

DISCRIMINATING SOME PROSPECTIVE CULTIVARS OF MAIZE (*ZEA MAYS* L.) FOR DROUGHT TOLERANCE USING GAS EXCHANGE CHARACTERISTICS AND PROLINE CONTENTS AS PHYSIOLOGICAL MARKERS

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Abstract

A greenhouse experiment was conducted to discriminate maize (*Zea mays* L.) cultivars for drought tolerance using gas exchange characteristics and proline contents as physiological markers. Seven maize cultivars viz., Sahiwal-2002, Sadaf, EV-5098, Pak-Afgoyee, Agaiti-2002, Agaiti-85 and EV-1098 were grown under well watered or water deficit condition (60% of field capacity). Imposition of water deficit condition decreased the shoot and root fresh and dry weights, relative water content, photosynthetic and transpiration rates, stomatal conductance, while increased root/shoot ratio, chlorophyll *a*, *b* and chlorophyll *a/b* ratio, proline content and shoot N and K in all maize cultivars. Effect of drought stress was non-significant on shoot or root P and shoot N. Of all maize cultivars, cv. Agaiti-85 was found to be relatively better in growth, proline accumulation and gas exchange characteristics under drought stress as compared to the other cultivars.

Introduction

Maize is one of the major world food crops. However, in areas where water availability is limited, maize grain losses may reach up to 24 million tons per year that is equivalent to 17% of well watered production in the world (Edmeades *et al.*, 1999). In Pakistan, water stress is also a major obstacle for maize production. Approximately, 65% of the maize in Pakistan has access to irrigation, while the remaining portion strictly depends on natural rain (Anon., 2007). Under mild drought stress, the reduction in maize crop is up to 10-13%, but under severe drought, the loss may increase many times. The problem becomes more alarming under arid conditions covering an area of 25-30% of major crops planted in Pakistan (Farooq *et al.*, 2007).

In view of all these problems, it is crucial to understand the mechanisms that plants use to adapt under water limited conditions (Ober & Sharp, 2003). It is now well established that, water deficit reduces plant growth by decreasing leaf area, net CO₂ assimilation rate, transpiration rate, closure of stomata, chlorophyll fluorescence (F_v/F_m), lower chloroplast activity and nutrient imbalance (Akram *et al.*, 2007; 2008; Ashraf *et al.*, 2007; Ali *et al.*, 2008). Plant growth directly depends on availability of water and water use efficiency (Edwards & Walker, 1983) and can be increased by increasing WUE which is affected by photosynthetic rate (Ehleringer & Monson, 1993). Net CO₂ assimilation rate is positively correlated with net plant production and yield (Lawlor, 1995). Photosynthetic activity is directly related to dry mass production of plants and affects plant growth and yield (Natr & Lawlor, 2005). A positive correlation between photosynthetic rate and growth rate has been observed by many scientists in various crops like tomato and turnip (Makela *et al.*, 1998, 1999), *Brassica* species (Nazir *et al.*, 2001), and wheat (Ashraf & Bashir, 2003).

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Proline being the low molecular weight osmoprotectant, accumulates in larger amounts than other amino acids in water stressed plants (Pedrol *et al.*, 2000; Tamura *et al.*, 2003) and plants accumulate high concentration of proline under water deficit conditions (Parida *et al.*, 2007). Proline accumulation is thought to be a key adaptation under severe water deficit conditions (Akram *et al.*, 2007). The role of proline as a protective agent against reactive oxygen species is also very important (Hare *et al.*, 1999). High accumulation of proline is effective in chlorophyll stability (Ashraf *et al.*, 1995) and helps in stress tolerance of plants (Bartels & Nelson, 1994; Hanson *et al.*, 1994). Drought tolerance is positively correlated with high accumulation of proline in many crops like wheat and barley (Nayyar & Walia, 2003), rice (Hsu *et al.*, 2003) and *Brassica juncea* (Madan *et al.*, 1995). Transgenic tobacco plant also showed high drought resistance by having high proline content (Kishor *et al.*, 1995). Under drought stress, high accumulation of proline in the cytoplasm helps in osmotic adjustment of maize plants (Ketchum *et al.*, 1991; Voetberg & Sharp, 1991).

Maize having great economic importance, both for humans and animals as food and forage, the development of maize with high and stable yield under low moisture is an important priority for today's needs, as access to drought tolerant cultivars may be the only reasonable alternative to many small scale farmers (Tabassum, 2004). In view of the importance of maize, the principle objective of the present study was to appraise the differential resistance of 7 maize cultivars to drought stress using gas exchange characteristics and proline contents as physiological markers.

Materials and Methods

To discriminate some prospective maize (*Zea mays* L.) cultivars for drought tolerance using gas exchange characteristics and proline contents as physiological markers, 7 maize cultivars (Sahiwal-2002, Sadaf, EV-5098, Pak-Afgoyee, Agaiti-2002, Agaiti-85 and EV-1098) were grown in a net house in the Old Botanical Garden, University of Agriculture Faisalabad, during September to November 2007. The average day and night temperatures were $31 \pm 2^\circ\text{C}$ and $19 \pm 3^\circ\text{C}$, respectively. The relative humidity ranged from 32.6 to 51.4% and day length from 11-12 h.

The seed materials of maize cultivars, used in this study, were obtained from Maize and Millets Institute, Yousafwala (Sahiwal). Ten seeds were sown in each plastic pot (21 cm diameter and 25 cm depth) containing 10 kg air-dried soil. Saturation percentage of the soil used was 35.08, pH, 7.89 and ECe, 1.63 dS m^{-1} . The plants were allowed to establish for 12 days after sowing before the start of drought stress. The plants were thinned to 6 plants per pot. Drought stress treatments were control (normal watering) or 60% of field capacity. Plants were harvested, 15 days after drought stress. The data for the following attributes were recorded.

Plant biomass: Two plants from each pot were uprooted carefully, washed with distilled water and fresh weights of both shoots and roots recorded. Then, plant samples were oven-dried at 65°C up to constant weight and dry weights recorded.

Chlorophyll contents: Chlorophyll 'a' and 'b' contents were determined according to Arnon (1949). Fresh leaves (0.5g) were ground in 80% acetone and centrifuged at $10,000 \times g$ for 5 minutes. Absorbance of the supernatant was read at 645 and 663 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Chlorophylls *a* and *b* were calculated using the following formulae:

$$\text{Chl. } a \text{ (mg g}^{-1} \text{ f.wt)} = [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] \times V/1000 \times W$$

$$\text{Chl. } b \text{ (mg g}^{-1} \text{ f.wt)} = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times V/1000 \times W$$

V = volume of the extract (ml), W = weight of the fresh leaf tissue (g)

Relative water contents (RWC): Relative water contents of fully expanded third leaf from top were determined following Turner (1986) using the following equation:

$$\text{RWC} = [\text{FW} - \text{DW}] \times 100 / [\text{TW} - \text{DW}]$$

FW = Fresh weight, DW = Dry weight, TW = Turgid weight

Proline determination: A fully expanded second leaf was used to determine proline according to the method of the Bates *et al.* (1973) after extraction with 3% 5-sulphosalicylic acid at room temperature. Proline concentration was calculated on fresh weight basis as follows:-

$$\mu\text{mole proline g}^{-1} \text{ fresh weight} = (\mu\text{g proline mL}^{-1} \times \text{mL of toluene}/115.5)/(\text{g of sample})$$

Gas exchange characteristics: An open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England) was used for the measurement of net CO₂ assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (*g_s*) and sub-stomatal CO₂ concentration (*C_i*) of a fully expanded youngest leaf of each plant. These measurements were made from 10:15 to 11:45 a.m. with the following specifications/adjustments: leaf surface area 11.35 cm², ambient CO₂ concentration (*C_{ref}*) 354.4 μmol mol⁻¹, temperature of leaf chamber varied from 31.5 to 37.8°C, leaf chamber gas flow rate (*v*) 392.8 mL min⁻¹, Molar flow of air per unit leaf area (*U_s*) 404.84 mol m⁻² s⁻¹, ambient pressure (*P*) 99.2 kPa, water vapor pressure (*e_{ref}*) into chamber ranged from 20.5 to 23.1 mbar, *PAR* (*Q_{leaf}*) at leaf surface was maximum up to 1048 μmol m⁻² s⁻¹.

Determination of mineral elements in plant tissues: The dried ground leaf or root material (0.1 g) was digested with a digestion mixture according to the method of Allen *et al.*, (1986). Nitrogen was estimated by micro-Kjeldhal method (Bremner, 1965). Phosphorus was determined by spectrophotometer (Hitachi-220) using Barton reagent as described by Jackson (1962). Potassium was determined with a flame photometer (Jenway, PFP-7).

Statistical analysis: Two-way (cultivars and drought stress) analysis of variance technique was employed for the statistical analysis of the data collected (Steel & Torrie, 1980). The mean values were compared with the least significance difference test (LSD) following Snedecor & Cochran (1980).

Results

Imposition of drought stress treatment (60% of field capacity) for a period of 15 days to 12 day-old plants of 7 maize cultivars viz., Sahiwal-2002, Sadaf, EV-5098, Pak-Afgoyee, Agaiti-2002, Agaiti-85 and EV-1098 had a significant reducing effect on shoot and root fresh and dry biomass. The difference among cultivars with respect to the above-mentioned attributes was non-significant. Cultivars differed significantly for root/shoot ratio. Cultivars Sahiwal-2002 and Agaiti-85 were higher while Sadaf and Pak-Afgoyee

lower in root/shoot ratio than the other cultivars. Root/shoot ratio increased with the imposition of water deficit conditions (60% of field capacity) in all cultivars except in Sadaf where the response was reverse (Table 1; Fig. 1).

There was a significant increase in chlorophyll *a* and *b* contents ($p \leq 0.001$; $p \leq 0.01$, respectively) under water deficit conditions in all cultivars except Sahiwal-2002 in which the response was reverse. A maximum increase in chlorophyll *a* and *b* contents was observed in EV-5098 and Agaiti-2002 (Table 1; Fig. 1). Chlorophyll *a/b* ratio was also increased under water deficit conditions, except in cvs. Sahiwal-2002 and Pak-Afgoyee in which the ratio was decreased (Table 1; Fig. 1).

Relative water content (RWC) decreased significantly ($p \leq 0.001$) in all maize cultivars due to drought stress. Cultivar Sahiwal-2002 was the highest of all the cultivars in RWC (Table 1; Fig. 2). Drought stress significantly ($P \leq 0.001$) increased the proline contents of all maize cultivars. However, difference in all cultivars in proline contents was consistent in all 7 maize cultivars but no significant difference was observed among the cultivars under well watered or water limited conditions (Table 1; Fig. 2).

There was a significant decrease ($p \leq 0.001$) in both photosynthetic and transpiration rates under water deficit conditions. Photosynthetic and transpiration rates were higher in cv. Pak-Afgoyee under both well watered and drought stressed conditions as compared to other cultivars, while the reverse was true for cv. Agaiti-2002 under well watered conditions (Table 1; Fig. 2).

Stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) were significantly decreased in all 7 cultivars of maize under water deficit condition. Cultivars also differed significantly in the two gas exchange characteristics. However, stomatal conductance and sub-stomatal CO_2 were lower in cvs. EV-5098 and EV-1098 than those in the other cultivars under water deficit conditions. In contrast, under well-watered conditions, g_s and C_i were higher in cvs. EV-5098 and EV-1098 than those in the other cultivars (Table 1; Fig. 2).

Water use efficiency (A/E) varied significantly among the cultivars. Response of the cultivars was different under control or water stress conditions. Under non-stressed conditions, cv. EV-5098 was the highest in WUE, while the same was true for cv. Agaiti-85 under water deficit conditions (Table 1; Fig. 2).

C_i/C_a ratio increased significantly in all cultivars under water limited conditions. A maximum increase in C_i/C_a ratio was observed in cv. Sahiwal-2002. The interaction between drought and cultivars for C_i/C_a ratio was also significant ($p \leq 0.001$) (Table 1; Fig. 2).

Shoot N decreased significantly due to drought stress in all maize cultivars except Pak-Afgoyee, while there was no significant effect of drought stress on root N content. Cultivar EV-1098 suppressed all the cultivars in root N content under water deficit conditions (Table 1; Fig. 3).

Water deficit condition had no significant increasing or decreasing effect on shoot or root phosphorous concentration, while the cultivars showed variable behaviour in root P. Overall, cv. Sahiwal-2002 was the highest of all cultivars in root P (Table 1; Fig. 3).

There was a highly significant ($p \leq 0.001$) effect of water deficit conditions on shoot and root K^+ concentrations. Water deficit conditions caused a significant increase in shoot K^+ in all cultivars. However, cultivar difference was not prominent for root K^+ (Table 1; Fig. 3).

Table 1. Mean squares from analyses of variance of data for growth, chlorophyll pigments, gas exchange characteristics and mineral nutrients of seven maize cultivars when 12 day-old plants were subjected to water deficit conditions for 15 days. ($n = 4$)

Source of variation	Degrees of freedom	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Cultivars (Cvs)	6	202.52ns	2.945ns	8.22ns	0.396**
Drought (D)	1	45873.6***	374.67***	820.65***	6.681***
Cvs x D	6	164.18ns	0.933ns	3.795	0.055ns
Error	42	7.31	0.50	3.21	0.113
Source of variation	Degrees of freedom	Root/ Shoot ratio	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll <i>a/b</i> ratio
Cultivars (Cvs)	6	0.011*	0.221**	0.055***	0.505ns
Drought (D)	1	0.036**	1.056***	0.067**	2.322*
Cvs x D	6	0.005ns	0.274**	0.037**	0.609ns
Error	42	0.004	0.067	0.009	0.391
Source of variation	Degrees of freedom	Relative water content	Proline contents	A	E
Cultivars (Cvs)	6	1823.2***	2.542ns	180.26***	1.446**
Drought (D)	1	9880.3***	625.62***	2746.4***	39.254***
Cvs x D	6	643.21*	2.357ns	145.36***	0.332ns
Error	42	245.7	1.601	9.374	0.372
Source of variation	Degrees of freedom	g_s	C_i	A/E	C_i/C_a
Cultivars (Cvs)	6	4996.2***	7059.5***	40.64***	0.053***
Drought (D)	1	108504.9***	105668.1***	1.865ns	0.877***
Cvs x D	6	3955.5**	8483.5***	86.92***	0.065***
Error	42	1064.7	044.7	3.906	0.009
Source of variation	Degrees of freedom	Shoot N	Shoot P	Shoot K	
Cultivars (Cvs)	6	28.888**	28.585ns	914.40***	
Drought (D)	1	823.09***	129.71ns	13801.2***	
Cvs x D	6	29.834**	69.73ns	1140.6***	
Error	42	8.683	32.59	59.36	
Source of variation	Degrees of freedom	Root N	Root P	Root K	
Cultivars (Cvs)	6	10.138***	55.761*	7.955ns	
Drought (D)	1	2.755ns	74.062ns	199.75***	
Cvs x D	6	3.779ns	66.799*	4.845ns	
Error	42	1.994	20.805	9.615	

*, **, *** = Significant at 0.05, 0.01, and 0.001 level, respectively.

ns = Non-significant

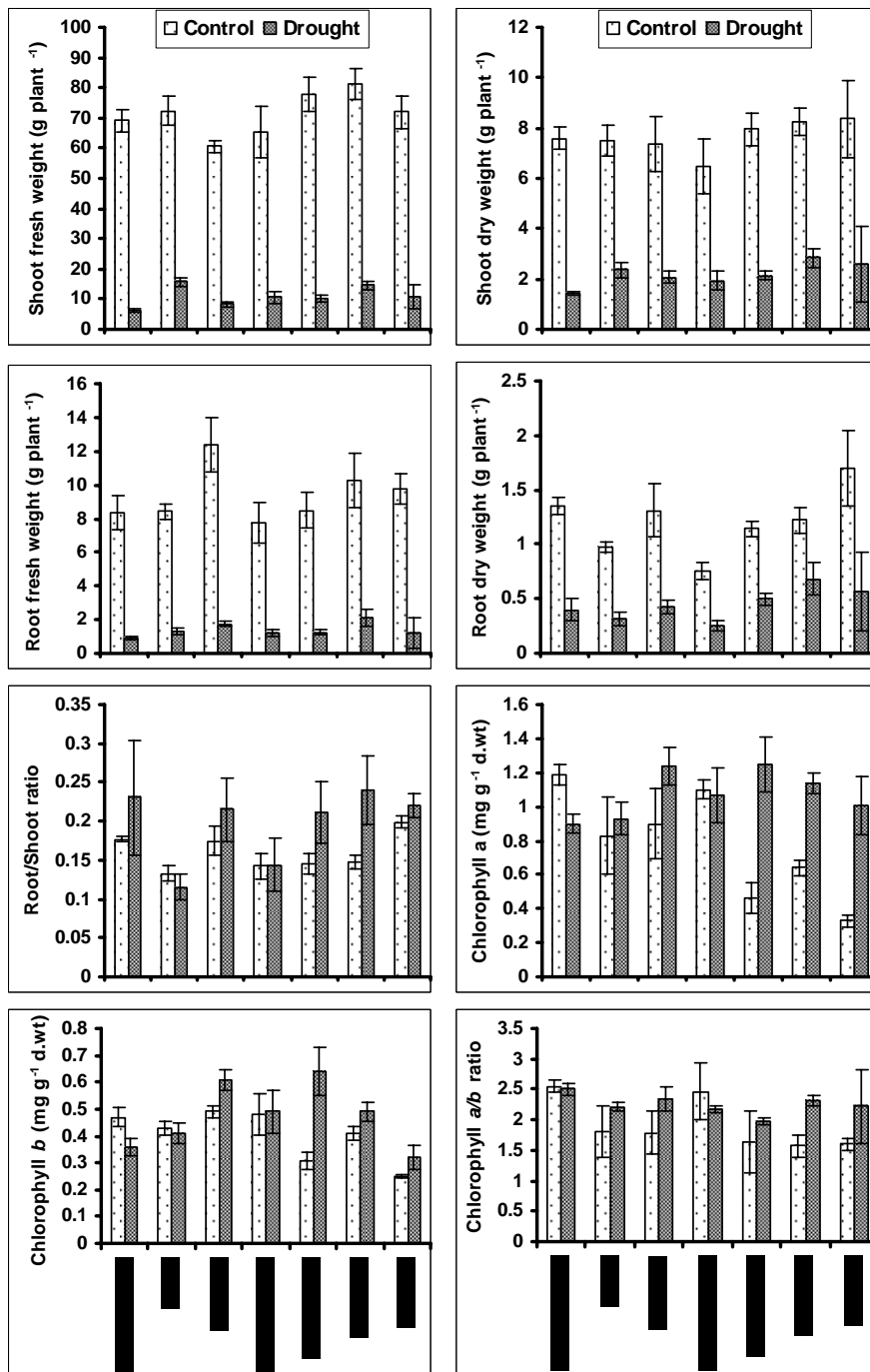


Fig. 1. Growth attributes and chlorophyll contents of 7 maize (*Zea mays* L.) cultivars when 12 day-old plants were subjected to water deficit conditions for 15 days.

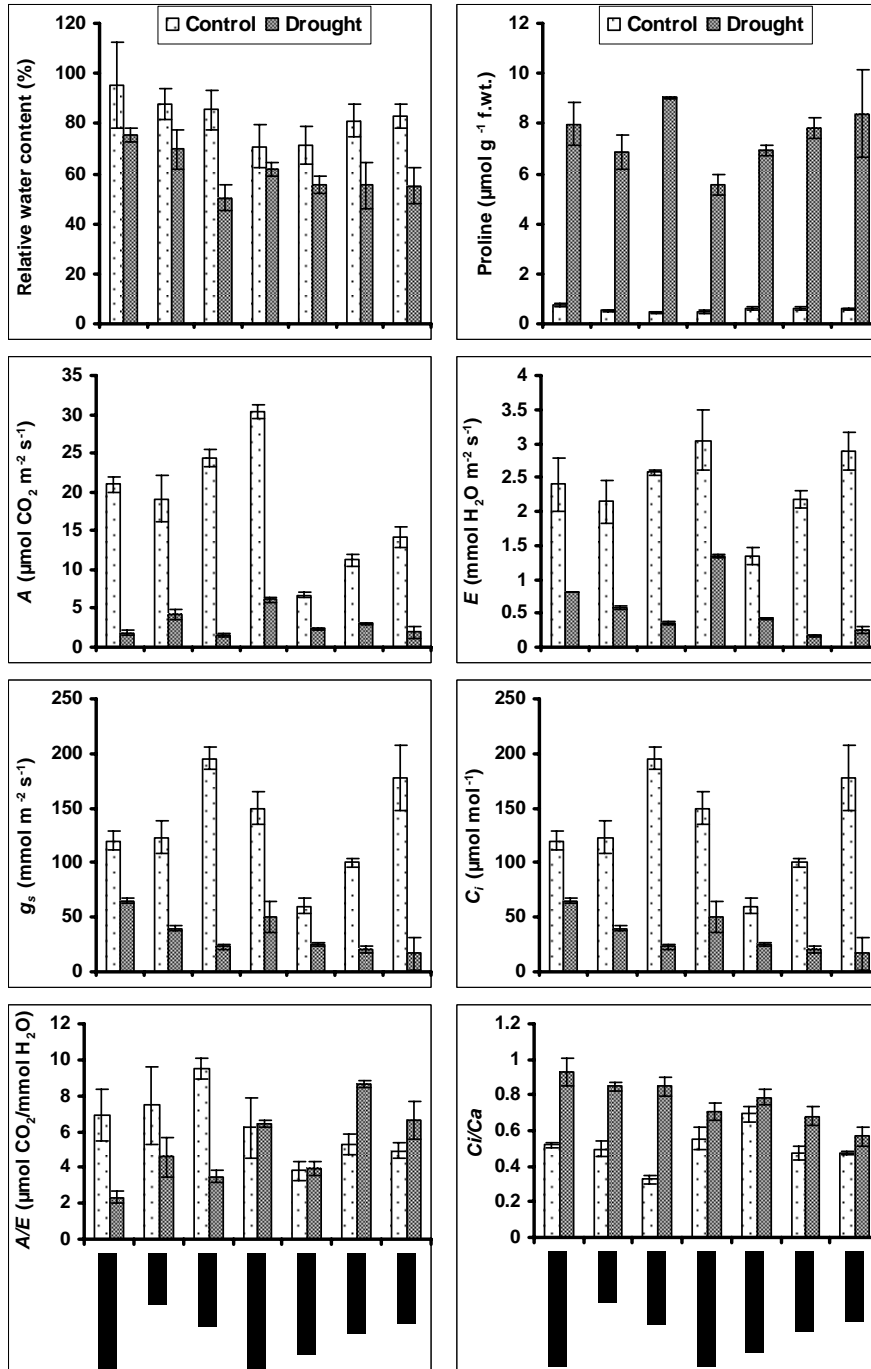


Fig. 2. Relative water content, proline content and gas exchange characteristics of 7 maize (*Zea mays* L.) cultivars when 12 day-old plants were subjected to water deficit conditions for 15 days.

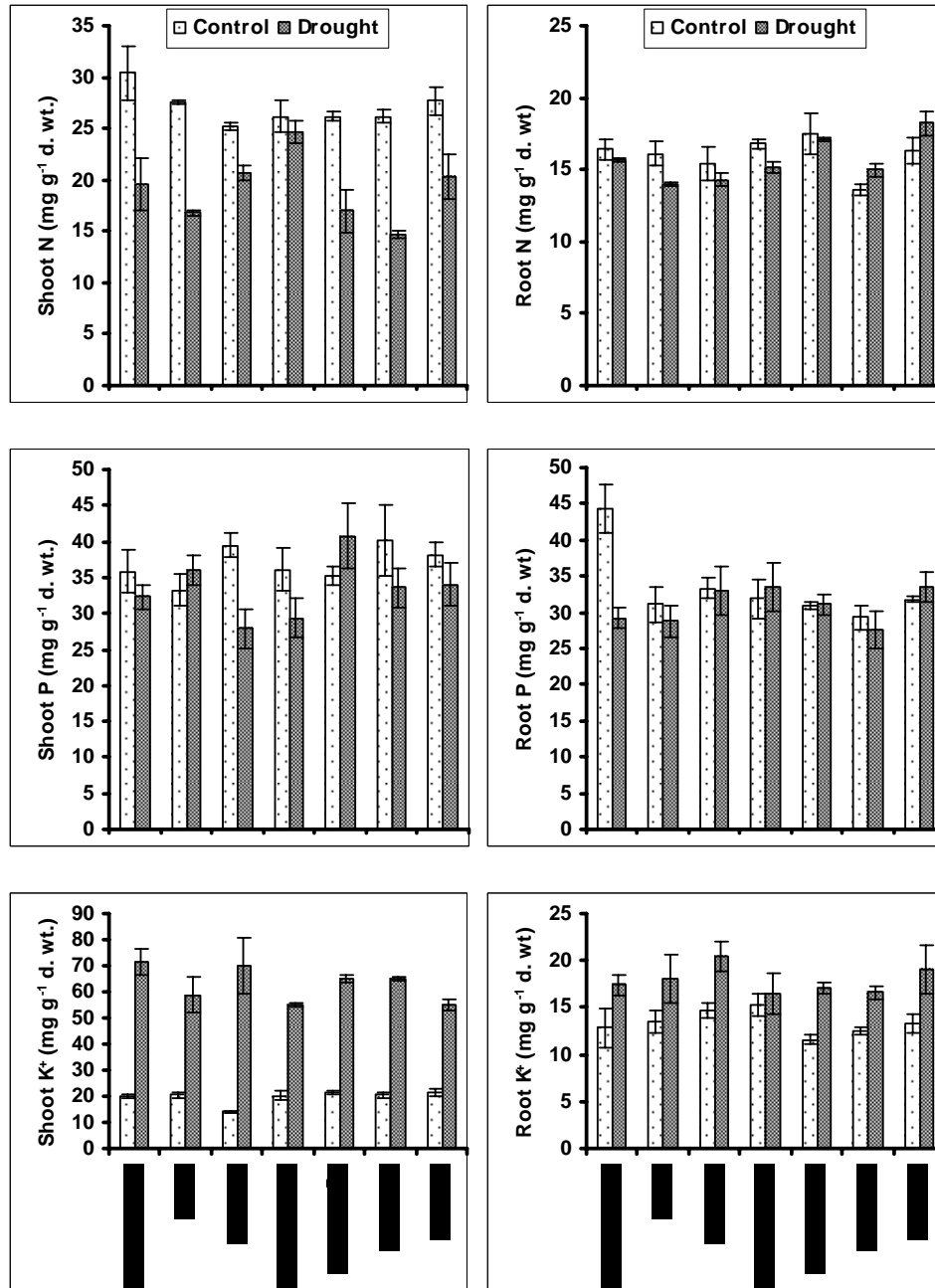


Fig. 3. Shoot and root mineral nutrients of 7 maize (*Zea mays* L.) cultivars when 12 day-old plants were subjected to water deficit conditions for 15 days.

Discussion

Drought effects on plants are complex, variable and are expressed by a number of primary and secondary factors (Boyer, 1996). A common adverse effect of low water potential or water deficit stress is the reduction in fresh and dry biomass production in different crops such as grasses (Ashraf & Yasmin, 1995), wheat (Peschke *et al.*, 1997; Ashraf *et al.*, 1998), maize (Abrechit & Carberry, 1993) and rice (Manabendra & Baruah, 1998). Similarly, in our study water deficit conditions (60% of field capacity) imposed for a period of 15 days to 12-day-old plants had a significant detrimental effect on the growth of 7 maize cultivars viz., Sahiwal-2002, Sadaf, EV-5098, Pak-Afgoyee, Agaiti-2002, Agaiti-85 and EV-1098). These results are in agreement with some earlier studies in which it was reported that progressive drought during early growth stage suppressed the shoot dry matter production, root development and water uptake in rice (Banoc *et al.*, 2000) and wheat (Lopez *et al.*, 2003).

Huang *et al.*, (1997) were of the view that maintenance of root growth for nutrient and water uptake during drought is vital for plant tolerance to drought as water deficit stress was suggested to be the primary cause of root death under field conditions (Smucker *et al.*, 1991; Huang & Nobel, 1992) especially in the surface soil. In the present study, reduction in fresh and dry biomass production (shoot and root) was observed in all maize cultivars with the imposition of water stress treatment. However, there was an increase in root/shoot ratio under water deficit conditions (Hamblin *et al.*, 1990) that may have been due to increased accumulation of assimilates diverted to root growth (O, Toole & Bland, 1987), differential sensitivities of the roots and shoots to endogenous ABA or to a greater osmotic adjustment in roots compared with shoots (Sharp & Davies, 1989).

In our study, water stress significantly increased chlorophyll *a* and *b* contents in most of the maize cultivars under investigation. Plants under water shortage have evolved mechanisms to protect against photodamage. One such mechanism for protection entails changes in chlorophyll content in order to reduce the extent of absorbed light (Giardi *et al.*, 1996; Murchie & Horton, 1997). So the increase in chlorophyll contents under water deficit conditions is a common observation (Estill *et al.*, 1991; Hamada, 1996). In the present study, chlorophyll “*a*” was less affected than chlorophyll “*b*” under water deficit conditions. These findings are parallel to what was earlier observed in maize (Garcia *et al.*, 1987).

One of the most important responses of plants to drought and other abiotic stresses is an over-production of different types of compatible solutes (Serraj & Sinclair, 2002; Ashraf & Harris, 2004). Of these organic solutes, proline being the low molecular weight accumulates greatly in water stressed plants (Tamura *et al.*, 2003; Parida *et al.*, 2008). Moussa & Abdel-Aziz (2008) also observed that proline contents increased in maize seedlings with the application of drought stress. Proline accumulates in all parts of the maize seedlings at low water potentials (Raymond & Smirnoff, 2002). Our results for high accumulation of proline in all maize cultivars are in agreement with a number of past studies including some cited earlier. Thus, proline accumulation is not just a sign of cellular injury resulting in response to water shortage but is a marker of stress tolerance having a definite osmoregulatory role in plants subjected to stressful conditions.

Decline in the growth of many plant species subjected to a water limited environment is often associated with a reduction in photosynthetic capacity (Lawlor, 2002; Dubey, 2005). Reduction in photosynthetic rate has earlier been reported in different crops e.g., in tomato (Srinivasa-Rao *et al.*, 2000), wheat (Molnar *et al.*, 2002) and sunflower (Tezara *et al.*, 2002). With the increase in the intensity of water stress, a

biochemical decline of photosynthetic process is observed. Decline in photosynthetic rate might be due to stomatal closure, which reduces CO_2/O_2 ratio in leaves and inhibits photosynthesis (Janson *et al.*, 2004; Moussa, 2006) which is in agreement with our findings that g_s decreased under water deficit conditions in all maize cultivars. Of various responses, stomatal closure is one of the earliest responses to drought, protecting the plant from extensive water loss (Chaves *et al.*, 2003). Drought stress leads to a noticeable decrease in stomatal conductance (g_s) and mesophyll conductance, and increase in intercellular CO_2 concentration along with the decrease in photosynthetic rate (A) (Siddique *et al.*, 1999). Leaf stomatal conductance (g_s) is also of great importance under the conditions when water stress increases (Flexas & Medrano, 2002) thereby restricting CO_2 entrance into the leaf (Cornic, 1994). The extent of stomatal conductance can be used as an indicator to assess stomatal or non-stomatal limitations to photosynthesis under water deficit conditions (Flexas *et al.*, 2002a; 2002b; Medrano *et al.*, 2002).

In the present study, transpiration rate (E) declined severely in all maize cultivars under water-limited condition. Plants can compensate water deficit condition with the closure of stomata to avoid further loss of water through transpiration (Lawlor, 1995).

Values for water use efficiency (WUE calculated as A/E) showed significant differences among all the cultivars. It showed an increasing trend in some cultivars and decreasing in some others under water stress. Increase in WUE under reduced water availability is critical for plant survival (Chaves *et al.*, 2004). The increasing trend in WUE is in agreement to what has earlier been observed by Ashraf *et al.*, (2002) in okra (*Hibiscus esculentus*).

Leaf relative water content has been emphasized as a better indicator of water status of a plant than water potential (Sinclair & Ludlow, 1985). Our results for RWC are similar to what Moussa & Abdel-Aziz (2008) found in maize seedlings under different levels of water stress. They also reported a decline in RWC due to drought stress.

It is well established that plants subjected to water stress can accumulate inorganic solutes e.g., N, P, and K etc. Analysis of macronutrients N, P and K in the maize cultivar clearly indicates that water stress increased the shoot and root potassium (K^+) concentrations in all maize cultivars. It is known that vacuolar cation (K^+) could balance the cytoplasmic free proline osmotically (Weimberg *et al.*, 1982) but potassium made only a small contribution to osmotic adjustment in water stressed plants (Voetberg & Sharp, 1991). So, in the present study, the increased accumulation of potassium (K^+) in maize seedlings might have played a significant role in plant survival under drought stress by playing an important role in osmotic adjustment.

Shoot nitrogen concentration decreased in all maize cultivars due to drought stress. In fact, soil drying induces a decrease in nutrients, particularly nitrogen with strong interactive effects on plant growth and functions (McDonald & Davies, 1996). However, the differential accumulation of root nitrogen and shoot and root phosphorous in all the cultivars under water stress cannot be related to their drought tolerance, which is in contrast to what has earlier been observed in different crops. The decrease in N concentration due to water stress has been reported in various crops including wheat (McDonald & Davies, 1996; Singh & Usha, 2003), in soybean and rice (Tanguilig *et al.*, 1987) and in maize (Premachandra *et al.*, 1990). On the other hand, Sarwar *et al.*, (1991) studied the response of different wheat varieties to water stress and reported a significant increase in N content under water stress.

In conclusion, plant biomass, photosynthetic rate, transpiration rate, stomatal conductance, relative water contents, and shoot N concentrations decreased under water deficit conditions in all maize cultivars. However, root/shoot ratio, chlorophyll *a*, *b* contents, chlorophyll *a/b* ratio, sub-stomatal CO₂ concentration, proline contents and potassium concentrations increased under water limited conditions. Of all maize cultivars examined in the present study cv. Agaiti-85, was found to be relatively resistant to drought stress.

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