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Evaluation of Maize Cultivars for Drought Tolerance Based on Physiological Traits Associated with Cell Wall Plasticity

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ABSTRACT

Maize is negatively affected by many environmental factors during growth, with drought stress being one of the most common causes for reduction in maize yield world-wide. There is wide variation in stand establishment for various maize cultivars to water deficit condition, such as occur in in arid and semiarid regions of the world. An experiment was carried out to evaluate five cultivars of maize viz; EV-1097, Agaiti-2002, Sawaan-3, Islamabad Gold and EV-1098 for tolerance to water deficit based on their cell wall plasticity characteristics. The crop was sown in pots; each treatment consisted of five pots having three plants each, and each pot was watered uniformly up to soil capacity from germination to the three leaf stage as required. Then, the water supply was reduced to half soil capacity level for one week, and then to one-third capacity for the coming two weeks. After 35 days, irrigation was stopped for one week. After one week without water, the plants showed symptoms of drought stress, at which time data was recorded on leaf growth rate, chlorophyll content, leaf water potential, protein content, proline content, relative water content, specific leaf weight, and sugar content. Control plants were maintained at full soil capacity. The experiment was laid out by using Completely Randomized Design with three replications and five treatments (cultivars). The response of the five cultivars examined under these water deficit stress treatments differed dramatically. Cultivars EV-1097 and Agaiti-2002 were amongst the best performers, showing maximum cell wall plasticity, having the highest leaf growth rate, proline, protein, sugar and relative water contents, as well as the highest specific leaf weight, leaf water potential, and chlorophyll content when compared with other cultivars. EV-1097 and Agaiti-2002 were thus the most tolerant cultivars to these water deficit treatments. The Islamabad gold and EV-1098 cultivars were the least tolerant to water stress conditions.

Keywords: Maize, Leaf Growth, Proline, Sugar, Water Stress, Protein, Chlorophyll, Water Potential.

INTRODUCTION

Maize occupies 147.6 million hectares, with 701.3 million metric tons of grain produced annually, world-wide. In Pakistan, in the year 2009-10, maize production was 4,271 thousand tones, and the area under cultivation was 1,083 thousand hectares (GOP, 2012). Maize occupies the third most important place after wheat and rice for human and animal utilization (Araus *et al*., 2002). Drought causes many morphological and physiological changes in plants such as a decrease in photosynthesis, reduction in leaf area, reduction in starch content, reduction in leaf water content, and effects on carbohydrate partitioning leading to the synthesis of new compounds such as hexoses, sucrose, and acyclic and cyclic polyols (Bartels and Nelson, 1994).

Maize is very sensitive to climatological drought, and regardless of the stage in maize's life cycle, drought can adversely affect yield. For example, if drought arises during the sensitive seedling stage, then overall crop stand can be poor or completely fail (Ibrahim *et al*., 2001). Moderate drought stress causes stomatal closure and reduction in gas exchange, while the severe drought can causes shut down basic metabolism all together leading to plant death (Jaleel *et al*., 2007). In maize, drought stress has been shown to alter sugar levels, such as raffinose which play important roles in maintaining cell wall plasticity. The cell wall also takes part in cold and desiccation protection of the plant (Pattanagul and Madore, 1999). Mechanisms involved in drought tolerance in maize include accumulation of the solutes such as proline, betaines, polyols, and sugars, compounds that not only maintain cell wall plasticity but also contribute to maintenance of leaf turgor (Yancey *et al*., 1982).

Water deficit can cause growth inhibition, which leads to a decrease in cell wall plasticity, and causes destruction to a variety of other essential cellular structures (Nonami and Boyer, 1990). Even if plants are watered soon after experiencing water deficit stress, they will not recover the previous levels of cell wall extensibility and leaf growth as before the stress treatment. For example, maize seedlings exposed to water stress had reduced cell wall plasticity in elongating areas an overall decrease in leaf growth (Chazen and Neumann, 1994).

Assessment of the drought tolerance in maize varieties can be accomplished in a many ways, such as on the basis of nutrient uptake efficiencies, plant emergence characteristics, water use efficiency, radiation use efficiency, deep root development, hormonal regulation, canopy temperature, osmotic adjustment, delayed senescence, grain number maintenance, grain filling duration rate, and harvest index under drought, among others (Lin *et al*., 2004). Cell wall plasticity is one of the key indicators used to evaluate relative drought tolerance among varieties. Cell wall plasticity is the ability of the wall to adjust or maintain its shape in such a manner that enhances cell wall turgor and improves the plant's ability to tolerate drought (Ralph *et al*., 2004). Drought tolerant varieties, for example, show relatively less reduction in leaf area relative to sensitive lines, associated in large part with maintenance of cell wall plasticity and continued leaf growth (Chazen and

Neumann, 1994). The objective of this study was to evaluate five maize cultivars for drought tolerance on the basis of their cell wall plasticity at the vegetative stage.

MATERIALS AND METHODS

A pot experiment was carried out to evaluate five maize cultivars for tolerance to water deficits based on measures of cell wall plasticity. The experiment was carried out in the Crop Physiology Laboratory, Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. The seeds of the five maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold, and EV-1098) were obtained from the National Agriculture Research Council, Islamabad, Pakistan. Two levels (control and water stress) of soil moisture were maintained for five maize cultivars adopting completely randomized design with two factors (cultivars and moisture levels) repeated thrice. Ten kg of dry soil was placed in every pot and seeded @ 5 seeds pot^{-1}. Thinning was performed to maintain three plants per pot and five pots were maintained for each cultivar as one replicate. Pots were watered uniformly up to full soil water holding capacity from germination to the three-leaf stage to maintain near full soil water capacity. Monitoring of soil moisture was done after every two days through the gravimetric method, for maintaining the required amount of water. Then the water supply was reduced to half of soil capacity level for one week, and one-third for the coming two weeks. To determine the field capacity, prepared the saturated soil paste and shifted to a porous Buckner funnel for removing the extra water. The moisture retained represents the field capacity, was recorded by gravimetric method (Anderson and Ingram, 1993). After 35 days, the water supply was ended for one week, and then, when plants showed symptoms of water stress, then data was recorded for various parameters. The control plants were watered regularly to maintain near full soil water capacity.

1. **Leaf Growth Rate (cm/day):**

Leaf growth rate was measured with the help of measuring scale at 3-4 days intervals, for five total measurements, from the upper and lower most leaf. Leaf growth rate was measured in cm/day after the water stress application.

2. **Chlorophyll Content (mg/ml):**

Chlorophyll contents were measured as in Witharn *et al*. (1971). One gram of fresh leaves from each plant in the experiment was excised and ground with 4 ml of 80% acetone and allowed to settle in the dark at room temperature for 10 min. The extract was then centrifuged at 2000 rpm for 5 min to clear the suspension. A Schimadzu UV-260 spectrophotometer was used to determine the absorbance of supernatant at 645 nm for chlorophyll ‘a’ and 663 nm for chlorophyll ‘b’ against 80% acetone blank. Total chlorophyll was determined as:

$$\text{Total Chlorophyll Content} = 20.2 \times \text{Chlorophyll a} + 8.02 \times \text{Chlorophyll b}$$

3. Leaf Water Potential (-MPa):

Leaf water potential was measured as in Turner (1974). A single fully expanded leaf was sealed in a pressure chamber with a cut surface. Pressure was applied to the leaf from the cylinder until xylem sap appeared at the cut surface. The balancing pressure was regarded as the tension originally existing in the xylem sap and approximately equal to the water potential of cells. Measurements were made on fully expanded upper leaf (L1) and the next leaf (L2) of each plant to determine the mean leaf water potential.

4. Total Soluble Protein Content (mg/g):

Total soluble protein contents were determined based on the method described by Lowry *et al*. (1951) by using Bovine Serum Albumin (BSA) as standard. Fresh leaf material (0.1 g) was homogenized in 5 ml of phosphate buffer solution and centrifuged at 15000 rpm for 15 min. Then, 0.5 ml of extract was placed in a test tube with, 0.5 ml distilled water and 3 ml of bio-red color dye. The extract was shaken in orbital shaker and absorbance of samples was read at 595 nm (Schimadzu UV-260 spectrophotometer). Phosphate buffer was used as a blank sample**.**

$$\text{Total Soluble Protein Content} = \frac{\text{Absorbance of sample} \times \text{K Value} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 100}$$

5. Proline Content (mg/g):

Proline content was determined as in Bates *et al*. (1973). Fresh leaves (0.5 g) were blended in 3 % sulfosalycylic acid and allowed to settle. Then, 2 ml of the filtrate was placed in a test tube and mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. At 100 ^{0}C, the mixture was boiled for 1 hr. After that reaction was terminated in an ice bath, 4 ml of toluene was added. The organic phase was collected and the absorbance was read at 520 nm (Schimadzu UV-260 spectrophotometer). Toluene was used as blank sample.

$$\text{Proline Content} = \frac{\text{Absorbance of Sample} \times \text{K Value} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 100}$$

6. Relative Water Content (%):

Relative water content was determined following the method of Barrs and Weatherly (1962). Leaves were cut from plants and weighed immediately as fresh weight (FW). Then these leaves were soaked in deionized water for 24 hr, and weighed again to determine turgid weight (TW). At the end leaves were dried at 85 ^{0}C and their dry weight (DW) was measured. Relative water content (RWC) was calculated as:

$$RWC = (FW-DW) / (TW-DW) \times 100$$

7. Specific Leaf Weight (mg/cm^2):

Specific leaf weight was determined as in Yang *et al*. (2003). From each plant 2-3 leaves were cut and their surface area was measured. Then the leaves were dried at 70 ^{0}C to a constant dry weight and leaf weight was calculated as dry weight per unit leaf area.

$$\text{Specific Leaf Weight} = \text{Dry Weight} / \text{Unit Leaf Area}$$

8. Total Soluble Sugar Content (mg/g):

Total soluble sugar content was determined as in Dubois *et al*. (1951). Fresh leaves (0.1 g) of each sample were homogenized in 5 ml of 80 % ethanol in test tubes. Test tubes were kept in water bath of 80 ^{0}C for 1 hr, and sample extracts of 0.5 ml were transferred to another test tube, and 0.5 ml distilled water and 1 ml of 5 % phenol then added and allowed to incubate for 1 hr. Finally after 1 hr, 2.5 ml sulphuric acid was added to the test tubes, and shaken well on an orbital shaker. Absorbance was read at 490 nm on Spectrophotometer (Schimadzu UV-260 spectrophotometer) and glucose was used as standard.

$$\text{Total Soluble Sugar Content} = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{Dilution Factor}}{\text{Weight of sample} \times 100}$$

Statistical Analysis:

Analysis of variance was determined and means were compared by employing Least Significant Difference (LSD) Test at 5 % level of probability. The statistical work was done using the computer based statistical package MSTATC according to Steel *et al*. (1997).

RESULTS AND DISCUSSION

The effect of soil water deficit on diverse maize cultivars was determined by studies of cell wall plasticity (CWP), specifically impacts associated with leaf growth rate during the juvenile stage. The cultivar EV-1097 showed the maximum value of leaf growth rate under water deficit stress conditions (4.16 cm/day); as well as in standard irrigation treatments (5.87 cm/day) (Fig. 1). The lowest leaf growth rate (3.26 cm/day) was recorded in Islamabad Gold. Drought tolerant cultivars are described here as those having more leaf growth during water deficit conditions when compared to susceptible lines. The cultivar EV-1097 showed the maximum leaf growth rate so, it is considered under terminology used here as having more CWP, while Islamabad Gold having the minimum CWP associated with the lowest mean leaf growth rate. Nonami *et al*. (1997) revealed how water stress creates hydraulic limitations to water uptake, cell wall plasticity, and subsequent growth rates. As reported by Neumann (1997), we report here that that water stress plays an important role in the growth of maize, wherein even moderate water stress leads to reduction of cell wall plasticity and inhibition of leaf growth. And the significant variation in CWP among the five cultivars examined here reveals potential genetic variation for this trait that could be exploited in a maize breeding program targeting arid and semi-arid regions of the world.

Besides CWP, the chlorophyll content of maize cultivars at the vegetative stage differs significantly (Fig. 2). Maximum chlorophyll content was observed in EV-1097 (44.18 mg/ml) and the minimum value was observed in Islamabad Gold (19.27 mg/ml). Relative chlorophyll content has a positive relationship with photosynthetic rate, and leaf chlorophyll content can be affected by many abiotic factors like heat, drought, and salt. Higher the drought stress, chlorophyll content will be affected severely (Guo, 1996). Chlorophyll contents are also directly related to leaf growth rate, and our results showing

that EV-1097 had both the highest leaf growth rate and highest leaf chlorophyll content is consistent with previous studies linking chlorophyll content, photosynthesis, and growth. Likewise, the lowest chlorophyll content of Islamabad Gold is constituent with our findings that it also has the lowest leaf growth rate among cultivars. Our results are in line with those of Cicek and Cakirlar (2008) who reported that drought stress leads to a strong reduction of the uptake of CO_2 due to a reduction in leaf growth rate, cell wall plasticity of the leaf, photosynthetic rate, and ultimately a reduction in chlorophyll synthesis.

Leaf water potential also differs significantly in maize cultivars (Fig. 3). The highest leaf water potential among the cultivars examined was observed in EV-1097 (-1.41 MPa), whereas Islamabad Gold showed the lowest water potential of -1.61 MPa at the same vegetative stage. Leaf water potential is the potential energy of water per unit volume relative to pure water in reference conditions, and is directly related to plant growth, with more negative potentials associated with less growth. Stresses caused by water and high temperature stress can also lower leaf water potentials. EV-1097 has highest leaf water potential, and as expected, this is associated with the highest leaf growth rate, while Islamabad Gold has the lowest leaf growth rate and water potential. Just as described by Hall (2001) and Perdomo *et al*. (1996), our maize plants placed under water deficit stress conditions exhibited lower leaf water potentials and leaf growth rates, which are associated with reductions in cell wall plasticity.

Total soluble proteins differ significantly in maize cultivars (Fig. 4). The highest amounts of total soluble proteins were observed in the cultivar EV-1097 at 163.20 mg/g, whereas Islamabad Gold showed the minimum of 137.83 mg/g at the same vegetative stage. Salekdeh *et al*. (2002) revealed how the leaves in which the protein level was higher exhibited higher leaf water potentials, leaf growth rate, and maintenance of cell wall plasticity when compared to the leaves of cultivars having lower leaf protein contents. Our results are consistent with those of Salekdeh *et al*. (2002), and reveal significant variation among the five cultivars tested in this study.

Proline contents do not differ significantly in the maize cultivars examined here (Fig. 5). The highest proline contents were observed in EV-1097 at 3.45 mg/g, whereas Islamabad Gold showed the lowest values of 3.24 mg/g at the same vegetative stage. Ashraf and Foolad (2006); Qayyum *et al*. (2011) observed that in response to environmental stresses including drought, salinity extreme temperature, UV radiation and heavy metals, proline and glycine betaine are two major organic osmolytes that accumulate in a variety of plant species. Srinivas and Balasubramanian, (1995) described that proline acts as an osmolytes for osmotic adjustment and enhances leaf growth rate that ultimately enhances the CWP. Our findings lend support to the supposition that the EV-1097 cultivar can perform better under water stress conditions because of its high proline contents, this tolerance to water deficit being influenced also by the combination of other physiologically beneficial attributes described above.

Relative water content of the leaf is the relative proportion of the fresh weight, dry weight and the turgid weight of the stressed plant (Fig. 6). The relative water content of the

maize cultivars at vegetative stage differs significantly. The highest relative water content was observed in EV-1097 at 86.34 % and Islamabad Gold showed the minimum of 74.27 % at the same vegetative stage. Decov *et al*. (2000) described that relative water content and water potential in leaves of maize decreases significantly when leaves are subjected to drought, which reduces the leaf growth rate and ultimately its cell wall plasticity. EV-1097 has the highest relative water content associated with highest leaf growth rate among the cultivars, while Islamabad Gold exhibited the lowest relative water content and leaf growth rate, having the lowest cell wall plasticity and tolerance to water deficit.

Specific leaf weight is the proportion of leaf dry weight to unit leaf area. Specific leaf weight differs significantly in maize cultivars (Fig. 7). The highest specific leaf weight was observed in EV-1097 at 0.57 mg/cm^2, whereas Islamabad Gold showed the minimum of 0.34 mg/cm^2 at the same vegetative stage. Agaiti-2002 and Sawaan-3 also showed significance differences. In maize, it has been shown that specific leaf weight decreases as water stress increases, and this is associated with reduced assimilate formation in leaves, leaf growth rate, and CWP (Cutler *et al*., 1980). Our results also showed that specific leaf weight decreased under the drought condition in the five maize cultivars. EV-1097 showed the highest specific leaf weight (along with maximum leaf growth rate described above), and so it may be expected to have high tolerance to climatological drought, whereas Islamabad Gold showed the lowest specific leaf weight and leaf growth rate, indicating it likely will have higher susceptibility in the field to drought stress conditions.

Total soluble sugar content is an important physiological indicator of a plants ability to survive under drought stress. Total soluble sugar of maize cultivars at vegetative stage differed significantly (Fig. 8). The highest total soluble sugar was observed in EV-1097 at 50.32 mg/g, whereas Islamabad Gold showed the lowest of 40.30 mg/g at the same vegetative stage. Trouverie *et al*. (2003) reported that accumulation of simple sugars such as glucose and fructose could be associated with an increase in the leaf cell wall plasticity and membrane turgidity. Our results were consistent with those of Trouverie *et al*. (2003), revealing that the cultivar EV-1097 has the highest leaf growth rate associated with higher sugar accumulation under drought stress.

The maize cultivars examined here differed significantly in response to water deficits based on measures of many physiological characteristics associated with differences in cell wall plasticity. The cultivar EV-1097 had the highest leaf growth rate, chlorophyll content, leaf water potential, protein content, proline content, relative water content, specific leaf weight, and total soluble sugars, and so it is likely that the EV-1097 cultivar would have the highest drought tolerance under field conditions. The cultivar Agaiti-2002 is the next best variety of maize behind EV-1097 for predicted drought tolerance. The cultivar Islamabad Gold produced the least proline, protein, sugar and chlorophyll contents, as well as the lowest specific leaf weight, leaf water potential, and relative water content under water deficit, and is likely the most susceptible cultivar to drought. The maize variety EV-1097, as well as the similarly performing Agaiti-2002, may be excellent cultivars to use in growing regions where climatological drought occurs regularly.

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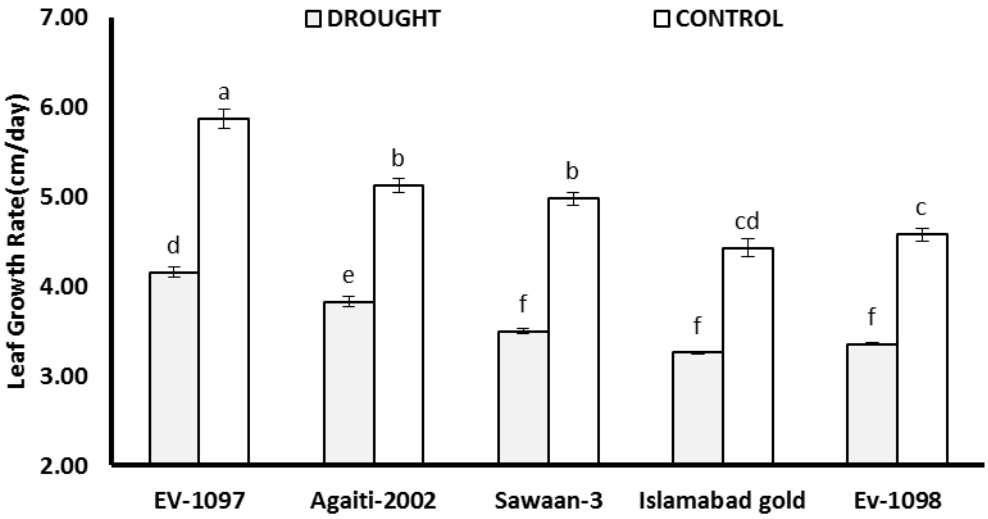

Figure 1: Leaf growth rate (cm/day) of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage (56 days after sowing) under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

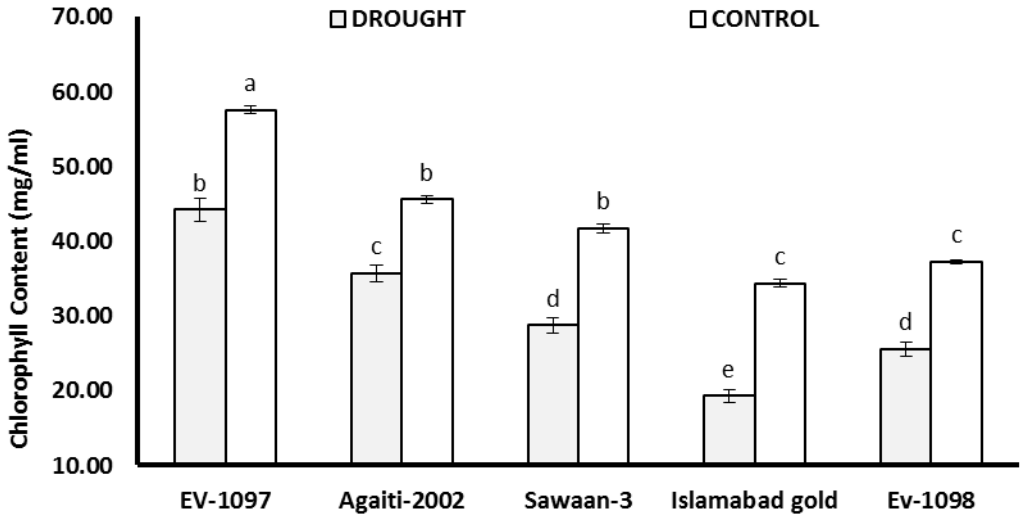

Figure 2: Total leaf chlorophyll content of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

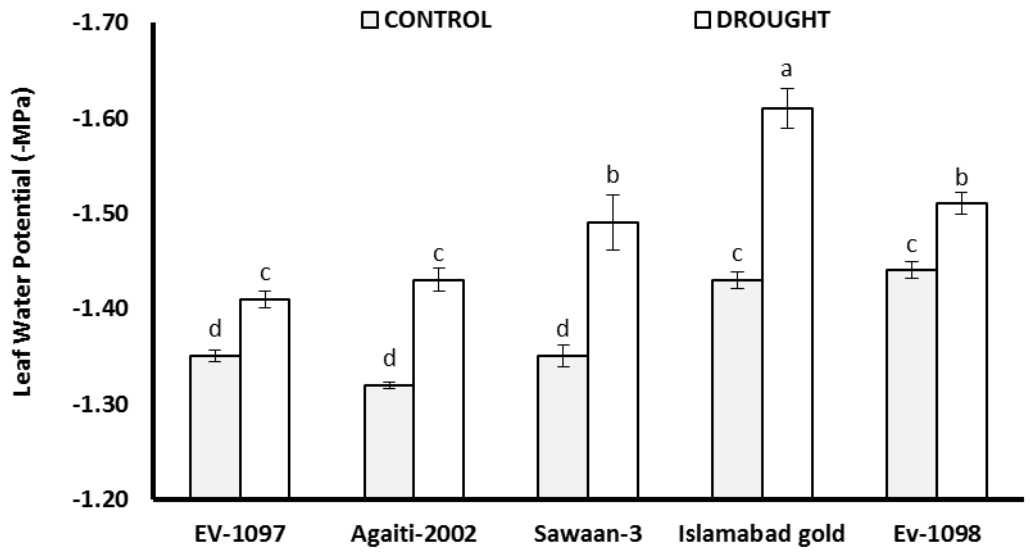

Figure 3: Leaf water potential of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

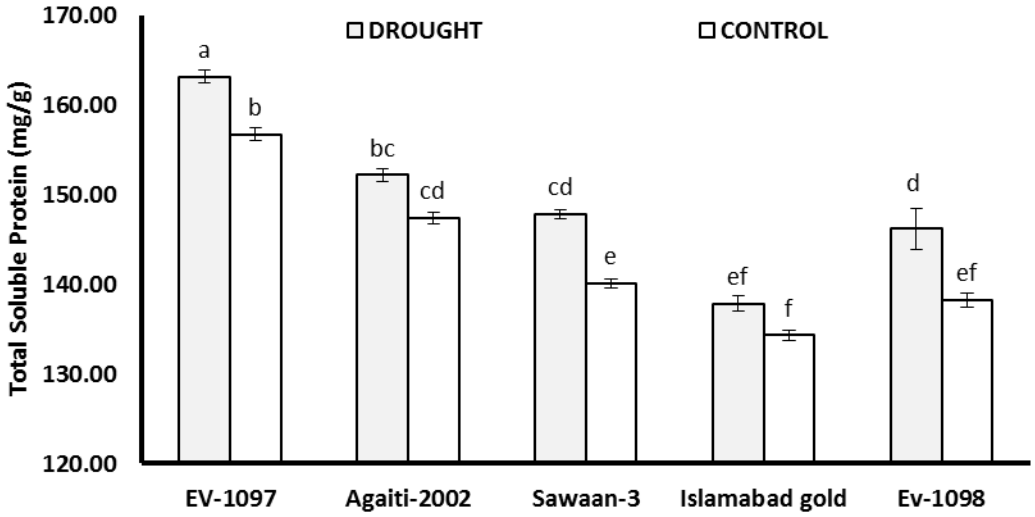

Figure 4: Total soluble protein contents of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

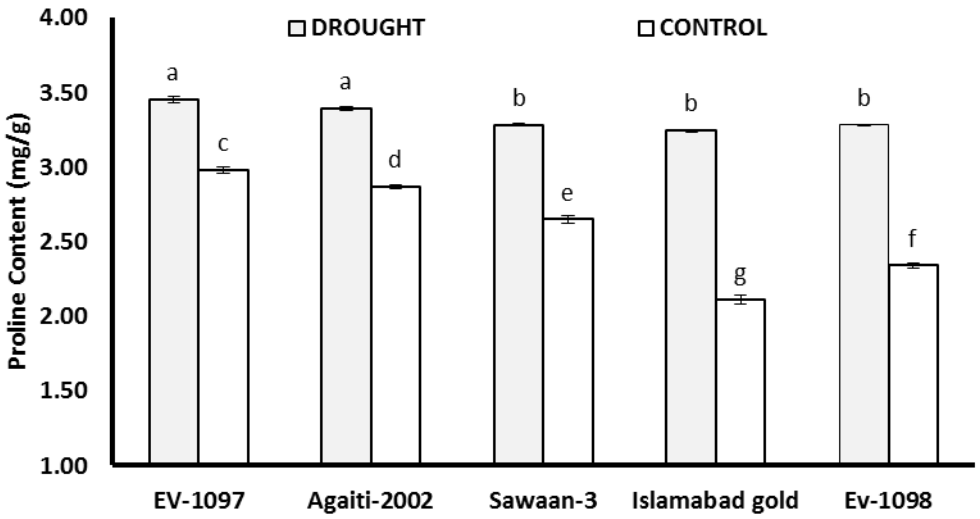

Figure 5: Proline content of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

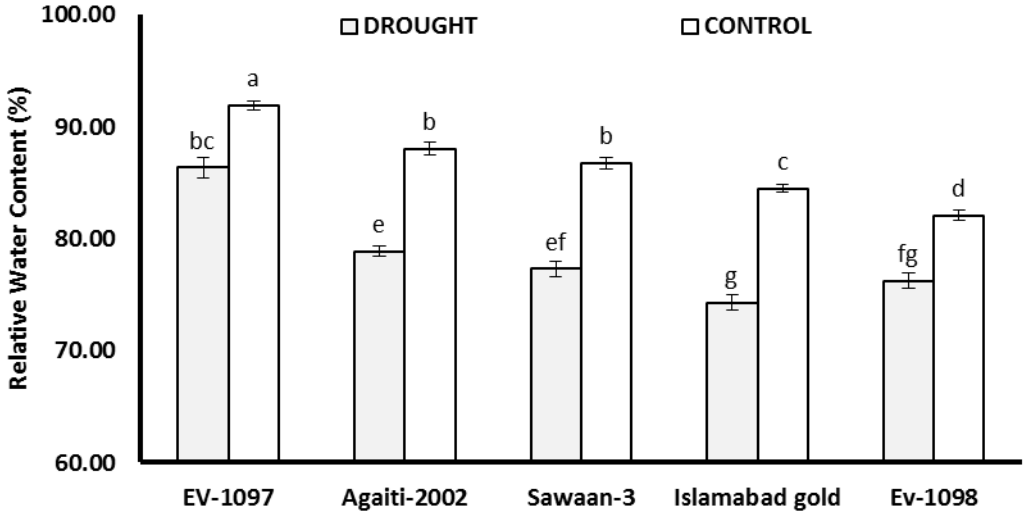

Figure 6: Relative water content of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

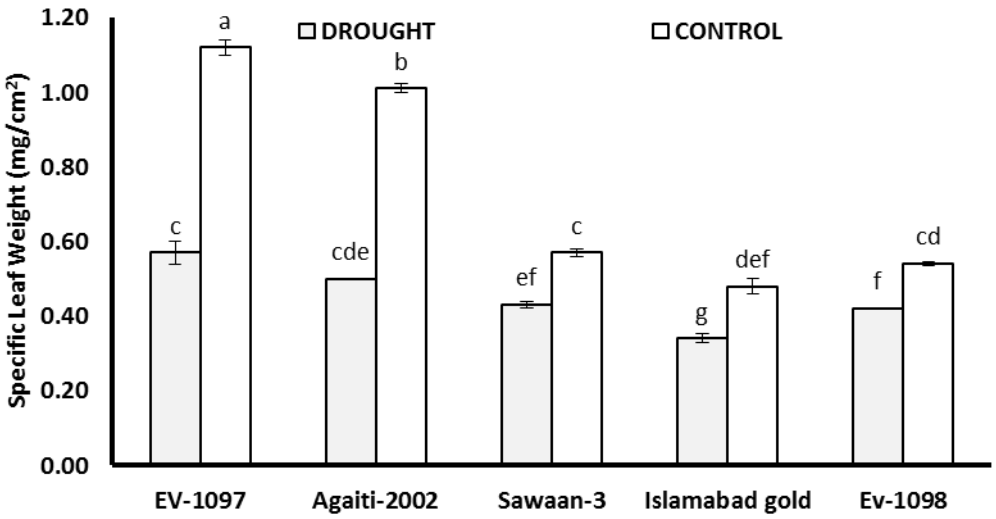

Figure 7: Specific leaf weight of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).

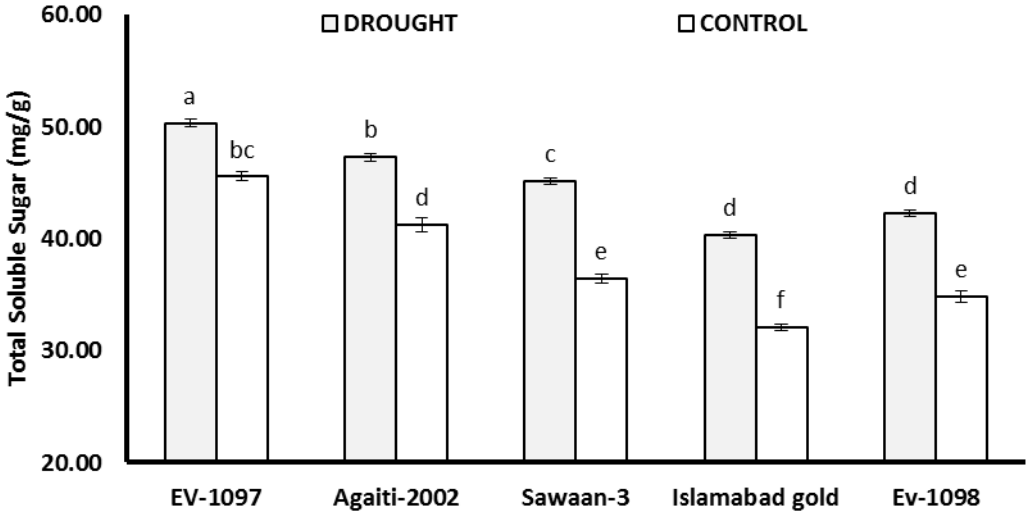

Figure 8: Total soluble sugars of maize cultivars (EV-1097, Agaiti-2002, Sawaan-2003, Islamabad Gold and EV-1098) at vegetative stage under water deficit stress and control conditions (all means [± s.d.] are significantly different at P<0.05).