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ARE CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHETIC CAPACITY POTENTIAL PHYSIOLOGICAL DETERMINANTS OF DROUGHT TOLERANCE IN MAIZE *(ZEA MAYS* L.)

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Abstract

In order to assess as to whether traits related to plant photosynthetic capacity such as chlorophyll fluorescence and net CO_2 assimilation rate could be used as indicators for drought tolerance in maize, 5 synthetic and 2 hybrids were subjected to PEG-induced water stress for 3 weeks. Although the growth of all maize cultivars was significantly reduced by PEG-induced water stress, they differed significantly in producing shoot biomass under water stress conditions. However, cv. Sahiwal-2002 was higher in growth under water stress conditions. The photosynthetic capacity (*A*) of all maize cultivars was also reduced under water deficit conditions. Since, there was a positive correlation between biomass production and net CO_2 assimilation rate so photosynthetic capacity could be used as a potential selection criterion for drought tolerance in maize. In contrast no such relationship of drought tolerance of the cultivars with photosystem-II efficiency measured as Fv/Fm. Thus, it did not prove to be a viable criterion for drought tolerance in maize.

Introduction

Water is one of the main abiotic factors limiting crop production in several regions of the world (Araus *et al*., 2002). Similarly, in Pakistan heavy crop losses occur due to low and irregular rainfall (less than 100 mm) resulting in shortage of water (Anon., 2003). Furthermore, by extrapolating the results of various climatic change models, different researchers have projected that crop losses due to unpredictable changes in rainfall or low availability of fresh water resulting in aridity will further increase (Athar & Ashraf, 2005; Parry *et al*., 2006; Tambussi *et al*., 2007). In view of this alarming situation, different effective measures need to be adopted to reduce crop losses, particularly for cereals whose demand is growing at 2% per year with growing urban population (Owen, 2001; Skovmand *et al*., 2001). Maize is one such crop whose demand is increasing day by day. According to one of the global food supply-demand model, the demand of maize will increase from 526 M tons to 784 M tons from 1993 to 2020, particularly in developing countries (Rosegrant *et al*., 1999). However, being an efficient moisture user, it requires 500-800 mm of water during life cycle of 80-110 days (Critchley & Klaus, 1991). Furthermore, under water scarce conditions the growth and yield of maize decrease due to reduction in photosynthetic capacity (Bruce *et al*., 2002; Ma *et al*., 2005). Secondly, its initial growth stage is more sensitive to water stress than the later growth stages. These characteristics of maize make it an excellent model plant to examine the physiological basis of water stress tolerance and to identify some key traits in improving drought tolerance. Thus, the development of drought-tolerant maize cultivars through selection and breeding program has been a major concern of crop scientists for many years. However, it requires the identification of key traits and their incorporation into high-yielding varieties using conventional or biotechnological tools (Bruce *et al*., 2002; Reynolds *et al*., 2005a).

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Most of cereal plants respond to water stress through a range of morpho-physiological adaptations or processes. However, these physiological attributes could be used as reliable indicators for the selection of genotypes/cultivars for drought tolerance (Akram *et al*., 2007; Parry *et al*., 2006; Tambussi *et al*., 2007), such as photochemical activity of photosystem II (PS-II) calculated as F_v/F_m and chlorophyll content (Kauser *et al*., 2006), water use efficiency (Araus *et al*., 2002; Tambussi *et al*., 2007), stomatal conductance (Flexas *et al*., 2004), osmotic adjustment (Turner & Jones, 1980), $\delta^{13}C$ discrimination (a measure of the extent to which photosynthesis is maintained while stomatal conductance decreases) (Richards *et al*., 2002), and cell membrane stability (CMS) (Aslam *et al*., 2006). Although selection and breeding for drought tolerance using these traits are certainly scientifically sound, there are some reports that these physiological traits cannot be used as efficient selection criteria and thus needs to be further elucidated before their recommendation to researchers (Parry *et al*., 2003; Mitchell *et al*., 2004).

Keeping in view all the above information, the present study was conducted to screen maize accessions for water stress tolerance using physiological attributes like chlorophyll fluorescence and gas exchange characteristics and to assess whether the lines/cultivars screened using these physiological attributes also show the same pattern of their drought tolerance with respect to their growth performance under water deficit conditions.

Materials and Methods

The experiment was conducted in the growth room of the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The seed material of maize collected from the Maize and Millet Institute of Yousuf Wala, Sahiwal, Pakistan contained two experimental (EV-1098 and EV-5098) and five synthetic (Sahiwal-2002, Sadaf, Pak-Afgoee, Agaiti-2002 and Agaiti-85) lines. Twenty sterilized seeds of each maize line were germinated in Petri plates double lined with filter paper moistened with 5 mL of Hoagland's nutrient solution. The seedlings of each maize line were allowed to establish for 7 days. Ten healthy and of uniform size 7-day old maize seedlings of each line were transplanted to foam-plugged holes in polystyrene sheets (thermopore sheets) floated over 2 L Hoagland's nutrient solution in plastic containers (28 x 16 x 8 cm). Three days after transplanting, plants of each line were subjected to 0 or -0.66 MPa (water stress) without or with $PEG_{(8000)}$ (-0.66 MPa, 18%) in Hoagland's nutrient solution, respectively. Plants were grown at 26/20 °C with a 16/8 h light-dark period. Irradiance at leaf level was 450-470 mmol m^{-2} s^{-1} and 70% RH. Two weeks after imposition of water stress treatment, plants were harvested and separated into shoots and roots and then blotted dry before recording their fresh weights. All plant parts were dried at 65ºC until constant dry weight, and their dry weights measured. However, before harvest following physiological attributes were recorded:

Chlorophyll fluorescence: The polyphasic rise of fluorescence transients of intact leaves of non-stressed and water stressed plants were measured by a Plant Efficiency Analyzer (PEA, Handsatech Instruments Ltd., King's Lynn, UK) according to Strasser *et al*., (1995). For the measurement of the chlorophyll fluorescence all the samples were covered with clips, kept in dark for 30 minutes before fluorescence measurements. The

transients were induced by red light of 3000 μmol m^{-2} s^{-1} provided by an array of six light emitting diodes (peak 650 nm), which focused on the sample surface to give homogenous illumination over exposed area of sample surface and maximal quantum yield of PS II (Fv/Fm) measured.

Chlorophyll contents: Chlorophyll '*a*' and '*b*' contents, and 'chlorophyll *a/b*' ratio were determined according to the method of Arnon (1949). Fresh leaves (0.2 g) were ground in 80% acetone and centrifuged at 10,000 x *g* for 5 minutes. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Gas exchange parameters: Measurements of gas exchange attributes were made on the 2nd intact leaf from top of each plant using an ADC LCA-4 portable infrared gas analyzer (Analytical Development, Hoddesdon, UK). These measurements were made from 11:00 to 14:00 h. Following specifications/adjustments were observed/maintained during measurement of all gas exchange parameters: leaf surface area, 6.25 cm^2; water vapor pressure into chamber ranged from 0.0006.0 to 0.00089 MPa, ambient CO_2 concentration, 361 μmol mol^{-1}; temperature of leaf chamber varied from 28.4 to 32.4 °C; leaf chamber gas flow rate (U), 257 μmol s^{-1}; molar flow of air per unit leaf area 227.56 mol m^{-2} s^{-1}; RH of the chamber ranged from 35.4 to 39.9 %; *PAR* (Q_{leaf}) at leaf surface during noon was maximum up to 1368 μmol m^{-2} s^{-1}; and ambient pressure 97.3 kPa.

Statistical analysis of data: The data for various morpho-physiological attributes were subjected to analysis of variance using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test following Snedecor & Cochran (1980).

Results

Analysis of variance of the data for shoot fresh and dry weights of 7 maize lines show that imposition of water stress caused a significant reduction in shoot biomass of all maize lines (Table 1). However, considerable variation was observed among the maize lines when grown under normal or PEG-induced water stress (Fig. 1). Cultivars Agaiti-2002 and Sahiwal-2002 produced higher shoot fresh and dry biomass than the other cultivars under normal growth conditions, whereas under water stress conditions Sahiwal-2002 followed by EV-1098 were the higher biomass producers. In contrast, cv. Pak-Agfoee was the lowest in producing shoot fresh and dry biomass under both normal and water stress conditions. Similarly, Agaiti-85 was intermediate in shoot fresh and dry weight among all cultivars under both normal and water stress conditions.

Water stress reduced photosynthetic capacity (*A*) only in cv. EV-1098, while that of all other lines remained unaffected due to water stress. However, cvs. Sahiwal-2002, Sadaf and EV-5098 had greater photosynthetic rate than that of all other lines.

A marked reduction in stomatal conductance (g_s) was observed in all maize lines due to drought stress, however, this water stress-induced reducing effect was more in cvs. EV-5098, EV-1098, and Agaiti-85 than that in the other cultivars. In contrast, cvs. Sadaf and Pak-Agfoee were the highest of all cultivars in stomatal conductance under water stress conditions.

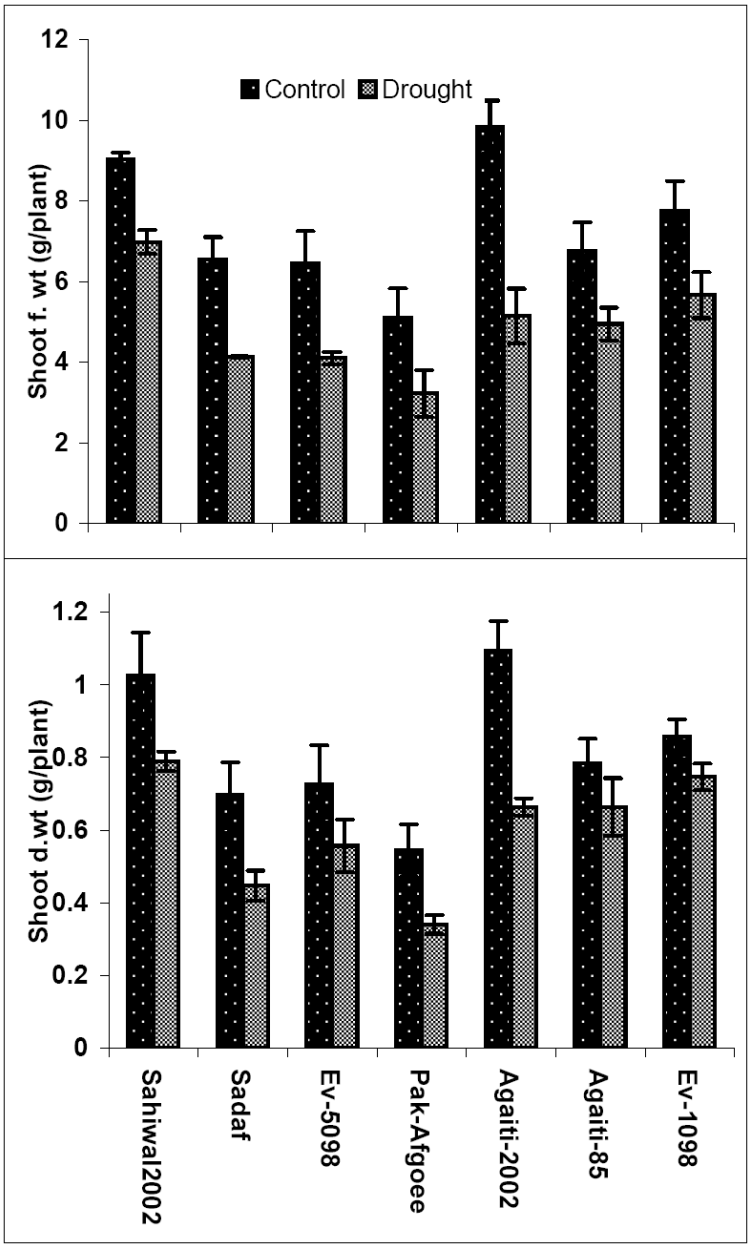

Fig. 1. Shoot fresh and dry weights of maize cultivars when 10 day-old plants were grown at 0 or -0.66 MPa for 15 days (n = 4).

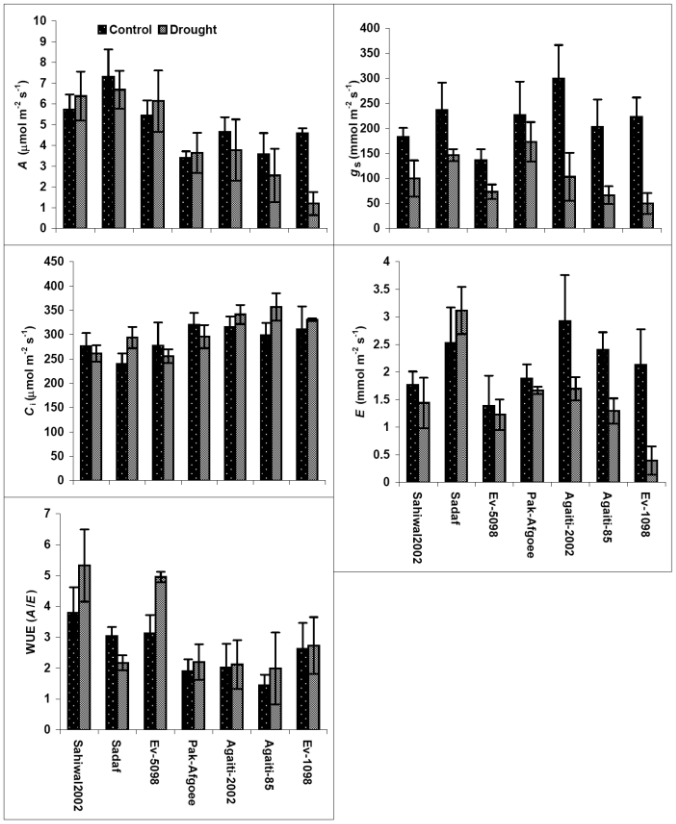

Fig. 2. Gas exchange attributes of maize cultivars when 10 day-old plants were grown at 0 or -0.66 MPa for 15 days (n = 4).

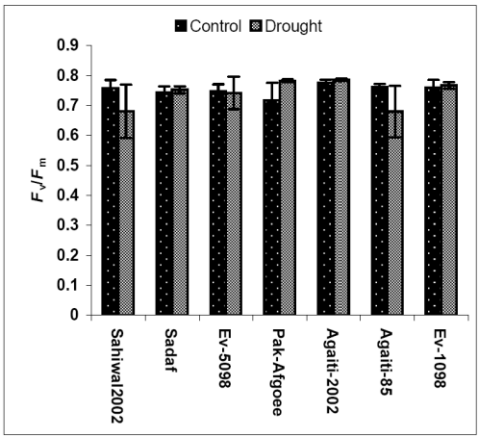

Fig. 3. Maximum quantum yield of PS-II as measured Fv/Fm of maize cultivars when 10 day-old plants were grown at 0 or -0.66 MPa for 15 days (n = 4).

Although transpiration rate was significantly affected due to imposition of PEG-induced water stress, cultivars differed significantly in this gas exchange attribute. Transpiration rate was markedly reduced in cvs Agaiti-2002, Agaiti-85, and EV-1098, while that in other lines it remained unchanged.

Water stress did not change the sub-stomatal CO_2 (C_i) in all maize cultivars. However, cultivars differed significantly in sub-stomatal CO_2 (C_i) and it was higher in cvs Agait-2002, Agaiti-85, and EV-1098 under both normal and water stress conditions. The lowest value for sub-stomatal CO_2 (C_i) was recorded in cv. Sahiwal-2002.

Water use efficiency (WUE) increased in cvs. Sahiwal-2002 and EV-5098 due to water stress, whereas it remained almost unchanged in all maize cultivars. Furthermore, WUE was also higher in cvs. Sahiwal-2002 and EV-5098 under normal growth conditions than that on other cultivars.

Maximum yield of PS-II efficiency calculated as Fv/Fm was not affected due to water stress. Furthermore, all maize cultivars did not differ significantly in this attribute.

Discussion

It is now well evident that selection of suitable plants from small or large germplasm collection using specific morpho-physiological traits is a viable way forward for crop improvement for water stress tolerance (Reynolds *et al*., 2005; Kiani *et al*., 2007; Tambussi *et al*., 2007). Hence a simple method of screening maize lines using two potential physiological selection criteria, phototosynthetic capacity and chlorophyll flourescence were tested in the present study.

The diverse set of maize cultivars, examined in the present study, exhibited a considerable variation for water stress tolerance at early growth stages. For example, on the basis of growth under water stress conditions cvs Sahiwal-2002 and EV-1098 proved to be more tolerant to drought, while Pak-Agfoee the most sensitive being the least biomass producer. Burke (2001) and Srikanthbabu *et al*., (2002) were of the view that genetic variability for stress response could only be observed upon plant exposure to water stress. Thus, higher water stress tolerance in Sahiwal-2002 and EV-1098 might have been due to expression of water stress-responsive genes that can be translated into certain physiological phenomena such as maintenance of relative water content, osmotic adjustment, photosynthetic rate, and water use efficiency (Bruce *et al*., 2002; Waseem *et al*., 2006). Of all these phenomena, photosynthetic rate is very viable that directly contributes to plant productivity (Lawlor, 2002). Using photosynthetic capacity (A) as a selection criterion it was possible to discriminate among the maize cultivars, because a highly significant and positive correlation was found between this physiological attribute and water stress tolerance in terms of biomass production. Thus, photosynthetic capacity could be used as an efficient selection criterion for secreening maize germplasm for water stress tolerance. Similarly, Runkulatile *et al*., (1993) found that land races of common beans adapted to dry areas had higher photosynthetic rate. Such a positive relationship between photosynthetic rate and water stress tolerance was also observed in sunflower genotypes (Kiani *et al*., 2007).

Photosystem II (PSII) plays a key role in the response of leaf photosynthesis to environmental perturbation (Baker, 1991), so significant changes in photosynthesis under water stress conditions are expected. In the present study, although maize cultivars substantially differed in photosynthetic rate under normal or water stress conditions, the

cultivars did not differ significantly in PS-II efficiency measured as F_v/F_m. Furthermore, parallels between *A* and F_v/F_m or shoot biomass of all cultivars cannot be drawn, which is in contrast to what has earlier been observed in canola (Kauser *et al*., 2006).

Although the correlation between net photosynthetic rate (*A*) and stomatal conductance (g_s) or transpiration rate (*E*) is a significant, positive association between *A* and C_i, g_s or C_i is not found. For example, cv. EV-1098 was the lowest in *A* but lowest in C_i of all cultivars. Similarly, cv. Sahiwal-2002 having higher photosynthetic rate was the lowest in C_i. Furthermore, in the present study, water stress caused a slight increase in C_i in cvs. Sadaf and Agaiti-85, while it remained unaffected in other cultivars. These results indicate that lower stomatal conductance and transpiration could increase intercellular CO_2 concentration (C_i) due to metabolic limitation of photosynthesis or by increased CO_2 production from respiration relative to photosynthesis (Lawlor, 1995; 2002; Baker *et al*., 2007; Sharkey *et al*., 2007; Tambussi *et al*., 2007). Furthermore, under water stress conditions plants rapidly adjust water loss through transpiration and absorption of CO_2 through stomatal regulation thereby resulting in increased water use efficiency (Baker *et al*., 2007; Sharkey *et al*., 2007; Tambussi *et al*., 2007). The high biomass producing cv. Sahiwal-2002, had higher photosynthetic rate as well as higher water use efficiency, but such kind of relationship among these traits was not found in other cultivars.

In conclusion *A* has a positive relationship with growth of most cultivars so it can be used as a potential selection criterion but not F_v/F_m.

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