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PHYSIOLOGICAL RESPONSE OF DIFFERENT ACCESSIONS OF *SESBANIA SESBAN* AND *CYAMOPSIS TETRAGONOLOBA* UNDER WATER DEFICIT CONDITIONS

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Abstract

Identification of leguminous crops and screening of accessions for drought tolerance are effective approaches to ensure food security and hence to increase soil fertility. To appraise the physiological response of *Sesbania sesban* L. Merril. and *Cyamopsis tetragonoloba* L. Taub., their different accessions were evaluated under different field capacities. The accessions were grown in pots, and irrigation was managed to attain 100%, 80%, and 60% field capacity. The photosynthetic attributes and biochemical markers such as photosynthetic pigments, proline, soluble sugars, total soluble proteins, amino acids, glycine betaine, and nutrient contents were employed for evaluation of drought tolerance of all accessions of both crops. All these attributes were adversely affected due to soil moisture deficit conditions, and the response of different accessions to water deficit varied significantly. The soil moisture deficiency significantly reduced the gas exchange attributes, chlorophyll contents, soluble proteins, free amino acids, and N and P contents, while it significantly increased carotenoids, proline, glycine betaine, and K contents. Accession BR99 of *Cyamopsis tetragonoloba* and accessions Sahianwala and Shahpur of *Sesbania sesban* showed better performance under soil moisture deficit conditions as compared to the other accessions, so these accessions could be cultivated under semiarid and arid conditions.

Key words: Sesbania; Guar; Water deficit; Photosynthesis; Metabolites.

Introduction

Water scarcity limits plant production in the semiarid and arid regions (Schwabe & Connor, 2012; Schwabe *et al*., 2013; Surendran *et al*., 2019). Optimum soil moisture is required to sustain growth, development and metabolic activities of plants (da Silva *et al*., 2013). Water scarcity disrupts the essential physiological processes resulting in inhibition of photosynthesis, stomatal opening and photosynthetic pigments (Ali *et al*., 2015).

Plants have developed certain mechanisms to perceive and respond to water deficiency by a series of physiological and biochemical processes (Chaves *et al*., 2009). Under moderate drought, plants close down their stomata to decrease the transpiration rate so as to maintain cell turgor (Cornic, 2000; Nawaz *et al*., 2013). The stomatal closure limits the diffusion of CO_2 in leaf thereby declining photosynthetic efficiency under drought stress (Xu *et al*., 2010). Thus, gas exchange attributes are most sensitive to water deficit stress, and a decline in photosynthetic traits depends on the duration, intensity of drought and types of plant species (Ali & Ashraf, 2011; Ashraf & Harris, 2013; Ali *et al*., 2015). Water scarcity causes a reduction in chlorophyll contents and induces metabolic changes (Khanzada *et al*., 2003; Guo *et al*., 2018).

Leguminous plants have ecological and economic significance as they improve soil fertility by nitrogen fixation and are a major source of protein (Sultan *et al*., 2012; Sohrawardy & Hossain, 2014). Sesbania (*Sesbania sesban* L. Merril.) and guar (*Cyamopsis tetragonoloba* L. Taub.) are ecologically significant leguminous plants of arid and semiarid areas due to their limited water requirement and enhanced nitrogen fixation capability. Guar and sesbania are multipurpose crops having food and fodder uses due to their nutritional properties (Sultan *et al*., 2012). Sesbania is also used as green manure and organic fertilizer (Mahmood *et al*., 2008; Nigussie & Alemayehu, 2013). Guar has industrial and medicinal uses being a source of galactomannan gum in seeds (Hosobuchi *et al*., 1999). It is a tolerant crop and can grow well under various abiotic stresses (Kuravadi *et al*., 2012; Alshameri *et al*., 2019).

Identification of drought-tolerant leguminous cover crops and cultivars is an efficient approach to ensure food security, reduce soil erosion and improve soil fertility of semiarid and dryland areas. Furthermore, the comparison of metabolic changes in the susceptible and tolerant genotypes may lead to devise effective breeding programs aiming at improving stress tolerance in these potential crops.

Materials and Methods

Different accessions of Sesbania (*Sesbania sesban* L. Merril) and guar (*Cyamopsis tetragonoloba* L. Taub.) were grown under natural conditions in a greenhouse at the Nuclear Institute for Agriculture and Biology, Pakistan. The seeds of four accessions of sesbania (Sahianwala, Sialkot, Shahpur, and Chiniot) and four accessions of guar (BR99, 5597, BWP5595, and Sialkot black) were collected from semiarid regions (Bahawalpur, Kalorkot, Bowana, Chiniot Mardan, Shahpur and Sialkot) of Pakistan. Seeds of the accessions of guar and sesbania were sown in soil-filled clay pots (12 cm diameter, 20 cm depth). After germination, three levels of field capacity, i.e., 100% (FC_{100}), 80% (FC_{80}) and 60% (FC_{60}) were attained by managing irrigation. The treatments were arranged in a completely randomized design (CRD) with four replications of each treatment.

Thinning was done after germination and two healthy plants were maintained per pot. To protect the experiment from rain, a transparent plastic sheet was fixed at the top of the wire-house. Photosynthetic traits, chlorophyll, and carotenoid pigments, and other physiological parameters were measured after 30 days of growth. At maturity, the plants were harvested and plant nutrient uptake was determined. The physicochemical properties of soil used for the experiment were recorded and presented in Table 1. Total N, available K and organic carbon content of the soil were determined using the micro-Kjeldhal method, flame-photometer, and Walkley & Black (1934) methods, respectively.

Table 1. Physico-chemical profile of the soil used in pot experiments.

Soil characteristics	Values
Soil texture	Sandy loam
Saturation percentage	39.5%
EC	0.77–0.97 dS m^{-1}
Soil pH	7.60–7.90
Organic matter	0.36–0.61%
NO_3-N	11.08–14.30%
Potassium	102–200 mg kg^{-1} soil
Phosphorus	7.80–10.9 mg kg^{-1} soil

Photosynthetic traits: Photosynthetic rate (*Pn*) and transpiration rate (*E*) were measured during daytime after 30 days of drought stress. A portable infrared gas analyser (IRGA) was used to determine the photosynthetic traits in the second fully expanded leaf of each plant.

Biochemical attributes: Chlorophyll, carotenoid, soluble proteins, total free amino acids, soluble sugars, glycine betaine and proline contents were determined using a spectrophotometer (Hitatchi, U-2800). Carotenoid and chlorophyll (a, b and total) contents in fresh leaves were determined using the Arnon (1949) method. Half gram of fresh leaves was mashed into pieces and then 5 mL of acetone (80%) were added to each of the samples, which were kept at 10 ºC overnight and centrifuged at 14000 × g for 5 min to get the clear supernatant. The absorbance of the supernatant was read at 480, 663, 645, and 652 nm using a UV-Visible spectrophotometer.

Total free amino acids were measured using the Hamilton & Van Slyke (1973) method and absorbance was recorded at 570 nm with a spectrophotometer. Different concentrations of leucine were prepared to make a standard curve. The method of Lowry *et al.* (1951) was used to appraise total soluble proteins in leaves. Absorbance was recorded at 620 nm spectrophotometrically and a standard curve was drawn using bovine serum albumin.

For the determination of total soluble sugars (Yemm & Willis, 1954), absorbance was recorded at 625 nm, and sugar contents were calculated by preparing a standard curve with different concentrations of glucose. Proline content in the leaves was measured using a method as described by Bates *et al*., (1973) and absorbance was recorded at 520 nm. For the estimation of glycine betaine, the leaves were chopped in a mixture of 0.05% toluene and deionized water. Leaf extract was filtered and 2N HCI and potassium tri-iodide were added to it. Then the samples were vortexed and incubated at 4°C overnight. The samples were centrifuged at 10,000 rpm for 20 min at 0°C and periodite crystals were dissolved in 1, 2-dichloroethane and vortexed. The absorbance was recorded at 365 nm with a spectrophotometer.

Nutrient composition: For the determination of nutrients in plants, the leaves of sesbania and guar were acid-digested using H_2SO_4 and H_2O_2 (Wolf, 1982). The extract was filtered and nitrogen (N), phosphorus (P) and potassium (K) contents were determined. The N content was determined using Micro–Kjeldhal's method as proposed by Bremner (1965). Potassium (K) and P were determined using a flame photometer (Jenway PFP 7) and a spectrophotometer (Jackson, 1962), respectively.

Results and Discussion

Physiological attributes: Photosynthetic rate (*Pn*) and transpiration rate (*E*) of sesbania and guar accessions were significantly influenced by the soil moisture level (Fig. 1, Table 2). *Pn* and *E* were maximum under 100% FC and minimum at 60% FC. A reduction in *Pn* and *E* in sesbania and guar accessions was due to the deficiency of available water in the soil. It is well reported that the deficiency of available soil water had a direct or indirect effect on the photosynthetic traits (Ashraf & Harris, 2013). The first response of plants under water deficit stress is the stomatal closure in order to reduce the transpiration rate, which in turn also reduces CO_2 uptake and photosynthesis rate (Pinheiro & Chaves, 2010). This is also manifested by the present results, as a significant reduction was observed in the transpiration rate in all accessions of both crops. Generally, the accessions that maintained a higher transpiration rate had a higher photosynthesis rate. The reduction in *Pn* of guar and sesbania accessions under low soil water could be attributed to stress-induced reduction in substomatal conductance (Iqbal *et al.*, 2011), decline in CO_2 utilizing efficiency (Karaba *et al*., 2007), and reduction in leaf turgidity and suppression in the chlorophyll contents (Chaves *et al*., 2009).

Photosynthesis showed a significant reduction due to soil water deficiency, and it varied significantly in different accessions. The Shahpur and Sahianwala accessions of sesbania and 5597 and BR99 accessions of guar had higher net photosynthesis and transpiration rate than those of the other accessions. Similarly, Alshameri *et al*., (2019) reported a significant variation in guar accessions under heat, drought and salinity stress, and accession BWP 5595 was found better in maintaining higher biomass, leaf area and stomatal conductance under drought stress than the accession 24320. In contrast, Kosar *et al*., (2015) observed that the reduction in soil water availability from 80% to 60% of field capacity did not influence the photosynthetic traits of two wheat cultivars. The variation in response to less soil moisture depends mainly on the intensity and duration of drought stress and also varies with the genetic potential of plant species or crop varieties/accessions (Pinheiro & Chaves, 2010; Xu *et al*., 2010).

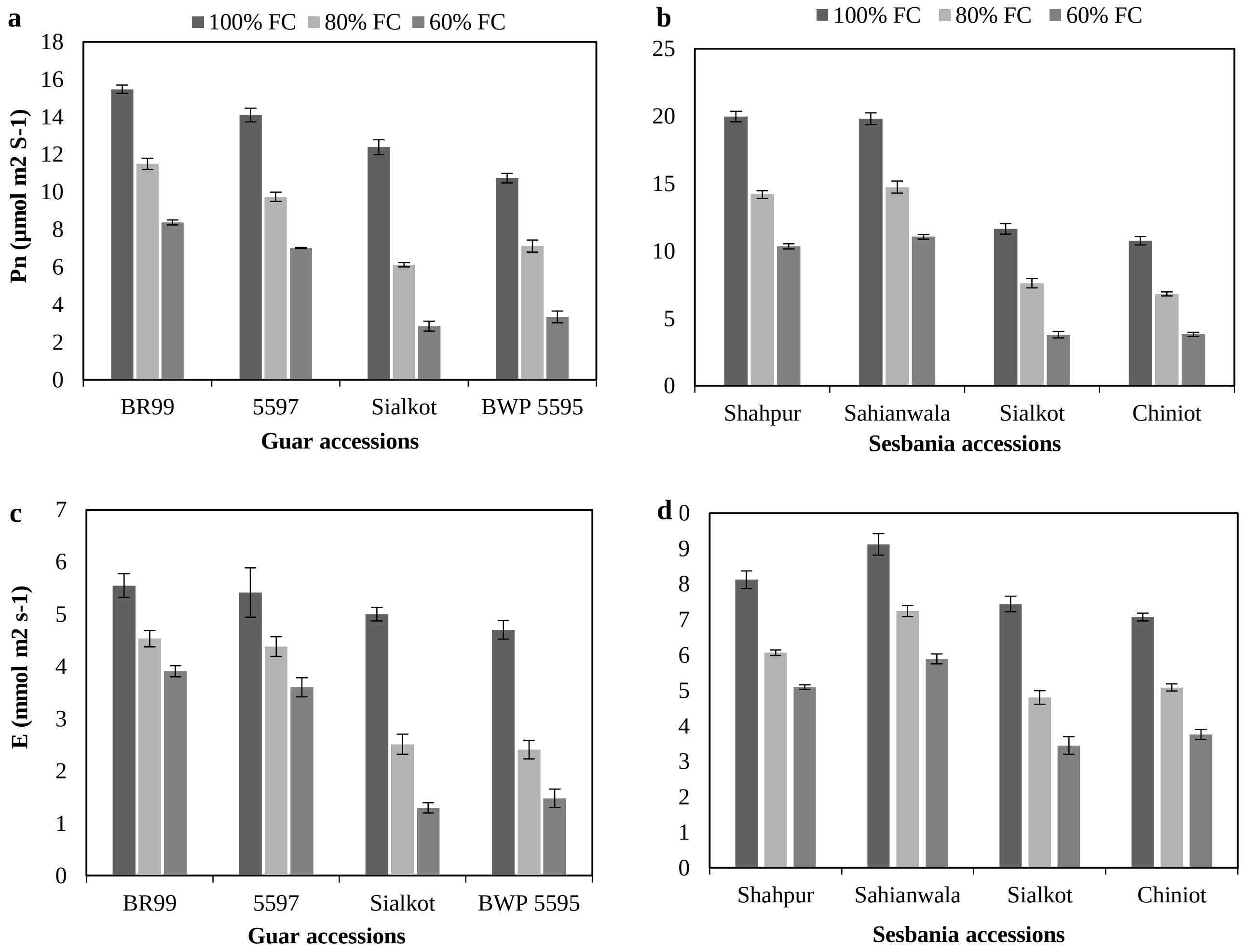

Fig. 1. Photosynthetic rate of (a) guar, (b) sesbania; and transpiration rate of (c) guar, (d) sesbania accessions grown under soil moisture conditions.

Table 2. Mean square values from analysis of variances of photosynthesis rate, transpiration rate, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents in different accessions of guar and sesbania grown under different soil moisture conditions.

S.O.V.	df	*P_n*	*E*	Chla	Chlb	Total Chl	CAR
Guar							
Treatment (T)	2	244.41**	27.81**	0.067**	0.032**	0.194**	0.23**
Accession (A)	3	66.45**	11.17**	0.097**	0.030**	0.233**	0.016**
T×A	6	1.99**	1.164**	0.002**	0.015**	0.014**	0.008**
Sesbania							
Treatment (T)	2	276.43**	47.11**	2.50**	1.79**	8.52**	6.75**
Accession (A)	3	232.76**	12.93**	0.68**	0.02**	1.66**	10.47**
T×A	6	1.52**	0.21**	0.17**	0.15**	0.58**	0.035ns

*, ** and *** indicated significance at 0.05, 0.01 and 0.001 probability levels; ns: non-significant; *Pn*: net photosynthesis; *E*: transpiration rate; Chl: chlorophyll

Biochemical attributes: Pigment (chlorophyll and carotenoid) contents of sesbania and guar accessions were significantly affected due to water deficit conditions (Table 2). Maximum chlorophyll contents were found at 100% FC followed by at 80% FC and 60% FC, while in contrary to chlorophyll, carotenoids were maximum in plants at 60% FC, while minimum in plants grown at 100% FC (Fig. 2). In comparison to guar accessions, BR99 had the highest chlorophyll content, whereas minimum chlorophyll content was recorded in BWP 5595. Accession BR99 maintained the highest chlorophyll contents at 100% FC, while these pigments were minimum in BWP 5595 accession under the highest drought level.

A decrease in chlorophyll contents under water deficit is also reported in previous findings (Kannan & Kulandaivelu, 2011; Ahmad *et al*., 2017). Drought-induced reduction in chlorophyll indicates substantial damages to photosynthetic pigments and thylakoid membranes (Din *et al*., 2011). The decline in leaf chlorophyll content may be due to the breakdown of already present chlorophyll in leaves due to increased activities of chlorophyllase and peroxidase enzymes or/and also by slow biosynthesis of chlorophyll (Ghandchi *et al*., 2016; Hörtensteiner & Kräutler, 2011).

The results showed a significant increase in carotenoids of plants of both sesbania and guar accessions grown under deficient soil water. The guar accession BR99 showed a maximum amount of carotenoids, while minimum carotenoid contents were found in accessions Sialkot and BWP5595. At 60% FC, the minimum carotenoid contents were found in BWP 5595, while maximum being in Sialkot. At 80% FC, maximum carotenoid contents were found in 5597, while minimum in BWP 5595 and Sialkot. Among sesbania accessions, Sahianwala had the highest chlorophyll content, followed by Shahpur, while Chiniot and Sialkot had the minimum chlorophyll contents (Fig. 2).

Increased carotenoid content as observed in Shahpur and Sahianwala accessions of sesbania and in 5597 and BR99 accessions of guar indicated their resistance to drought stress. This difference in the response of tolerant and sensitive accessions might have been due to the differences in their genetic makeup and differential adaptability to drought stress. In contrast to the present results, Mibei *et al*., (2017) reported a considerable reduction in carotenes and xanthophyll due to drought in eggplants. Carotenoids are not only the accessory pigments but are potent antioxidants as they scavenge free radicals and protect the photosynthetic apparatus (Grassmann *et al*., 2002). The results also indicate that the accessions with higher carotenoid contents showed less reduction in chlorophyll contents.

Total free amino acids, soluble proteins, and soluble sugars: Water stress significantly influenced the total soluble sugars (Fig. 3a & 3b), free amino acids (Fig. 3c & 3d), and soluble protein contents (Fig. 3e & 3f) in the sesbania and guar accessions (Table 3). Total free amino acids and total soluble sugars were found highest in plants under 60% FC followed by at 80% FC. Among guar accessions, BR99 had the maximum total soluble sugars, while BWP 5595 accession retained the minimum amount. Among sesbania accessions, Sahianwala had the highest soluble sugars, while Sialkot accession had the lowest content. Sugars are primary source of energy and precursors for the synthesis of several metabolites (Hedrich *et al*., 2015). Amino acids perform different important roles as a component of nucleic acids and proteins. Some researchers have documented that the accumulation of soluble sugars and amino acids helps plants to withstand water stress by osmotic adjustment (Izanloo *et al.*, 2008; Iqbal *et al*., 2011).

The soluble proteins were found to be reduced by reduction in soil moisture contents. Total soluble proteins were minimum in plants grown at 60% FC followed by at 80% FC. Among the guar accessions, BR99 and 5597 had the highest soluble proteins, whereas, Sialkot and BWP 5595 had the lowest protein content. Among sesbania accessions, Shahpur had the highest, while Chiniot had the lowest soluble proteins.

Reduction in the soluble proteins occurs due to the deficiency of available soil water and it is ascribed alteration in gene expression. Mostly, protein content of C_3 plants showed more reduction than C_4 plants (Feki & Brini, 2016). The tolerant guar accessions 5597 and BR99, and sesbania accession Shahpur had accumulated higher total soluble proteins than did BWP 5595 and Sialkot of guar and Chiniot and Sialkot accessions of sesbania. The maximum reduction in soluble proteins was recorded in BWP 5595 accession of guar and Chiniot accession of sesbania at 60% FC. Our results are in accordance with previous researchers indicating that under water deficit stress, plants adjust the soluble protein contents which vary with the species (Shabbir *et al*., 2016; Nawaz *et al.*, 2016). Whereas, other researchers reported that enhanced accumulation of proteins in drought stressed plants help increase drought resistance (Li *et al*., 2017).

Glycine betaine (GB) and proline contents: Proline and GB contents in the leaves of sesbania and guar accessions were significantly affected by the soil moisture regimes (Fig. 4). Proline and glycine betaine contents were highest at 60% FC followed by those at 80% FC, whereas, minimum were found at 100% FC. Among the guar accessions, 5597 and BR99 accumulated higher amount of proline and glycine betaine than that in BWP 5595 and Sialkot. Among the sesbania accessions, Sahianwala accumulated the highest amount of proline and glycine betaine; accession Shahpur had moderate, while Chiniot and Sialkot accumulated low amount of both osmotica (Fig. 4). In general, accumulation of proline content with the decline in soil moisture is a common response of most plants (Medeiros et al., 2012; Ali & Abdur, 2017).

Nutrient composition: The different soil water moisture had significantly affected the phosphorus (P), potassium (K), and nitrogen (N) contents of the guar and sesbania accessions (Table 3). Water deficit stress reduced the N and P content, whereas, K increased under water deficiency (Fig. 5). The highest P and N contents were observed in plants which received 100% FC, followed by at 80% and 60% FC, while K was maximum at 60% FC. The reduction in the uptake of nutrient under low soil moisture condition usually occurs due to the decrease in transpiration rate and less absorption and translocation of water to aboveground plants parts (Jabeen *et al*., 2008; Du *et al*., 2010; Nawaz *et al*., 2016), and reduction in membrane permeability and active transport (Raza *et al*., 2016). Furthermore, the low solubility of nutrients under soil moisture deficit conditions also results in alteration of physiological processes in plants (Fageria *et al*., 2002; Garg, 2003).

Among different guar accessions, BR99, Sialkot and 5597 accumulated the highest quantity of nutrients, while minimum amount of the nutrients was found in BWP 5595. The comparison among different sesbania accessions indicated that Shahpur accumulated the highest P and N contents, followed by Sahianwala, while Chiniot had the lowest. Under 60% FC, accession BWP 5595 maintained the highest K content, while minimum K content was recorded in accession 5597. From sesbania accessions, the accession Chiniot maintained the highest K content, while Shahpur had the lowest K content. The variation in different accessions in absorption and transportation of nutrients has been observed in plants under drought (Ali *et al*., 2008).

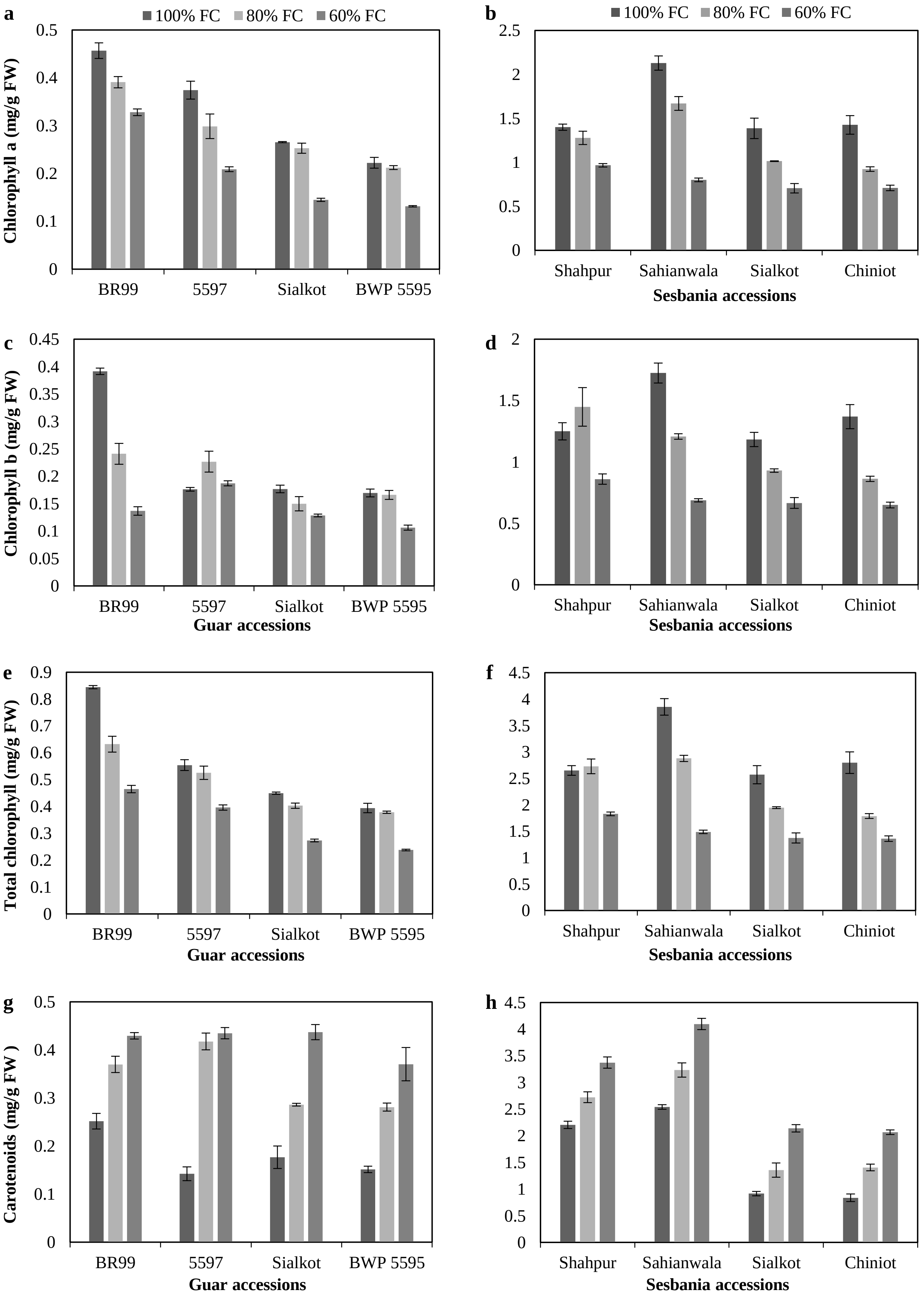

Fig. 2. Chlorophyll a (a) guar and (b) (sesbania); chlorophyll b (c) guar and (d) sesbania; total chlorophyll (e) guar and (f) sesbania; and carotenoids (g) guar and (h) sesbania of different accession grown under water deficit.

The sesbania accessions Chiniot and Sialkot, as well as guar accessions BWP5595 and Sialkot accumulated a higher amount of K than the other accessions. This increase in K contents in different plants has been previously observed by other researchers under various abiotic stress (Ashraf, 1998; Nawaz *et al*., 2016; Raza *et al*., 2016; Hussain *et al*., 2016). Azhar *et al*. (2011) reported that increased K concentration enhanced the stomatal conductance of sunflower. However, in this study, the increased K content did not enhance the stomatal conductance, similar to what has been earlier observed by Patakas *et al*., (2002).

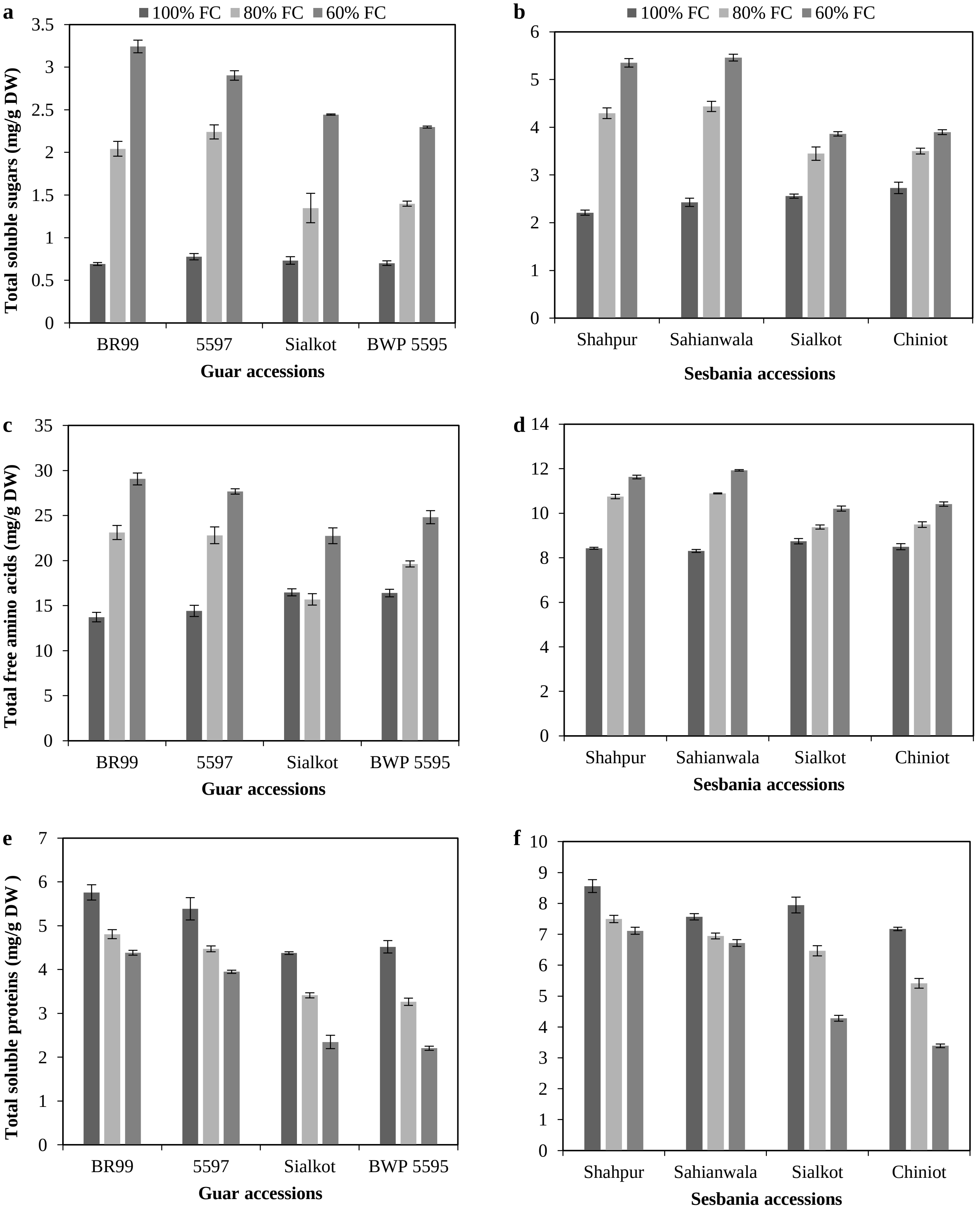

Fig. 3. Total soluble sugar (a) guar and (b) sesbania; total free amino acids (c) guar and (d) sesbania; and total soluble proteins (e) guar and (f) sesbania accession grown under water deficit.

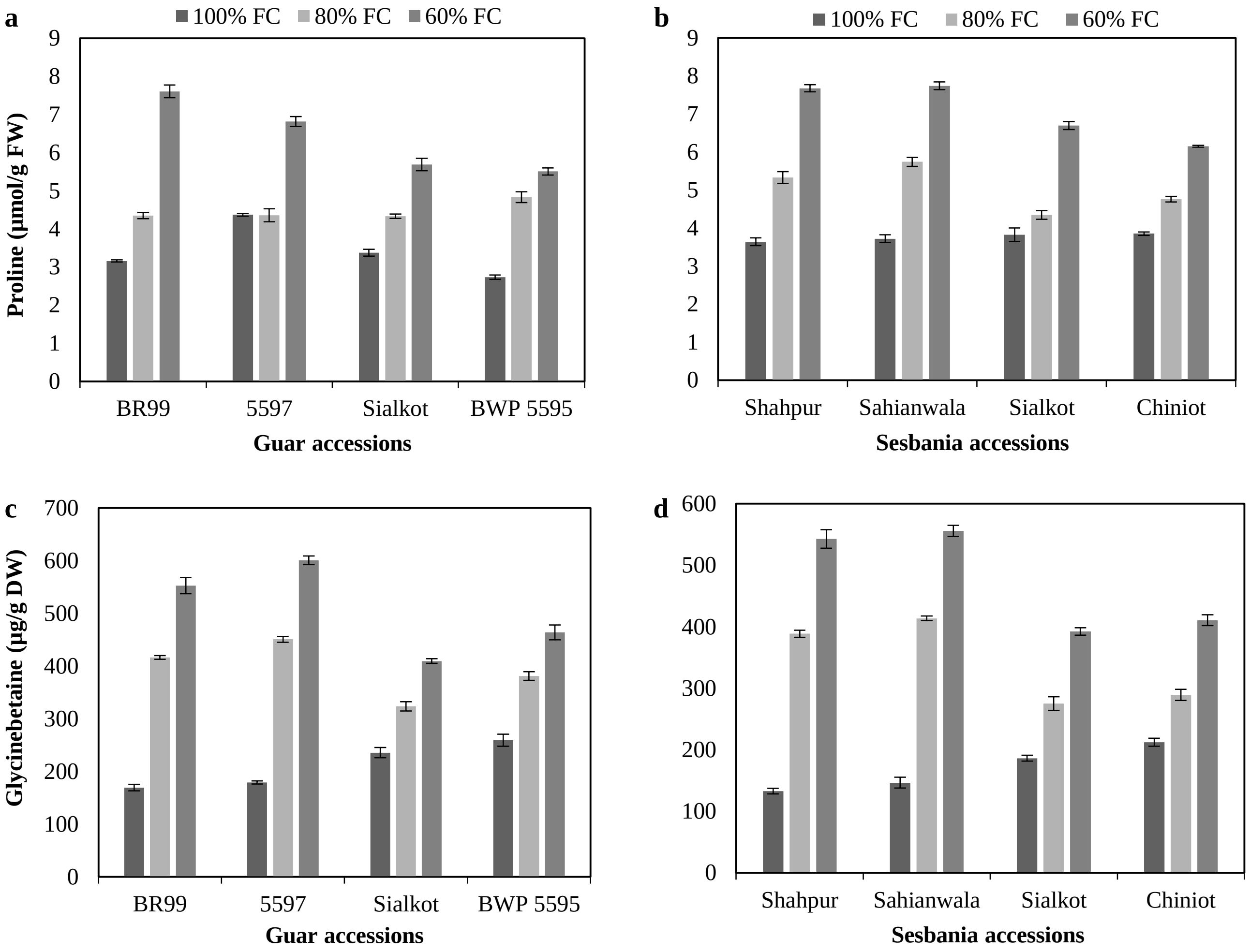

Fig. 4. Proline content of (a) guar and (b) sesbania; and glycinebetaine of (c) guar and (d) sesbania accession grown under water deficit.

Table 3. Mean square values from analysis of variances of total soluble sugars, free amino acids, total soluble protein, proline, glycinebetaine, nitrogen, potassium and phosphorus in different accessions of guar and sesbania grown under soil moisture conditions.

S.O.V.	df	TSS	AA	TSP	Proline	GB	N	K	P
Guar									
Treatment (T)	2	15.94**	469.4**	12.89**	36.88**	356538**	202.47**	180.23**	19.05**
Accession (A)	3	0.99**	33.1**	8.57**	2.01**	15715**	282.50**	33.53**	16.17**
T×A	6	0.29**	27.3**	0.23**	2.03**	16648**	24.89^{NS}	18.92**	0.42**
Sesbania									
Treatment (T)	2	19.37**	6.84**	23.73**	44.49**	376667**	472.67**	301.69**	26.82**
Accession (A)	3	2.01**	3.02**	12.98**	2.05**	20451**	185.74**	11.67**	8.40**
T×A	6	1.18**	1.32**	2.40**	0.95**	16892**	3.41ns	8.39**	0.49**

*, ** and *** indicate significance at 0.05, 0.01 and 0.001 probability levels; ns: non-significant; TSS: total soluble sugars; AA: total free amino acids; TSP: total soluble proteins; GB: glycine betaine; N: nitrogen; K: potassium; P: phosphorus

Conclusion

The response of different accessions was significantly different under water deficit stress. Reducing the soil moisture from 100% field capacity to 60% field capacity significantly influenced gas exchange traits, pigments, soluble proteins, amino acids, proline, and glycine betaine contents as well as nutrient composition of sesbania and guar accessions. The guar accessions, 5597 and BR99 and sesbnia accessions, Sahianwala and Shahpur, showed up-regulation of physio-biochemical attributes and contents of key inorganic nutrients under water deficiency. Thus, these accessions can be grown under low soil moisture regimes. These results further confirm that the physiological and biochemical indicators could be used to identify the drought tolerant germplasm. Tolerant genotypes could be utilized in breeding programs aiming at developing stress tolerant high yielding cultivars.

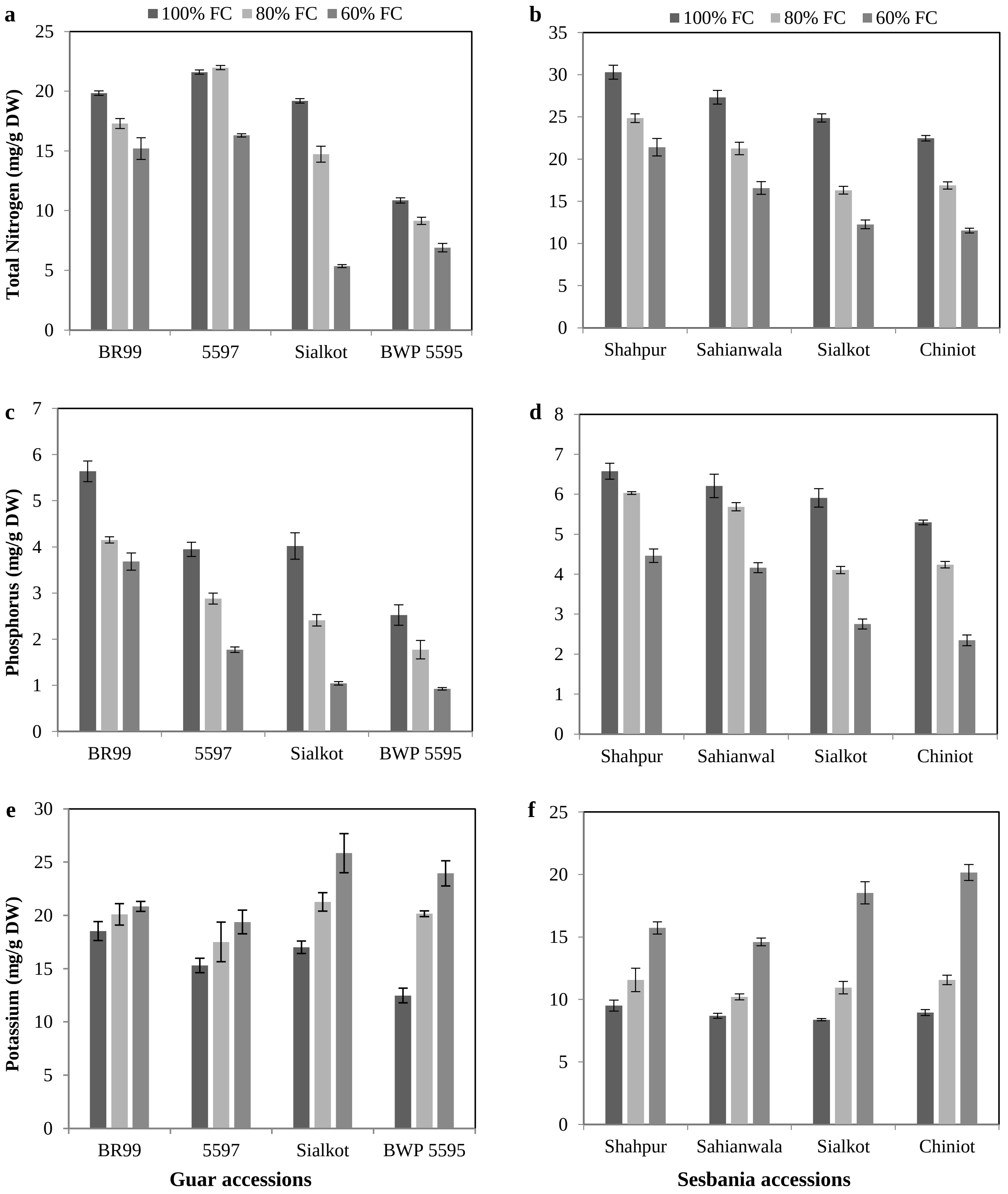

Fig. 5. Total nitrogen in (a) guar and (b) sesbania; phosphorus content in (c) guar and (d) sesbania; and potassium content in (e) guar and (f) sesbania accession grown under water deficit.

References

Ahmad, Z., E.A. Waraich, R. Ahmad and M. Shahbaz. 2017. Modulation in water relations, Chlorophyll contents and antioxidants activity of maize by foliar phosphorus application under drought stress. *Pak. J. Bot.*, 49: 11-19.

Ali, G. and R. Abdur. 2017. The influence of salinity and drought stress on sodium, potassium and proline content of *Solanum lycopercicum*. *Pak. J. Bot.*, 49: 1-9.

Ali, Q. and M. Ashraf. 2011. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. *J. Agron. Crop Sci*., 197: 258-271.

Ali, Q., M. Ashraf, M. Shahbaz and H. Humera. 2008. Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot*., 40: 211-219.

Ali, Z., M. Ashraf, F. Qurainy and M.S. Khan. 2015. Appraising drought tolerance in local accessions of sesbania (*Sesbania sesban* (L.) Merril.) using biomass production, relative membrane permeability and photosynthetic capacity as selection criteria. *Pak. J. Bot*., 47: 845-850.

Alshameri, A., F. Al-Qurainy, S. Khan, M. Nadeem, A.R. Gaafar, A. Alameri, M. Tarroum, S. Alansi and M. Ashraf. 2019. Morpho-physiological responses of guar [*Cyamopsis tetragonoloba* (L.) taub.] to multiple stresses of drought, heat and salinity. *Pak. J. Bot.*, 51: 817-822.

Arnon, D.T. 1949. Copper enzyme in isolated chloroplasts, poly phenaloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1-15.

Ashraf, M. and P.J. Harris. 2013. Photosynthesis under stressful environments: An overview. *Photosynthetica*, 5: 163-190.

Ashraf, M.Y. 1998. Yield and yield components response of wheat (*Triticum aestivum* L.) genotypes under different soil water deficit conditions. *Acta Agron. Hung*., 46: 45-5.

Azhar, N., B. Hussain, M.Y. Ashraf and K.Y. Abbasi. 2011. Water stress mediated changes in growth, physiology and secondary metabolites of Desi Ajwain (*Trachyspermum ammi* L.). *Pak. J. Bot.*, 43: 15-19.

Bates, L.S., R.P. Waldron and I.W. Teaxe. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.

Bremner, J.M. 1965. Total nitrogen and inorganic forms of nitrogen. In: (Ed.): Black, C.A. Methods of soil analysis, 2:1149- 1237. *Amer. Soc. Agron.*, Madison, Wisconsin.

Chaves, M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.*, 103: 551-560.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. *Trends Plant Sci*., 5: 187-188.

da Silva, E.C., M.B. de Albuquerque, A.D. de Azevedo Neto and C.D. da Silva Junior. 2013. Drought and its consequences to plants–from individual to ecosystem. In Responses of organisms to water stress. Intech Open. DOI: 10.5772/53833.

Din, J., S. U. Khan, I. Ali and A.R. Gurmani. 2011. Physiological and agronomic response of canola varieties to drought stress. *J. Anim. Plant Sci.*, 21: 78-82.

Du, N.W., G.X. Zhang and R. Wang. 2010. Morphological and physiological responses of *Vitex negundo* L. var. hetrophylla (Franch) Rahd. to drought stress. *Acta Physiol. Plant*., 32: 839-848.

Fageria, N.K., V.C. Baligar and R.B. Clark. 2002. Micronutrients in crop production. *Adv. Agron*., 77: 185-67.

Feki, K. and F. Brini. 2016. Role of proteins in alleviating drought stress in plants. U: Ahmad, P. (ur.) *Water stress and crop plants: A sustainable approach*, Vol: 1, First Edition, John Wiley & Sons Ltd, pp.165-176.

Garg, B.K. 2003. Nutrient uptake and management under drought: Nutrient-moisture interaction. *Curr. Agric*., 27: 1-8.

Ghandchi, F.P., G. Caetano-Anolles, S.J. Clough and D.R. Ort. 2016. Investigating the control of chlorophyll degradation by genomic correlation mining. *PloS One*, 11: p.e0162327.

Grassmann J., S. Hippeli and E.F. Elstre. 2002. Plant's defence mechanism and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiol. Biochem.*, 40: 471-478.

Guo, R., L. Shi, Y. Jiao, M. Li, X. Zhong, F. Gu, Q. Liu, X. Xia and H. Li. 2018. Metabolic responses to drought stress in the tissues of drought-tolerant and drought-sensitive wheat genotype seedlings. *AoB Plants*, 10: p.ply016.

Hamilton, P.B. and P.D. Vanslyke. 1973. Amino acid determination with ninhydrin. *J. Biol. Chem.*, 150: 231-232.

Hedrich, R., N. Sauer and H.E. Neuhau. 2015. Sugar transport across the plant vacuolar membrane: nature and regulation of carrier proteins. *Curr. Opinion Plant Biol*., 25: 63-70.

Hörtensteiner, S. and B. Kräutler. 2011. Chlorophyll breakdown in higher plants. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1807(8): 977-988.

Hosobuchi, C., L. Rutanassee, S.L. Bassin and N.D. Wong. 1999. Efficacy of acacia, pectin, and guar gum-based fiber supplementation in the control of hypercholesterolemia. *Nut. Res.*, 19: 643-649.

Hussain, R.A., M.Y. Ashraf, R. Ahmad, E.A. Waraich and M. Hussain. 2016. Foliar nitrogen and potassium applications improve photosynthetic activities and water relations in sunflower under moisture deficit condition. *Pak. J. Bot*., 48: 1805-1811.

Iqbal, N., M.Y. Ashraf and M. Ashraf. 2011. Modulation of endogenous levels of some key organic metabolites by exogenous application of glycine betaine in drought stressed plants of sunflower (*Helianthus annuus* L.). *Plant Growth Regul*., 63: 7-12.

Izanloo, A., A.G. Condon, P. Langridge, M. Tester and T. Schnurbusch. 2008. Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J. Exp. Bot*., 59: 3327-3346.

Jabeen, F., M. Shahbaz and M. Ashraf. 2008. Discriminating some prospective cultivars of maize (*Zea mays* L.) for drought tolerance using gas exchange characteristics and proline contents as physiological markers. *Pak. J. Bot*., 40: 2329-2343.

Jackson, M.L. 1962. Soil chemical analysis. Constable and Company, England.

Kannan, N.D. and G. Kulandaivelu. 2011. Drought induced changes in physiological, biochemical and phytochemical properties of *Withania somnifera* Dun. *J. Med. Plants Res*. 5: 3929-3935.

Karaba, A., S. Dixit, R. Greco, A. Aharoni, K.R. Trijatmiko, Marsch-Martinez and N.A. Pereira. 2007. Improvement of water use efficiency in rice by expression of HARDY, an *Arabidopsis* drought and salt tolerance gene. *Proc. Natl. Acad. Sci.*, 104: 15270-15275.

Khanzada, B., M.Y. Ashraf, M.U. Shirazi, S.M. Alam, K.B. Samo and S.M. Mujtaba. 2003. Study of photosynthetic efficiency of some guar (*Cyamopsis tetragonoloba* L. Taub.) genotypes grown under different water regimes. *Asian J. Plant Sci*., 2: 127-131.

Kosar, F., N.A. Akram and M. Ashraf. 2015. Exogenously applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. *South Afr. J. Bot.*, 96: 71-77.

Kuravadi, N.A., S. Verma, S. Pareek, P. Gahlot, S. Kumari, U.K. Tanwar, P. Bhatele, M. Choudhary, K.S. Gill, V. Pruthi and S.K. Tripathi. 2012. Guar: An industrial crop from marginal farms. Agricultural sustainability: Progress and Prospects in Crop Research, Academic Press, pp. 47-60.

Li, W., X. Zhang, U. Ashraf, Z. Mo, H. Suo and G. Li. 2017. Dynamic seed germination, seedling growth and physiological responses of sweet corn under PEG-induced water stress. *Pak. J. Bot.*, 49: 639-646.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 191: 265-275.

Mahmood, A., M. Athar, R. Qadri and N. Mahmood. 2008. Effect of NaCl salinity on growth, nodulation and total nitrogen content in *Sesbania sesban*. *Agric. Conspectus Sci.*, 73: 137-141.

Medeiros, D.B., E.C.D. Silva, H.R.B. Santos, C.M. Pacheco, R.D.S. Musser and R.J.M.C. Nogueira. 2012. Physiological and biochemical responses to drought stress in Barbados cherry. *Braz. J. Plant Physiol*., 24: 181-192.

Mibei, E.K., J. Ambuko, J.J. Giovannoni, A.N. Onyango and W.O. Owino. 2017. Carotenoid profiling of the leaves of selected African eggplant accessions subjected to drought stress. *Food Sci. Nut.* 5: 113-122.

Nawaz, F., M. Naeem, M.Y. Ashraf, M.N. Tahir, B. Zulfiqar, M. Salahuddin, R.N. Shabbir and M. Aslam. 2016. Selenium supplementation affects physiological and biochemical processes to improve fodder yield and quality of maize (*Zea mays* L.) under water deficit conditions. *Front. Plant Sci*., 27: 1438.

Nawaz, F., M.Y. Ashraf, R. Ahmad and E.A. Waraich. 2013. Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. *Biol. Trace Elem. Res.,* 151: 284-293.

Nigussie, Z. and G. Alemayehu. 2013. Potential uses of an underutilized multipurpose tree in Ethiopia. *Afr. J. Plant Sci.,* 7: 468-475.

Patakas, A., N. Nikolaou, E. Zioziou, K. Radoglou and B. No-Itsakis. 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grape vines. *Plant Sci.,* 163: 361-367.

Pinheiro, C. and M.M. Chaves. 2010. Photosynthesis and drought: can we make metabolic connections from available data?. *J. Exp. Bot*., 62: 869-882.

Pirzad, A., M.R. Shakiba and S. Zehtab-Salmasi. 2011. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L. *J. Med. Plants Res*., 5: 2483-2488.

Schwabe, K., J. Albiac, J.D. Connor, R.M. Hassan and L.M. González (Eds.). 2013. Drought in arid and semi-arid regions. Springer Dordrecht Heidelberg New York London, doi:10.1007/978-94-007-6636-5

Schwabe, K.A. and J.D. Connor. 2012. Drought issues in semi-arid and arid environments. *Choices* 27: 1-5.

Shabbir, R. N., E.A. Waraich, H. Ali, F. Nawaz, M.Y. Ashraf, R. Ahmad, M.I. Awan, S. Ahmad, M. Irfan, S. Hussain and Z. Ahmad. 2016. Supplemental exogenous NPK application alters biochemical processes to improve yield and drought tolerance in wheat (*Triticum aestivum* L.). *Environ. Sci. Pollut. Res.,* 23: 2651-2662.

Sohrawardy, H. and M.L. Hossain. 2014. Response of short duration tropical legumes and maize to water stress: A glasshouse study. *Adv. Agric.,* vol. 2014, Article ID 641319, 12 pages. https://doi.org/10.1155/2014/641319.

Sultan, M., M.N. Yousaf, M.A. Rabbani, Z.K. Shinwari and M.S. Masood. 2012. Phenotypic divergence in guar (*Cyamopsis tetragonoloba* L.) landrace genotypes of Pakistan. *Pak. J. Bot.,* 44: 203-210.

Surendran, U., B. Anagha, P. Raja, V. Kumar, K. Rajan and M. Jayakumar. 2019. Analysis of drought from humid, semi-arid and arid regions of India using DrinC model with different drought indices. *Water Resour. Mange.* 33: 1521-1540.

Walkley, A. and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.,* 37: 29-38.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Commun. Soil Sci., Plant Anal*., 13: 1035-1059.

Xu, Z., G. Zhou and H. Shimizu. 2010. Plant responses to drought and re-watering. *Plant Signal Behav.,* 5: 649-655.

Yemm, E.W. and A.J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.,* 57: 508-514.

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