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FIELD SCREENING OF GUAR [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.] ACCESSIONS FOR ENHANCED FORAGE PRODUCTION ON HOT DRYLANDS

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Abstract

To explore the high biomass producing accessions of guar [*Cyamopsis tetragonoloba* (L.) Taub.], under hot and dry conditions, an experiment was conducted under natural field conditions located at Research Station, University College of Agriculture, Sargodha, Pakistan using 36 different accessions. Twenty-day old plants grown under normal irrigation were subjected to control and drought (withholding of irrigation for 20 and 40 days) conditions. It was observed that plant fresh and dry biomass, plant height, net CO_2 assimilation rate (P_n), transpiration rate (E) and inter CO_2 conc. (C_i) of all guar accessions under study decreased considerably on withholding of irrigation for both 20 and 40 days. Of all guar accessions, Acc. BR99, 5597, BWP 5595, BWP 5596, BWP 5609, BWP 5611, 24323, Chiniot Black, Khushab Black were relatively higher in plant height and plant biomass (fresh and dry) under water stress conditions. However, a significant increase particularly in BR99, BWP 5595, BWP 5599, BWP 5609, 41671 followed by Sialkot White was observed in the relative membrane permeability of all guar accessions under water limited conditions. All accessions varied significantly in terms of P_n, E and C_i. Accessions BR90, BR99, BWP 5596, 24323, 24332, Chiniot Black, Khushab Black, 41671, 24323, BWP 5611, Khushab White, Mardan, Khanewal Local 2 and Sialkot White were better in P_n, C_i and E. No significant change was observed in the water-use efficiency of guar accessions under water-limited conditions. Overall, BR99, 5597, BWP 5595, BWP 5596, BWP 5609, BWP 5611, 24323, Chiniot Black and Khushab Black were higher, whereas accessions BWP 5595, 24321, Khushab White, Silanwali White, Sialkot Black and Khanewal Local lower in plant biomass and different gas exchange characteristics under water limited conditions, so the earlier mentioned accessions can be recommended as high biomass producing guar accessions for their cultivation on hot drought-prone areas.

Key words: Guar [*Cyamopsis tetragonoloba* (L.) Taub.], Drought stress, High Biomass, Gas Exchange Characteristics.

Introduction

Optimal water supply is essential for normal metabolic functioning of plant cells or tissues. However, deficiency of water can easily restrict plant vital physiological processes particularly in the hot dryland areas (Anjum *et al.*, 2011; Zlatev & Cebola-Lidon, 2012; Mahesh *et al.*, 2013). Water stress so called drought is a multidimensional stress that affects the plants at several levels of their cellular/tissue organization (Zlatev & Cebola-Lidon, 2012; Razzaq *et al.*, 2013). Drought disrupts biochemical, morphological and physiological processes of plants resulting in inhibition of photosynthesis, energy storage/mobilization, membrane stability, enzymatic activities, stomatal opening, photosynthetic pigments, protein contents, nutrient acquisition as well as hormonal regulations (Razzaq *et al.*, 2013; Shafiq *et al.*, 2015; Akram *et al.*, 2015).

Responses of plants to a stress depend upon the cultivar genetics, severity and duration of the stress and plant developmental stage (Khan *et al.*, 2011; Razzaq *et al.*, 2013). Plants react to the particular set of circumstances and control at a given time in response to their changing environment (Shinozaki & Yamaguchi-Shinozaki, 2007; Almeida *et al.*, 2013). Through alterations in gene expressions, plants amend their metabolism that varies according to severity and duration of a stress (Almeida *et al.*, 2013). Due to stomatal closure, photosynthetic efficiency of most plants undergoes suppression under drought stress, which limits diffusion of CO_2 in leaf (Ali & Ashraf, 2011).

Legumes play a key role in the economy of arid and semiarid areas of the world as they are a major source of protein (Sohrawardy & Hossain, 2014). To attain better yield, physiological and morphological understanding of legumes could be helpful (Martinez *et al.*, 2007; Sohrawardy & Hossain, 2014). Legumes help improve soil fertility because of their inherent capability to fix atmospheric nitrogen (Sohrawardy & Hossain, 2014). It has been observed that nitrogen fixation and nodulation are more sensitive to environmental stresses including water stress. Water stress has been reported to suppress O_2 flux in nodules or supply of photosynthates, consequently causing a decrease in nitrogen fixation (Silvente *et al.*, 2012). Lotter *et al.* (2014) observed that net photosynthetic rate decreases under water limited conditions that can affect nutrient allocation and biomass in a legume *Aspalathus linearis*.

Guar (*Cyamopsis tetragonoloba* L.) is a summer annual legume crop and commercially known as cluster bean and guar gum (Kobeasy *et al.*, 2011; Rao & Shahid, 2011). It is mainly cultivated in Asia as forage for cattles and as a vegetable for humans (Rao & Shahid, 2011) under arid/semiarid areas of the world preferring hot dry environment (Sharma & Gummagolmath, 2012; Sultan *et al.*, 2012). It is a highly drought tolerant and multi-used crop because it is used in gum industries, green manure, animal feed and fodder (Sharma & Gummagolmath, 2012). Like other legume crops, guar is also an exceptional crop to enhance soil fertility as it can fix atmospheric nitrogen (Bewal *et al.*, 2009; Sultan *et al.*, 2012).

Genetic diversity is one of the important factors to improve many crops including guar (Sultan *et al*., 2012). It was observed that little water requirements of guar make it showing more potential to salinity consequently, obtained fast-growing high quality forage (Rao & Shahid, 2011). Stafford and McMichael (1991) reported that yield of guar plants was more affected than seeds/pod, seed weight and racemes/plant under water limited conditions. To keep in mind the significance of guar as a fodder crop, we focused to identify high biomass producing guar cultivars for hot arid and semi-arid areas using some potential physiological indicators such as gas exchange characteristics and relative membrane permeability.

Materials and Methods

Thirty six accessions of guar [*Cyamopsis tetragonoloba* (L.) Taub.] were evaluated for forage production under hot dry conditions using plant growth (fresh and dry biomass; plant height), different gas exchange characteristics and relative membrane permeability. The seeds of 36 accessions of guar were collected from different places of Faisalabad, Sargodha, Khushab, Bhakkar, Bahawalpur, Sialkot, and Jhang districts, Pakistan. The experiment was arranged in a RCBD with four replications (plot size 1.5 × 3 m) under natural field conditions, University College of Agriculture, Sargodha, Pakistan during summer, 2014. For soil physico-chemical properties, 12 samples of soil were collected randomly, homogenized and analysed for average soil texture (loam), TSS 0.53%, pH 7.5, organic matter 1.21%, P 8.1 mg/kg, saturation percentage 43, and K 132 mg/kg. The seeds were sown with a drill in line with a distance of 25 cm (line-line). For uniform plant growth, thinning was done after 7 days of seed germination. Normal irrigation was applied up to 20 days after germination. After it, plants of each control plot (with irrigation) were watered normally while the water-deficit plants in the other plot were kept without watering for 20 days. After which time, data for plant height, plant fresh and dry weights, relative membrane permeability and different gas exchange characteristics were recorded. The remaining plants were allowed to grow and second irrigation was applied only to the control (with irrigation) plants and again water stressed plants remained without irrigation. Then again data were collected for growth and photosynthetic attributes after 20-day of 2nd irrigation. For plant dry weight, the fresh plants were sun-dried, and then shifted to an oven for 72 h set at 65°C. The detailed procedures for the following attributes were adopted:

Relative membrane permeability (RMP): Following the protocol proposed by Yang *et al*. (1996), the youngest 3rd leaf from top (0.5 g) was collected, cut in to small pieces and placed in deionized water (10 ml). Then, it was vortexed and electrical conductivity (EC_o) of the extract noted. After it, all samples were kept overnight at 4°C and EC recorded as EC_1. Then, all samples were autoclaved for 60 min and again EC denoted as EC_2 which was measured after cooling at room temperature. The RMP in percent was calculated as:

$$\text{RMP (\%)} = (\text{EC1}-\text{EC}_o/\text{EC2}-\text{EC}_o) \times 100$$

Photosynthetic attributes: The data for transpiration rate, P_n, and internal CO_2 concentration were collected at daytime with maximum sunshine having *PAR* varied from 716.3-1165.6 µmol m^{-2} s^{-1}. For this a portable infrared gas analyzer (Model Cl-340; ADC, Hoddesdon, England) was used.

Statistical analysis: The data collected for different variables were subjected to analysis of variance using SAS 9.1 (SAS Inc., North Carolina, USA) and the means were separated using the least significant difference (LSD) test.

Results

After first irrigation: Analysis of variance of data showed that there was a significant reduction in the plant fresh and dry biomass of all 36 accessions (Acc.) of guar grown under water deficit conditions created by withholding water supply for 20 days (Fig. 1a). All accessions of guar varied significantly and of all accessions, BR99, 5597, BWP 5596, BWP 5609, BWP 5611, 24323 and Chiniot Black were relatively higher, while Acc. BWP 5595, 24321, Khushab White, Silanwali White, Sialkot Black and Khanewal Local lower and the remaining were moderate in plant biomass (fresh and dry) under water stress conditions.

Under water stress, a significant suppression was observed in plant height of all guar accessions under study. However, more reduction in plant height was observed in accessions 24321, 22159, Chiniot White and Hafizabad White. Of all accessions, plant height of accessions BR99, BWP 5595, 5597, 24323 and Khushab Black was less affected under water deficit conditions (Fig. 1a).

A significant ($p \leq 0.001$) increase in the relative membrane permeability (RMP) was observed in all guar accessions under water limited conditions (Fig. 1a). Under water stress, a maximum increase in the RMP was observed in accessions BR99, BWP 5595, BWP 5599, BWP 5609, 41671 followed by Sialkot White.

Photosynthetic rate (*A*), sub-stomatal CO_2 concentration (C_i) and transpiration rate (*E*) of guar accessions decreased considerably under water stress (Fig. 1b). Of all accessions, BR90, 24323, 24332, Chiniot Black, Khushab Black and Sialkot Black were superior in P_n, accessions BR99, BWP 5611, Khushab White and Mardan White in *E,* and Acc. BWP 5611, 41671, Khushab White and Sialkot White in $C_{i.}$ However, a significant increase or decrease was observed in the water-use efficiency (WUE) of different accessions of guar under study (Fig. 1b). Under water-deficit conditions, an increase was observed in the WUE of accessions 22157, 24323, 24333, Kalorkot White, Chiniot Black, Khushab Black and Sialkot Black, however, that of the remaining accessions decreased significantly under water-limited environment (Fig. 1b).

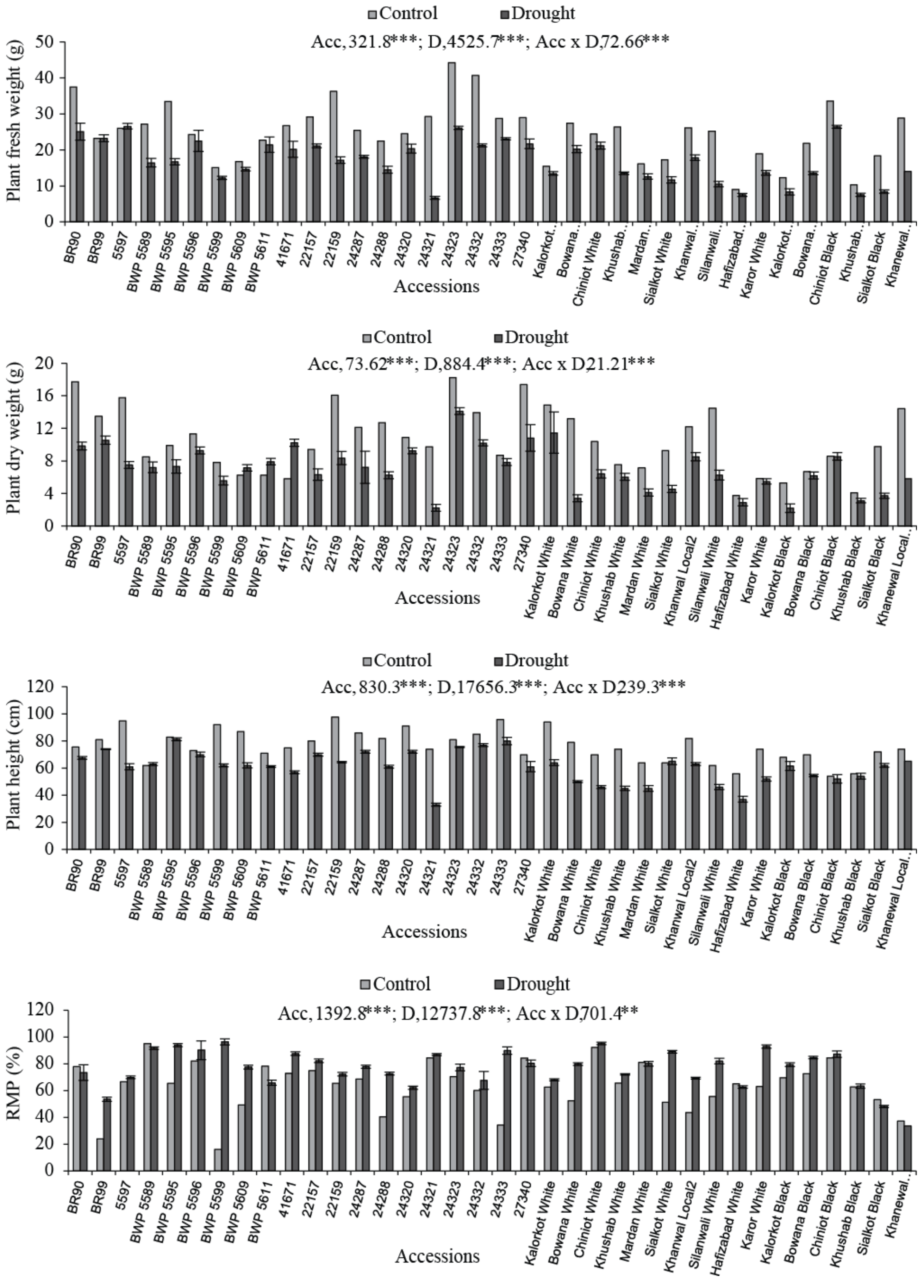

Fig. 1a. Plant fresh and dry biomass, plant height and relative membrane permeability (RMP) of 36 different accessions of guar (*Cyamopsis tetragonoloba*) grown for 20-day with or without irrigation under field conditions (Mean ± S.E.); Acc, Accessions; D, Drought; ** and ***, significant at 0.05, 0.01 and 0.001 levels.

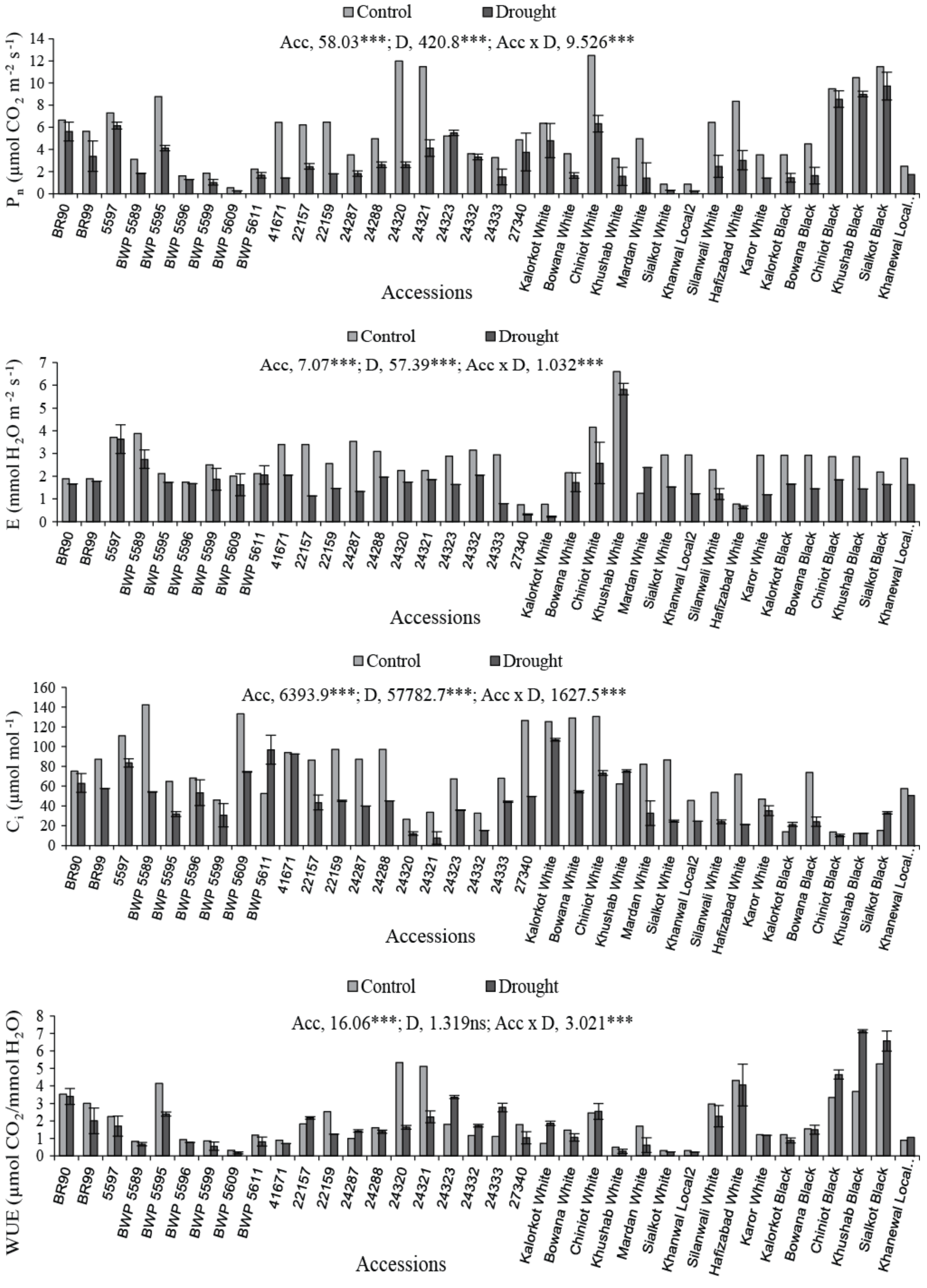

Fig. 1b. Net photosynthetic rate (P_n), transpiration rate (E), sub-stomatal CO_2 conc. and water-use efficiency (WUE) of 36 different accessions of guar (*Cyamopsis tetragonoloba*) grown for 20-day with or without irrigation under field conditions (Mean ± S.E.); Acc, Accessions; D, Drought; ns, non-significant; ***, significant at 0.001 level.

After second irrigation: Withholding irrigation for another 20-day, induced a significant ($p \leq 0.001$) reduction in the plant fresh and dry biomass as well as plant height of all accessions of guar (Fig. 2a). Of all accessions, the most sensitive accessions to water stress in terms of plant fresh and dry biomass were Acc. BWP 5599, 24320, 24321, 24332, Kalorkot White, Hafizabad White, Bowana Black, Khushab Black followed by Sialkot Black, while in terms of plant height were Acc. 24321, Khushab White followed by Hafizabad White, Acc. BR99, BWP 5589, 24287, Bowana White, BWP 5609, 22159 followed by Sialkot White while all the other accessions were moderate in terms of plant fresh and dry biomass as well as plant height under water-deficit conditions (Fig. 2a).

A considerable ($p \leq 0.001$) reduction was observed in A, E as well as C_i of all accessions of guar due to withholding of irrigation for 40 days. All guar accessions responded differentially to water stress conditions in terms of P_n, E and C_i. It was observed that of all accessions, Acc. BR99, BWP 5596, 41671, 24323 followed by Khanewal Local were superior in P_n, Acc. BR90, BR99, BWP 5595, BWP 5596, 41671, 22159, 24333 and Khanewal Local 2 in E, and Acc. BR99, 5597, 24320, 24333, 27340 and Khanewal Local in C_i under water-deficit conditions (Fig. 2b).

No significant change was observed in water-use efficiency (WUE) of guar accessions due to imposition of water stress conditions (Fig. 2b). However, WUE varied significantly in the set of genetically different accessions of guar. Of all accessions, Acc. BWP 5595, Khanewal Local 2, Silanwali White and Bowana Black were lower, while Acc. BR90, BR99, BWP 5596, BWP 5599, BWP 5611, 41671, 22159, 24321, Karor White, Kalorkot Black, Chiniot Black and Sialkot Black were higher in WUE under withholding of irrigation conditions (Fig. 2b).

Discussion

A number of studies can be deciphered from the literature in which the adverse effects of drought stress on different conventional or non-conventional crop plants have been determined using various morphological, physiological and biochemical attributes (Boutraa *et al.,* 2010; Ashraf *et al*., 2011; Shafiq *et al*., 2015; Kosar *et al*., 2015). Most of the studies clearly indicate that water stress-induced plant growth suppression can be due to several metabolic disorders including imbalance in macro/micro-nutrients and hormones, alteration in ultrastructure of vital proteins and enzyme activities, cellular membrane leakage, disturbance in water or turgor potentials, stomatal or non-stomatal changes in the gas exchange characteristics, etc. (Zhu, 2002; Ashraf *et al*., 2011; Iqbal *et al*., 2014; Bhardwaj *et al*., 2015). For example, while working with two cultivars of canola, Shafiq *et al*. (2014) observed that water stress reduced shoot and root growth of both canola cultivars and they attributed this plant growth reduction to the suppression in photosynthetic pigments as well as P and K contents in the shoots and roots. Similarly, Boutraa *et al.* (2010) grew four wheat cultivars under moisture levels of 50 or 30% field capacities and found growth reduction, which was ascribed to water-stress-induced decrease in relative water contents and water-use efficiency. We observed that all 36 accessions of guar varied significantly in terms of plant fresh and dry biomass as well as plant height under withholding of irrigation for either 20 or 40 days. Of all guar accessions, Acc. BR99, 5597, BWP 5595, BWP 5596, BWP 5609, BWP 5611, 24323, Chiniot Black, and Khushab Black were relatively higher in plant fresh and dry biomass and plant height under water stress conditions. These findings show that water deficit effect on different guar accessions varied significantly, which could be ascribed to differential genetic make-up of the germplasm used as has been earlier reported in different studies on different crops (Arshad *et al*., 2008; Ashraf *et al.,* 2011; Shafiq *et al*., 2014).

Cell membrane leakage of intact plants is one of the preliminary indicators of a stress effect. Stress-induced increase in membrane permeability/leakage has been observed in different plant species, e.g. cauliflower (Batool *et al*., 2013), wheat (Beltrano & Ronco, 2008), radish (Akram *et al*., 2015). Analogous to all these studies, in the current study, a significant increase in the relative membrane permeability (RMP) was observed in all guar accessions under water limited conditions. However, the response of all accessions varied considerably and a maximum increase in the RMP was observed in accessions BR99, BWP 5595, BWP 5599, BWP 5609, 41671 and Sialkot White. The response of all tolerant/sensitive (high/low biomass producing) guar accessions was not consistent in terms of RMP, because the data presented in Fig. 1a clearly indicate that RMP of some high biomass producing guar accessions increased considerably as compared to that of low biomass producing guar accessions. So, in the present study, change in RMP cannot be considered as a potential indicator of drought tolerance in the guar accessions used. So, there is a need to determine different factors which regulate membrane stability/functioning of differential responsive guar accessions. It has already been well established that cell membrane stability depends on K^+ flux, accumulation of reactive oxygen species (ROS) as well as synthesis of osmolytes (Akram *et al*., 2011, 2015; Demidchik *et al*., 2014).

Drought-induced suppression in photosynthesis has already been observed in a number of plant species including cereals, vegetables, grasses, forages as well legumes (Ashraf & Harris, 2013; Ambavaram *et al*., 2014). It is evident that stomatal as well as non-stomatal limitations are believed to be involved in restricting the rate of photosynthesis in stressed plants of different crops, e.g. wheat (Kosar *et al*., 2015), maize (Ali & Ashraf, 2011), barley (Iqbal *et al.,* 2014), sorghum (Massacci *et al*., 1996), *Cynodon dactylon* (Akram *et al*., 2007), and so on. We observed a considerable reduction in net A, E and C_i of all guar accessions due to withholding of irrigation for 20 or 40 days. It was observed that of all accessions, Acc. BR99, BWP 5596, 41671, 24323 followed by Khanewal Local were superior in P_n, accessions BR90, BR99, BWP 5595, BWP 5596, 41671, 22159, 24333 and Khanewal Local 2 in E, and accessions BR99, 5597, 24320, 24333, 27340 and Khanewal Local in C_i under water-deficit conditions. Most of the high biomass producing guar accessions under water stress conditions showed better performance in terms of P_n and $C_{i,}$ so in extension to previous observations it can be suggested that rate of photosynthesis (CO_2 assimilation) has a direct influence on biomass production of plants grown on drought prone areas.

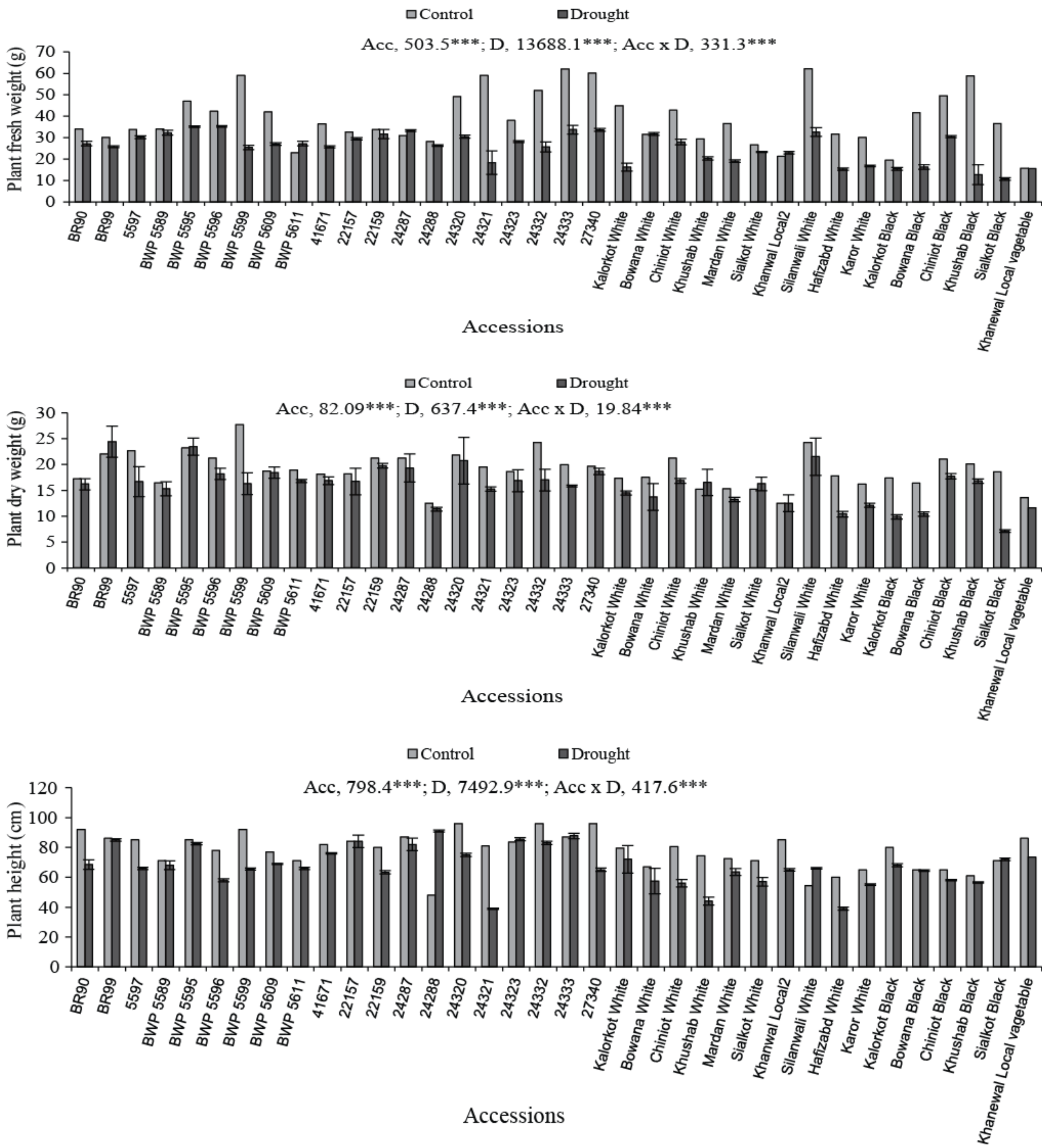

Fig. 2a. Plant fresh and dry biomass and plant height of 36 different accessions of guar (*Cyamopsis tetragonoloba*) grown for 40-day with or without irrigation under field conditions (Mean ± S.E.); Acc, Accessions; D, Drought; ***, significant at 0.001 level.

In conclusion, the plant fresh and dry biomass, plant height, P_n, E and C_i of all guar accessions under study decreased considerably on withholding (20 and 40 days) of irrigation. Of all guar accessions, Acc. BR99, 5597, BWP 5595, BWP 5596, BWP 5609, BWP 5611, 24323, Chiniot Black, and Khushab Black were relatively higher in plant fresh and dry biomass as well as plant height under water stress conditions. However, a significant increase particularly in accessions BR99, BWP 5595, BWP 5599, BWP 5609, 41671 followed by Sialkot White was observed in the RMP of all guar accessions under water limited conditions. All accessions varied significantly in different gas exchange variables such as P_n, E and C_i. Accessions BR90, BR99, BWP 5596, 24323, 24332, Chiniot Black, Khushab Black, 41671, 24323, BWP 5611, Khushab White, Mardan, Khanewal Local 2 and Sialkot White were better in P_n, C_i and E than the other accessions. Overall, accessions BR99, 5597, BWP 5595, BWP 5596, BWP 5609, BWP 5611, 24323, Chiniot Black and Khushab Black performed better in plant biomass and different gas exchange characteristics under water limited conditions, so they can be recommended as high biomass producing guar accessions for their cultivation on hot drought-prone areas.

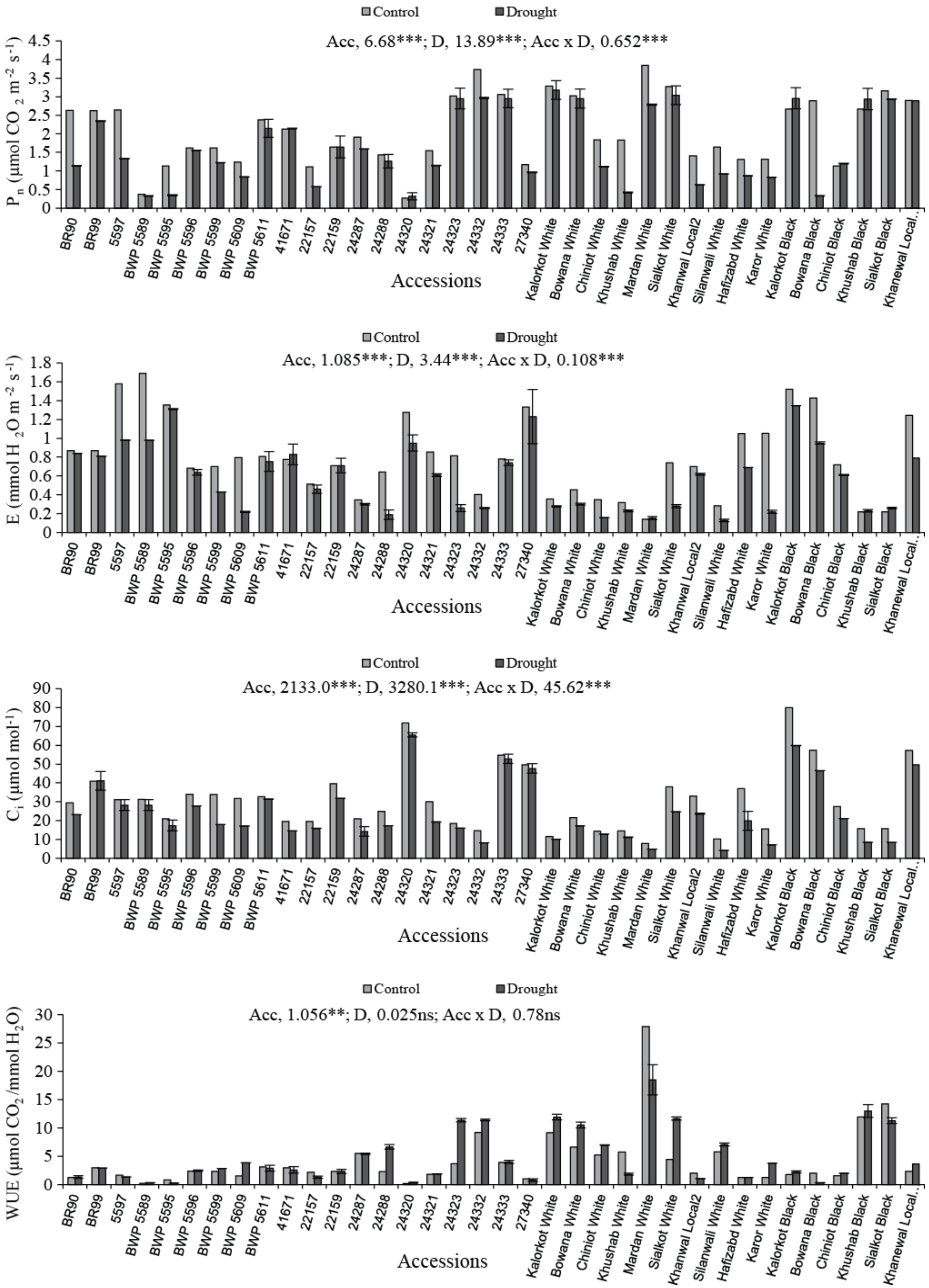

Fig. 2b. Net photosynthetic rate (P_n), transpiration rate (E), sub-stomatal CO_2 conc. and water-use efficiency (WUE) of 36 different accessions of guar (*Cyamopsis tetragonoloba*) grown for 40-day with or without irrigation under field conditions (Mean ± S.E.); Acc, Accessions; D, Drought; ns, non-significant; ** and ***, significant at 0.01 and 0.001 levels.

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