline accumulates in response to a wide range of stresses, such as drought and salinity (Nanjo et al., 1999; Jain et al., 2001, Serraj and Sinclair, 2002; Safarnejad, 2004). In this experiment, proline concentration was shown to be generally higher in stressed maize where in YSC which treated with T4 and TSC treated with T3 are relatively higher as compared to the control treatment (T1) at day 65. Proline accumulation in maize might be enough for osmotic adjustment under stress conditions. Increasing proline content of leaves with decreasing available water means an efficient mechanism for osmotic regulation, stabilizing sub-cellular structures and cellular adaptation to water stress was provided (Valentovic et al., 2006, Gunes et al., 2008). Proline accumulation under stress conditions supplies energy for survival and growth and thereby helps the plant to tolerate stress (Yoshibat et al., 1997; Sankatet et al., 2007; Aktaset et al., 2007).

Protein Content:

The first observed response is a decrease in its normal metabolic activities when a plant is subjected to any biotic or abiotic stress, with a consequent reduction of growth. Protein synthesis is one of the most negatively affected anabolic processes together with photosynthesis, transport of metabolites and uptake and translocation of ions (Gan and Amasino, 1997). The protein content among water stress treatments (T2, T3, T4, T5 and T6) in both varieties reduced with increasing water stress as compared to control treatment. Water deficit in plant tissue may cause reduction in polyribosome levels or lead to increase in the protein breakdown. Leaf protein function not only as a catalyst but also a major storage sink for nitrogen. Some proteins such as RuBP carboxylase act as both catalyst and as storage protein. The turn over characteristic of individual protein depends a great deal on their intracellular location and their accessibility to leaf protease (Onyango, 1996).

Chlorophyll Content:

Both varieties showed decreasing value in chlorophyll content due to low photosynthesis rate resulting from water stress. The chlorophyll content in maize treated with T2 and T3 in both varieties was apparently lower than the control maize. The observed reduction of chlorophyll in water stressed plants may be due to a reduction in the lamellar content of the light harvesting chlorophyll a/b protein (Randall et al., 1977). Under more prolonged water deficit, dehydration of plant tissue can result in an increase in oxidative stress, which causes deterioration in chloroplast structure and an associated loss of chlorophyll. This leads to a decrease in the photosynthetic activity (Jafaret et al., 2004). Reduction in chlorophyll concentration in water stressed plants could
indirectly lead to a decrease in photosynthetic activity. Increase in chlorophyll concentration in well watered plants, could have in turn led to increased protein synthesis and this has a direct consequence on the plant growth and photosynthesis hence increase in shoot dry weight (Muthomi and Musyimi, 2009).

**Lipid Peroxidation:**

The level of lipid peroxidation was measured in terms of MDA content which is a product of lipid peroxidation. MDA content of leaves started to increase by the time stress was applied. Noticeably, the MDA content in T1, T2 and T3 for both varieties did not increased appreciably during the experiment. However, there was an increasing trend in YSC subjected to T4. For the maize treated with T5 and T6 in both varieties, the MDA content decreased due to plant death. Higher lipid peroxidation and lower relative water content have also been reported in water stress conditions by Sairam and Saxena (2000). The increase in MDA content under stress conditions suggests that water stress could induce membrane lipid peroxidation by means of reactive oxygen species (ROS). Under water shortage, decreasing CO₂ assimilation coupled with changes in photosystem activities and photosynthetic electron transport capacity results in excessive ROS production by the chloroplast. The hydrogen peroxide content increased linearly with increasing level of water stress in both the genotypes (Sairam and Srivastava, 2000). As a consequence of ROS, lipid peroxidation can lead to cellular membrane rupture in plants subjected to stress (Hernandez et al., 2000). Therefore, lipid peroxidation measured as the MDA content has been used as an indicator of stress-induced damage at the cellular level (Parvanova et al., 2004; Shao et al., 2005).

**Membrane Stability Index (MSI):**

In comparison to T1 maize, T2 and T3 maize for both varieties showed lower MSI at day 65 as the water stress increased in these treatments. The plasma membrane is generally protected from desiccation-induced damage by the presence of membrane-compatible solutes, such as sugars and amino acids. Therefore, a link may exist between the capacities for osmotic adjustment and the degree of membrane protection from the effects of dehydration (Liley and Ludlow, 1996). The increased activities of antioxidant enzymes act as a damage control system and thus provide protection from oxidative stress, which otherwise could cause lipid peroxidation resulting in damage to the cell membrane and organelles, protein and DNA structure and inhibit photosynthesis and other enzyme activities (Iqbal and Bano, 2009). The lower membrane stability index reflects the extent of lipid peroxidation, which in turn is a consequence of higher oxidative stress due to water stress conditions. The MSI value decreased linearly with increased water stress level. Maintenance of membrane integrity and function under a given level of dehydration stress has been used as a measure of drought tolerance by Premchandra et al. (1990).

**Conclusion:**

Maize shows significant reduction in biochemical parameters which are protein content, MDA content, chlorophyll content and membrane stability index but increases in proline content during water stress, in comparison with the control treatment (T1). YSC shows highest proline content compared to TSC. Therefore, YSC can adapt to the progressive water deficit conditions. However, further investigation on the resistance mechanisms in YSC at the molecular level is required.

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**References**


