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APPRAISING DROUGHT TOLERANCE IN LOCAL ACCESSIONS OF SESBANIA [*SESBANIA SESBAN* (L.) MERRIL.] USING BIOMASS PRODUCTION, RELATIVE MEMBRANE PERMEABILITY AND PHOTOSYNTHETIC CAPACITY AS SELECTION CRITERIA

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Abstract

In order to identify accessions/lines of sesbania [*Sesbania sesban* (L.) Merril.] which could produce high quantity of forage for livestock in hot and arid environments, eight diverse accessions (Shahpur, Sialkot, Khanewal 1, Khanewal 2, Sahianwala, Bhowana 1, Bhowana 2 and Chiniot) collected from different regions of Punjab, Pakistan were screened for drought tolerance using relative membrane permeability and photosynthetic capacity as selection criteria. Plant fresh and dry biomass and gas exchange characteristics [net photosynthetic rate (P_n), transpiration rate (E), sub-stomatal CO_2 conc. (C_i) and water-use efficiency (A/E)] of all sesbania accessions were affected adversely due to water shortage, but the response of all sesbania cultivars was not consistent to dry conditions appraised using different physiological parameters. On the basis of plant biomass production under dry conditions, accessions Shahpur, Khanewal 2, Sahianwala and Bhowana 2 were ranked as superior, whereas Sialkot was ranked as the poorest performer of all accessions, so the earlier accessions could be grown on arid or semi-arid soils with some plausible management practices.

Key words: Drought tolerance, Photosynthetic capacity, *Sesbania sesban*.

Introduction

Among all environmental constraints, drought stress is the most limiting factor for plant productivity and distribution both in agricultural and natural systems (Ashraf *et al*., 2011; Srivastava & Kumar, 2014; Shafiq *et al*., 2015), because water in optimum quantity is essential for plant growth, development as well as for essential metabolic activities (Razmjoo *et al*., 2008; Abdulla *et al*., 2011). Limiting water availability or plant cell/tissue water uptake resulting in osmotic stress, nutritional imbalance, stomatal closure, hormonal changes (ABA production), over-production of reactive oxygen species (ROS), disturbance in key CO_2 as well as carbohydrate assimilation processes (Ashraf & Harris, 2013; Srivastava & Kumar, 2014; Kosar *et al*., 2015), altogether are critically involved in suppression of plant biomass and seed yield (Demirevska *et al*., 2008; Guarnaschelli *et al*., 2003; Srivastava & Kumar, 2014). Physiological, biochemical and molecular mechanisms such as repression in transpiration loss, improved water-use efficiency, maintenance of turgor potential, deep root system, upregulation of antioxidants, stomatal regulation, and photosynthetic rate at low water potential, and synthesis of osmolytes/osmoregulation help plants to sustain growth and biomass production under limited irrigation systems (Farooq *et al*., 2012; Shafiq *et al*., 2014). Physiological attributes such as membrane stability, tissue water and chlorophyll contents may be considered as indicators of better growth and yield under drought stress conditions (Razzaq *et al*., 2013). All stages of plant growth are not susceptible to water deficit conditions. So, well-organized and purposeful use of water regimes is crucial under stress conditions (Akram, 2011; Ashraf *et al*., 2011).

Sesbania [*Sesbania sesban* (L.) Merril.] belongs to family Fabaceae (Manjusha *et al*., 2012) and is a multipurpose small stature tree (Nigussie & Alemayehu, 2013). It is grown as a short duration perennial green manure (Mahmood *et al*., 2008), deep rooting shrub with high-quality foliage, and it serves as a protein supplement (Nigussie & Alemayehu, 2013). The seeds of *Sesbania sesban* contain 5-6% crude lipid, 30-40% crude protein and 2.7-3.3% ash (Hossain & Becker, 2002; Nigussie & Alemayehu, 2013). The root of sesban is bitter, hot and carminative, which helps cure fever, diabetes, ulcer, tuberculous glands and lecoderma (Manjusha *et al*., 2012), while bark is used in inflammation, spleen enlargement and diarrhea (Usman *et al*., 2013). It is found that root and stem of sesban contain protein, carbohydrates, alkaloids, flavonoids, phytosterol, phenol as well as gum and fixed oil (Mythili & Ravindhran, 2012). It is grown as a nitrogen-fixing leguminous tree which can serve as a green manure when it incorporates into soil. It has also potential of rapid decomposition (Patra *et al*., 2006). It grows well in the subtropics and shows tolerance against waterlogging (Usman *et al*., 2013). Photosynthesis (Puthur & Saradhi, 2004), growth, nodulation and total nitrogen content (Mahmood *et al*., 2008) of *Sesbania sesban* under salinity stress has been documented, while, information on the performance of sesban in terms of growth and photosynthesis under water deficit conditions is lacking. So, the major aim of the present study was to assess the influence of withholding irrigations on plant biomass, photosynthesis and relative membrane permeability of different accessions of *Sesbania sesban* so as to appraise whether some of the accessions could be grown as potential forage under water limited conditions.

Materials and Methods

The study was conducted on sesbania [*Sesbania sesban* (L.) Merril.] under natural field conditions at the Research Area of University College of Agriculture, Sargodha, Pakistan during the summer season of 2014. Twelve sub-samples of soil from the experimental field were collected randomly and analyzed which showed that the overall texture of the field soil was loam having average pH 7.5, organic matter 1.21%, total soluble salts 0.53%, phosphorus 8.1 mg/kg, potassium 132 mg/kg and saturation percentage 43. The experiment was laid-out in the RCB design with four replications; each replicate represented a plot of 1.5 × 3 m. The seeds of 8 different accessions of sesbania were collected from different regions of the Punjab Province, Pakistan. The seeds were sown with the help of a drill keeping line to line distance of 25 cm. After germination thinning was done. All other cultural practices such as hoeing, irrigation and plant protection measures were kept normal during the entire experimentation period. The plants were irrigated normally after germination. After 20 days of seed germination, water stress treatment was started. During this, plants of each control plot were irrigated normally while the drought stressed plants in other were kept without irrigation. After 20 days of first irrigation, samples of plants were taken and their fresh weights and plant height measured. Data for relative membrane permeability (RMP) and some key photosynthetic attributes were recorded. After this, 2nd irrigation was done only to control plants and after 20 days of irrigation, again data were collected for growth, RMP and photosynthetic rate as mentioned earlier. The fresh plants were placed in an oven and after 72 hours, their dry weights recorded. The data for the following parameters were recorded:

Relative membrane permeability (%): Fresh leaf (0.5 g) tissue was chopped and placed in 10 ml of distilled water, vortexed for 5 sec and electrical conductivity (EC_o) of the extract measured. The test tubes containing leaf samples were covered with aluminum foil and kept at 4°C for 24 h. After it electrical conductivity (EC_1) of the samples was measured. Then, the test tubes containing samples were autoclaved for 1 h, cooled at room temperature and electrical conductivity (EC_2) of the dead tissues measured. The relative membrane permeability (%) was determined using the following formula:

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

Photosynthetic attributes: Net photosynthetic rate (P_n), transpiration rate (E) and sub-stomatal CO_2 concentration (C_i) of 3rd young leaf were measured using a portable infrared gas analyzer (Model Cl-340; Analytical Development Company, Hoddesdon, England). Water-use efficiency was calculated as *A/E* ratio.

Results and Discussion

After first irrigation: Withholding of irrigation for 20 days reduced (p<0.05) fresh and dry weights of all 8 accessions of sesbania (Fig. 1). Maximum reduction in plant fresh and dry biomass was observed in accessions Sialkot, Bhowana 1 and Chiniot. However, the other accessions including Sahianwala were highest in fresh and dry biomass under water limited regime. Overall, analysis of variance of data showed that the response of all sesbania accessions was consistent to water stress conditions in terms of fresh and dry biomass.

Plant height of all sesbania plants was suppressed significantly (p<0.001) due to limited water regimes. Of all accessions, accessions Shahpur, Sialkot and Bhowana 1 were superior in plant height under well-watered conditions. However, in contrast, accessions Khanewal 2, Sahianwala and Bhowana 2 were the highest and accessions Chiniot, Khanewal 1, Sialkot and Bhowana 1 the lowest under water stress conditions (Fig. 1).

Statistical analysis showed that relative membrane permeability (RMP) was almost unchanged in all sesbania plants due to shortage of water (Fig. 1). However, average RMP of accessions Shahpur, Bhowana 1 and Chiniot was greater than that of the other sesbania accessions under arid environment.

Net photosynthetic rate (P_n) was significantly (p<0.001) affected by water-deficit conditions. An increase in P_n was observed in accessions Khanewal 1, Khanewal 2, Sahianwala, Bhowana 2 and Chiniot as compared to the other accessions under water-deficit stress (Fig. 1). Transpiration rate (E) and sub-stomatal CO_2 concentration (C_i) were found to decrease in all sesbania accessions under study. Highest value of E was observed in accession Bhowana 2 and lowest in acc. Sialkot under water-deficit conditions (Fig. 1).

Water-use efficiency (WUE) arose considerably in all 8 sesbania accessions under water stress conditions. Of all accessions, Sialkot, Khanewal 1 and Khanewal 2 were better in WUE than the remaining accessions under arid conditions (Fig. 1).

After second irrigation: Data collected after 20 days of 2nd irrigation showed that plant fresh and dry weights of all accessions of sesbania decreased substantially (p<0.001) under water limited conditions. The response of all accessions varied significantly (p<0.001) and of all sesbania accessions, Khanewal 2, Sahianwala and Bhowana 1 were higher in plant fresh and dry biomass (Fig. 2).

Plant height of all sesbania accessions decreased markedly (p<0.001) due to water stress conditions (Fig. 2). Differential response to irrigation regimes in terms of plant height was observed in all sesbania accessions. Of all accessions, Sahianwala and Bhowana 2 were highest in plant height under water-deficit conditions.

A significant reduction in P_n and C_i was observed due to water deficiency in all sesbania accessions (Fig. 2). Of all sesbania accessions, Shahpur and Bhowana 2 were highest and Khanewal 2 lowest in P_n while Bhowana 1 was higher and Shahpur lower in C_i under water stress conditions. Transpiration rate increased significantly in accessions Shahpur, Bhowana 1, Bhowana 2 and Chiniot, while it decreased in accessions Sialkot, Khanewal 1, Khanewal 2 and Sahianwala under stress conditions (without irrigation). No considerable change was observed in WUE in all accessions of sesbania under water stress conditions. However, mean data showed that accession Khanewal 1 was highest and Chiniot lowest in WUE under water limited conditions (Fig. 2).

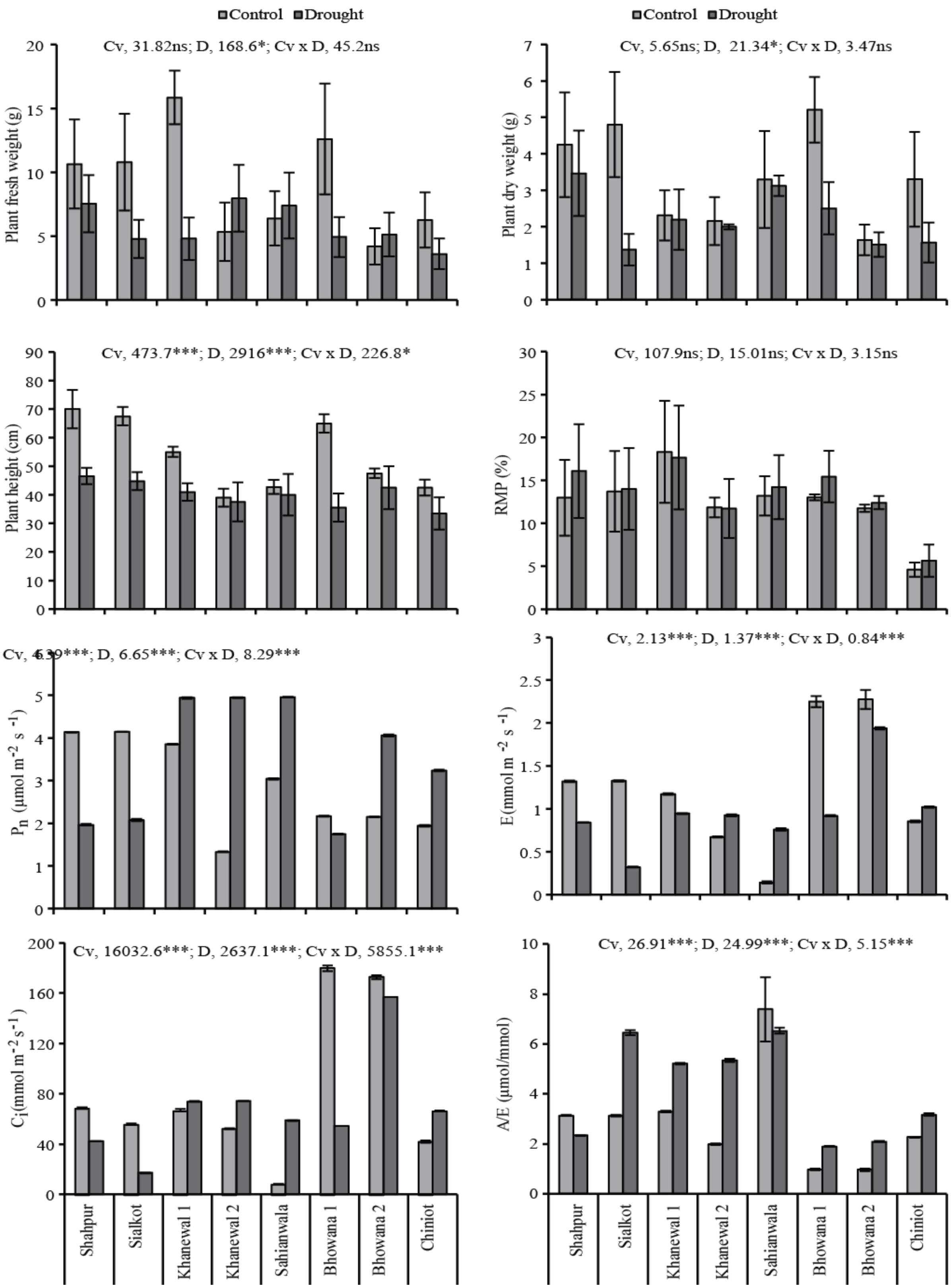

Fig. 1. Plant fresh and dry biomass, plant height, relative membrane permeability (RMP), net photosynthetic rate (P_n), transpiration rate (*E*), internal CO_2 concentration and water-use efficiency (*A/E*) of 8 accessions of 40-day old plants of *Sesbania sesban* subjected to control (one irrigations after 20 days of germination) and water stress (without irrigation) conditions (Mean ± SE). Cv, Cultivars; D, Drought, ns, Non-significant; *, ***, significant at 0.05 and 0.001 levels.

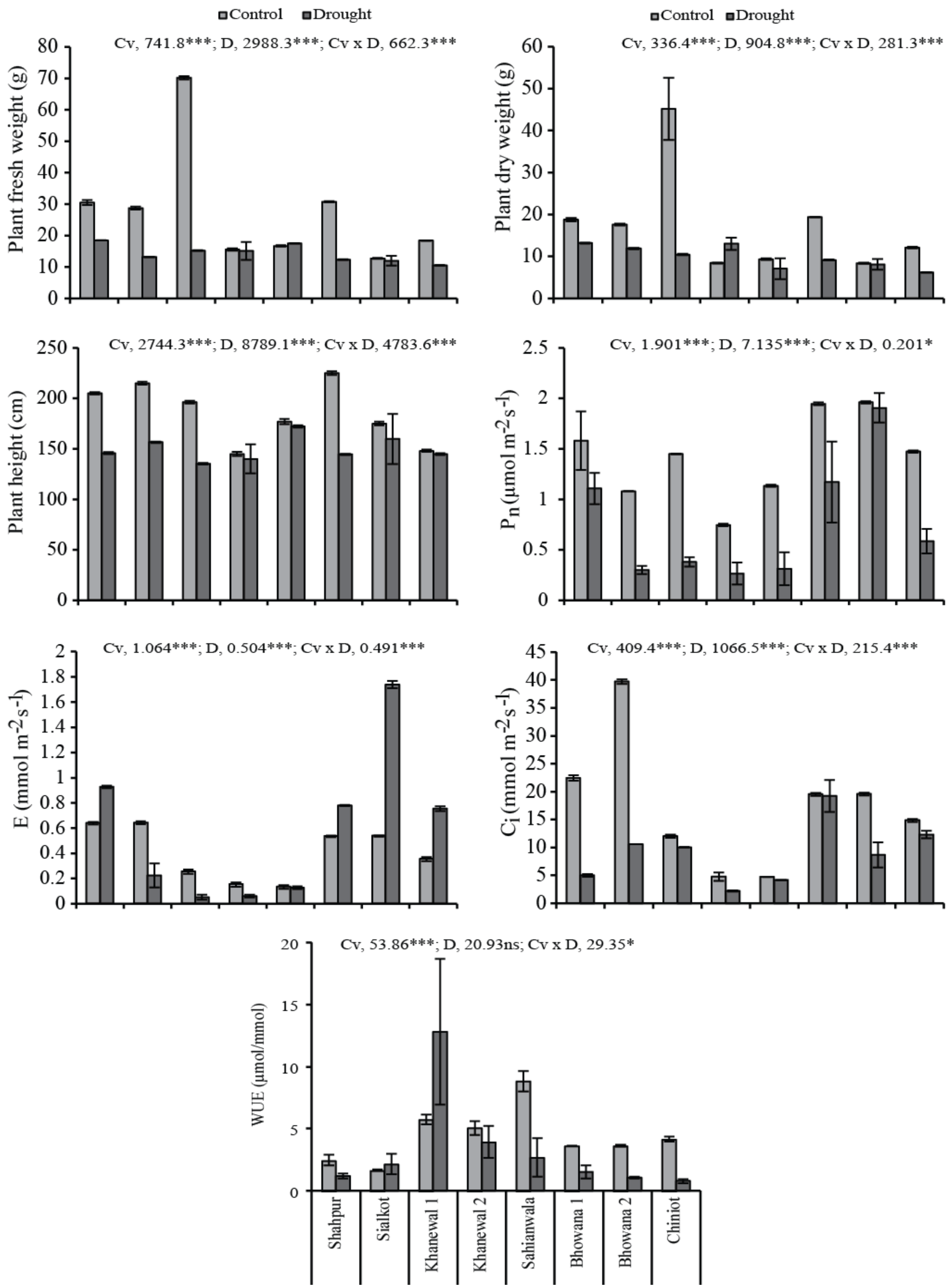

Fig. 2. Plant fresh and dry biomass, plant height, net photosynthetic rate (P_n), transpiration rate (*E*), internal CO_2 concentration and water-use efficiency (*A/E*) of 8 accessions of 60-day old plants of *Sesbania sesban* subjected to control (two irrigations at interval of 20 days) and water stress (without irrigation) conditions (Mean ± SE). Cv, Cultivars; D, Drought, ns, Non-significant; *, ***, significant at 0.05 and 0.001 levels.

Adequate quantity of water is one of the crucial entities to obtain maximum biomass or yield production of all agricultural crops world-over (Ashraf *et al*., 2011). Water stress could develop considerably over periods of a few days or months, so there is a possibility that key metabolic developmental changes may vary day-to-day in response to water shortage (Flexas *et al*., 2006; Gunes *et al*., 2008). However, reduction in plant growth is a common response of plants to deficiency of water rendered at cellular/tissue level or whole plant level (Wise *et al*., 1992; Kaya *et al*., 2006; Kosar *et al*., 2015). Water stress related to water depletion and/or high atmospheric vapor pressure deficit causes growth reduction due to disturbance in photosynthetic processes, stomatal closure, suppression in chlorophyll biosynthesis, sharp increase in membrane leakage, nutritional imbalance, decreased mesophyll conductance to CO_2 as well as utilization of end-product accumulation (Flexas *et al*., 2006; Gunes *et al*., 2008). In the present study, withholding of irrigation for 20 or 40 days reduced fresh and dry weights as well as plant height of all 8 accessions of sesbania. The response of all accessions of sesbania varied significantly to different irrigation regimes. A maximal reduction in plant growth was observed in accessions Sialkot, Bhowana 1 and Chiniot. However, the other accessions including Sahianwala were highest in fresh and dry biomass under water limited regime. Such type of genetic variation due to water deficit conditions has already been observed in different plant species, e.g. chickpea (Gunes *et al.,* 2008), rice (Saxena *et al*., 2002), cowpea (Agbicodo *et al*., 2009), and *Brassica* (Kumar & Singh, 1998), etc. From all these studies it was inferred that differential responses observed during stress conditions depends on a variety of physio-biochemical and agronomic traits which can be used in breeding programs aiming to enhance growth and yield production on rain-fed areas (Ashraf *et al*., 2011).

Relative membrane permeability/leakage (RMP) is one of the major indicators of stress conditions in plant cells/tissues (Gunes *et al*., 2008). In the present study, the response of all 8 accessions of sesbania varied significantly and overall low biomass producing sesbania accessions (Shahpur, Bhowana 1 and Chiniot) were relatively higher in RMP than that of high biomass producing accessions under water deficit conditions. Just analogous to this trend, Gunes *et al*. (2008) successfully screened 11 chickpea (*Cicer arietinum* L.) cultivars under drought stress conditions using RMP as an important physiological indicator. They found that high biomass producing chickpea cultivars were lower in relative membrane permeability as compared to drought-sensitive cultivars under water stress conditions.

It is well documented that water stress can adversely alter the rate of photosynthesis and related processes including reduction in CO_2 diffusion to the chloroplast and metabolic constraints those directly or indirectly involved therein (Ashraf & Harris, 2013). A significant reduction in net photosynthesis (P_n), transpiration rate (E) and sub-stomatal CO_2 concentration (C_i), while an increase in WUE was observed due to water deficiency in all sesbania accessions. The response of all sesbania accessions was not consistent with respect to different gas exchange characteristics under water limited environments. In contrast to our results, Kosar *et al*. (2015) found that water regimes (80 and 60% field capacity) did not alter C_i, P_n, E and stomatal conductance in the seedlings of two wheat cultivars. A comparison between 17 Vitis species performed by Padgett-Johnson *et al*. (2003) under water limited conditions showed that out of all, 6 Vitis species excelled in plant growth as well as A and g_s under drought conditions. Thus, in view of our results and those presented in the earlier mentioned studies suggest that the drought stress effects on gas exchange attributes strongly depend on drought intensity and/or duration along with type of plant species (Xu *et al*., 2010). Furthermore, the differential response of sesbania accessions to restricted irrigations could be due to some other metabolic limitations rather than those appraised in the present study.

Conclusion

In conclusion, plant fresh and dry biomass and gas exchange characteristics (P_n, E, C_i) of all sesbania accessions were affected adversely due to water shortage, but the response of all sesbania cultivars was not consistent to dry conditions appraised using different physiological parameters. On the basis of plant biomass production under dry conditions, accessions Shahpur, Khanewal 2, Sahianwala and Bhowana 2 were ranked as superior, while accession Sialkot was poorest, so the earlier mentioned accessions could be grown on arid or semi-arid soils with some appropriate management practices.

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