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Water deficit-induced regulation of growth, gas exchange, chlorophyll fluorescence, inorganic nutrient accumulation and antioxidative defense mechanism in mungbean [*Vigna radiata* (L.)Wilczek]

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Summary

The study was conducted to appraise the influence of water deficit conditions on growth, yield, gas exchange characteristics, and antioxidative defense system in two mungbean [*Vigna radiata* (L.) Wilczek] lines, 97001 and 97012. The plants of both lines were grown in equal weight plastic pots for 30 days under normal natural conditions, after which time two drought regimes [control (well-watering and 60 % field capacity)] were applied. Data for various attributes were recorded after 30 days of drought application while at maturity, yield attributes were recorded. Water deficit conditions caused a considerable reduction in growth attributes, net CO_2 assimilation rate, stomatal conductance, electron transport ratio, total phenolics, leaf Ca^{2+} and yield attributes. Imposition of water deficit conditions significantly increased leaf tocopherol contents and activity of catalase in both mungbean lines. Both lines showed a considerable variation in growth attributes, the line 97001 being better in performance compared with 97012 under water deficit conditions.

Introduction

Of various abiotic stresses, drought stress holds an important position on the globe (Farahani et al., 2009; Kusvuran et al., 2011). The main cause of this stress is the high rate of evapo-transpiration, particularly in arid and semi-arid regions having low precipitation rate (Jaleel et al., 2007; Nakayama et al., 2007; Shahbaz et al., 2011a, b). Drought is believed to influence plants from metabolic compartments to an individual organism (Choluj et al., 2004). It can also alter the morpho-physiological and biochemical attributes of plants (Ali et al., 2008; Rahbarian et al., 2011; Shahbaz et al., 2011a). Although drought stress has a marked inhibitory effect on plants at every growth and development phase, its imposition at early growth stages causes considerable reduction in both cell expansion and its elongation (Shao et al., 2008; Ashraf et al., 2013). Drought stress mainly hampers the rate of carbon assimilation which results in decreased growth and yield of most crops (Jabeen et al., 2008; Mafakheri et al., 2010) as well as it significantly reduces chlorophyll fluorescence attributes (Piper et al., 2007).

Under drought stress, a majority of plants accumulate active osmolytes in their cells, the process referred to as osmotic adjustment (Afkari et al., 2009). These osmolytes support the plant's defense mechanism to nullify the adverse effects of drought (Shao et al., 2005) as these osmolytes play a pivotal role in the maintenance of water absorption and cell turgor potential under stress conditions (Chaves et al., 2009; Mahmood et al., 2009). Osmotic adjustment is believed to occur in all plant parts like stem, leaf, root and fruit (Saglam et al., 2010).

Under drought stress, a variety of reactive oxygen species (ROS) are produced, which result in oxidative damage of cell membranes (Maheswari and Dubey, 2009). In order to scavenge these activated oxygen species, plants produce a number of enzymatic antioxidants including: superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT) and peroxidase (POD), as well as non-enzymatic antioxidants such as ascorbic acid (AsA), glutathione, α-tocopherol, flavonoids and carotenoids (Sgherri et al., 2000). It is reported in different studies that the oxidative cellular damage in drought stressed plants is related to the ability of their antioxidant systems (Liu et al., 2009; Basu et al., 2010).

Mungbean [*Vigna radiata* (L.) Wilzeck] is cultivated in many regions of the world because of its considerable nutritional value particularly for people encountering malnutrition (Allahmoradi et al., 2011). Despite this, being a leguminous plant it possesses considerable N fixing ability (Nadeem et al., 2004). Although mungbean crop requires less management practices (Sadeghipour, 2009), sufficient availability of water is attributed for better crop productivity and vice versa (Kramer and Boyer, 1997). Being a favorite crop of arid and semi-arid regions it must have to face drought conditions of varying intensity and time period (Ladrera et al., 2007). Mungbean production is adversely affected by the increase in drought prone area world-over (Postel, 2000). The use of drought resistant genotypes is one of the viable means of attaining better yield under water deficit conditions (Ennajeh et al., 2010). In order to develop drought resistant varieties/lines it is necessary to have the complete knowledge of plant behavior to drought stress (Jaleel et al., 2009). The variability of morpho-physiological attributes under water deficit conditions helps to detect resistant genotypes/lines for better productivity under stress environment (Nam et al., 2001).

In view of considerable inhibitory effects of drought stress on mungbean crop the premier objective of the present investigation was to examine how far this stress regulates some key physio-biochemical attributes involved in growth and development of mungbean plant.

Materials and methods

To assess the effect of water deficit conditions on mungbean, a pot experiment was conducted in Old Botanical Garden, Department of Botany, University of Agriculture, Faisalabad-Pakistan. Two lines of mungbean (lines 97001 and 97012) were obtained from the Ayub Agricultural Research Institute, Faisalabad. Fifteen seeds of each line of mungbean were sown in each of the plastic pots (24.5 cm diameter and 28 cm deep) filled with equal weight dry soil. Two drought stress treatments [Control (well-watered i.e. normal watering as per requirement of the crop) and 60 % field capacity (FC)] were applied on 30-day old plants. The experiment was laid out in a completely randomized design with four replications of each experimental unit. The weight of each plastic pots with filled soil and the water contents present at the time of sowing were already known, therefore moisture contents present in soil of pots equal to Control and 60 % FC were calculated for drought treatments. When water contents of each pot were at field capacity, then 15 seed of each line of pulse crop were sown. After 15 days of germination thinning of plants were done and 5 plants per pot were maintained. Plants were irrigated normally according to their requirements till 30 days before

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treatment start. After one month of normal growth of plants, drought stress levels were maintained. After 30 days of drought treatment, two plants were harvested from each of replicate, washed with distilled water and recorded data for shoot and root fresh weights and shoot and root lengths. The samples were oven-dried at 65 °C up to their constant weight and then dry weights recorded. In addition, data for following attributes were also recorded:

Gas Exchange Characteristics

A portable infra-red gas analyzer (IRGA) (ACD LCA-4 Analytical Development, Hoddesdon, UK) was used to determine the net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), water use efficiency (A/E), and internal CO_2 concentration (C_i) on fully expanded leaves. Following adjustments/values of the instrument were recorded/maintained during its operation: 403.3 mmol m^{-2} s^{-1} for molar flow of air, 99.9 kPa atmospheric pressure, 6.0 to 8.9 mbar water vapor pressure, 1711 µmol m^{-2} s^{-1} *PAR*, 28.4 to 27.9 °C leaf temperature and 352 µmol mol^{-1} ambient CO_2 concentration.

Water relation attributes

A fully expanded second leaf was excised at dawn and its mid rib was used in Scholander type pressure chamber (Arimad-2-Japan) to obtain water potential. The same leaf that used for water potential stored in freezer at -20 ºC to use it for osmotic potential. After one week the frozen leaf was thawed and the sap was extracted by pressing it with glass rod. The extracted sap was used to determine the osmotic potential by using the osmometer (Wescor 5520). Turgor potential was calculated as the difference between water potential and osmotic potential.

Chlorophyll fluorescence

Before measurements of Chlorophyll fluorescence, the leaf samples were kept in dark for 30 min by using light-exclusion clips to the surface of leaves. Chlorophyll fluorescence was determined using an OS5p Modulator Fluorometer (ADC BioScientific Ltd, Great AmwellHerts, UK) according to STRASSER et al. (1995).

Determination of mineral ions

The dried ground leaf or root material (100 mg) was digested with 2 ml H_2SO_4 following the method of WOLF (1982). The volume of the extract was brought up to 50 ml with distilled water, filtered and used determining mineral elements. Leaf and root potassium (K^+) and calcium (Ca^{2+}) contents were determined using a flame photometer (Jenway, PFP-7, UK). Nitrogen was determined following the KJELDAHL method as described by BREMNER et al. (1965) while phosphorus was determined spectrophotometrically following method of JACKSON (1962).

Extraction of antioxidant enzymes

Antioxidant enzymes were extracted from fresh leaf samples (0.5 g each sample) in 10 ml of phosphate buffer (50 mM with pH 7.8) at 4 ºC. The homogenate was then centrifuged at 12000 × g at 4 ºC for 20 min and it was centrifuged again at 15000 × g for 10 min. The supernatant was used for determining the activities of antioxidant enzymes.

Superoxide dismutase (SOD)

The protocol described by GIANNOPOLITIS and RIES (1977) was used for the determination of SOD activity. It was determined as the enzyme ability to inhibit photochemical reduction of nitrobluetetrazolium (NBT). The 3 ml reaction mixture consisted of 50 mM phosphate buffer of 7.8 pH, distilled water, methionine 13 mM, 50 µM NBT, 50 µl enzyme extract and 1.3 µM riboflavin. The reaction solutions were then kept under light (15 W fluorescent lamps) for 15 min at 78 µmol m^{-2} s^{-1}. The absorbance of the reaction mixture was read at 560 nm with a UV-visible spectrophotometer (U2020 IRMECO). One unit activity of SOD was defined as the amount of enzyme required to cause 50 % inhibition of the rate of NBT photoreduction as compared to the sample that lacked the plant enzyme extract.

Activities of catalase (CAT) and peroxidase (POD)

The method described by CHANCE and MAEHLY (1955) was used to appraise the activities of CAT and POD on protein amount basis. The reaction solution for CAT contained phosphate buffer and H_2O_2 of 50 and 5.9 mM, respectively. Addition of 0.1 ml enzyme extract to the reaction mixture initiated the reaction. After every 20 s the changes in the absorbance of the reaction mixture were observed at 240 nm. The reaction mixture for POD consisted of phosphate buffer, guaiacol, and H_2O_2 with molar values as 50, 20 and 40 mM, respectively, and 0.1 ml of the enzyme extract. At 470 nm, the absorbance was taken after every 20 s. The enzyme activity was assessed on protein basis, while one unit of CAT considered equivalent to 0.01 units per min change in absorbance and one unit of POD defined as the 0.01 units per min change in absorbance.

Determination of non-enzymatic antioxidants

Total phenolics

JULKENEN-TITTO (1985) proposed a method which was used to determine total phenolics. Leaf fresh material (50 mg) was homogenized in 80 % acetone. The homogenized material was centrifuged at 10,000 × g for 10 min, removed the pellet and the supernatant was used for the determination of phenolics. Then 100 µl of the supernatant were mixed with 1 ml of Folin-Ciocalteau's reagent. In addition, 2.0 ml distilled water and 5 ml of 20 % Na_2CO_3 were also added. The mixture was vortexed and absorbance read at 750 nm using a UV-Visible spectrophotometer (IRMECO U2020).

Leaf ascorbic acid contents

The amount of ascorbic acid in the mungbean leaves was determined following MUKHERJEE and CHOUDHRI (1983). Fresh leaves (0.25 g) were ground in 10 ml of 6 % TCA. The mixture was centrifuged for 10 min at 4 ºC at 1000 × g. An aliquot of 2 ml of 2 % dinitrophenyl hydrazine solution was added to 4 ml of supernatant. One drop of thiourea (10 % thiourea prepared in 70 % ethanol) was added to the mixture, and boiled the mixture for 20 min in a water bath. The mixture was placed in ice to reduce the temperature to about 25 ºC, then added 5 ml of 80 % sulphuric acid (v/v) at 0 ºC and the absorbance read at 530 nm. The ascorbic acid content was quantified against a standard curve which was prepared by known concentrations of ascorbic acid.

Estimation of leaf tocopherol content

The method of BAKER et al. (1980) was used for the determination of leaf alpha-tocopherol concentration. A mixture of 20 ml of petroleum ether and ethanol (2:1:6, v/v) was used to grind the fresh leaves (1.0 g each sample). The mixture was centrifuged at 10,000 × g for 20 min. An aliquot of 200 µl of 2 % 2, 2- dipyridyl (prepared in ethanol) was added to 1 ml of supernatant, mixed the mixture and placed it in the dark for 5 min. The absorbance was read at 520 nm using a spectrophotometer.

Yield attributes

Data for yield attributes like number of pods per plant, number of seed per plant, seed yield per plant and 100-seed weight were collected at the maturity of the mungbean crop.

Statistical analysis

A two-way analysis of variance (ANOVA) of data for all attributes was calculated using the COSTAT computer program.

Results

Imposition of water deficit conditions (normal watering and 60 % field capacity) significantly reduced shoot and root fresh and dry weights of two mungbean lines (97001and 97012) (Tab. 1; Fig. 1). Both lines showed a significant variation in root dry weight as line 97001 performed better than 97012, while in shoot fresh and dry weights and root fresh weight both lines showed a uniform behavior under both well-watered and drought stress conditions.

Imposition of water deficit conditions markedly ($P \leq 0.001$) reduced shoot length of the two mungbean lines i.e. 97001 and 97012 (Tab. 1; Fig. 1). Of both lines, 97001 showed better performance in shoot length as compared to 97012. In root length, the influence of drought stress was not prominent for both mungbean lines and the lines did not show any variation in this attribute under water deficit conditions (Tab. 1; Fig. 1).

Leaf water potential increased while osmotic potential decreased prominently due to imposition of drought stress regimes (Tab. 1; Fig. 1). Imposition of water deficit conditions caused an increase in leaf turgor pressure (Fig. 1). However, both lines showed a uniform behavior with respect to water relation attributes under well-watered and drought stress conditions.

Various drought stress regimes proved to be non-significant for photochemical quenching (q_P), co-efficient of non-photochemical quenching (q_N), non-photochemical quenching (*NPQ*) and efficiency of photosystem-II (*Fv*/*Fm*) in the two mungbean lines (97001 and 97012). In addition, the lines did not show any difference with respect to all these chlorophyll fluorescence attributes under various

Tab. 1: Mean squares from analyses of variance of data for morphological, physiological, biochemical and yield attributes of mungbean [*Vigna radiata* (L.) Wilczek] when 30 day old plants were subjected to drought stress

SOV	df	Shoot f. wt.	Shoot d. wt.	Root f. wt.	Root d. wt.	Shoot length	Root length	Water potential	Osmotic potential
Drought (D)	**1**	117.0**	2.772*	0.25*	0.024**	907.5***	5.881ns	0.035*	0.453*
Lines (L)	**1**	7.659ns	0.336ns	0.086ns	0.009*	247.3*	1.051ns	0.002ns	0.016ns
D × L	**1**	14.35ns	0.235ns	0.242*	0.004ns	83.26ns	1.626ns	0.0002ns	0.022ns
Error	**12**	11.68	0.466	0.045	0.002	28.83	4.027	0.004	0.050
SOV	**df**	**Turgor potential**	***A***	***E***	g_s	***A*/*E***	C_i	C_i/C_a	***Fv*/*Fm***
Drought (D)	**1**	0.695**	29.00***	0.360*	0.013*	7.777ns	17835.6**	0.144ns	0.006ns
Lines (L)	**1**	0.038ns	1.357ns	0.123ns	0.002ns	1.127ns	715.6ns	0.006ns	0.003ns
D × L	**1**	0.019ns	0.601ns	0.240ns	0.003ns	13.20ns	1410.0ns	0.012ns	0.149ns
Error	**12**	0.053	0.366	0.065	0.002	4.965	1559.1	0.013	0.039
SOV	**df**	***ETR***	***NPQ***	q_N	q_P	**Total phenolics**	**Leaf tocopherols**	**Leaf ascorbic acid**	**CAT**
Drought (D)	**1**	69.72*	0.000ns	0.001ns	0.234ns	3.597**	0.039*	0.0005ns	11.22*
Lines (L)	**1**	0.562ns	0.006ns	0.000ns	0.034ns	0.163ns	0.002ns	0.0001ns	25.49**
D × L	**1**	1.440ns	0.000ns	0.000ns	0.730*	0.007ns	0.024ns	0.00002ns	8.390ns
Error	**12**	8.642	0.017	0.006	0.091	0.295	0.005	0.0002	2.151
SOV	**df**	**POD**	**SOD**	**Total soluble proteins**	**MDA**	**Leaf K^+**	**Root K^+**	**Leaf Ca^{2+}**	**Root Ca^{2+}**
Drought (D)	**1**	0.899ns	0.291ns	4.155*	0.987ns	7.562ns	2.641ns	15.02**	1.891*
Lines (L)	**1**	13.28ns	0.238ns	0.049ns	3.822ns	9.00ns	9.766ns	5.641ns	0.141ns
D × L	**1**	429.4**	0.025ns	0.131ns	4.077ns	0.25ns	0.016ns	5.641ns	0.141ns
Error	**12**	29.22	0.125	0.929	3.43	4.302	3.620	1.411	0.2243
SOV	**df**	**Leaf P**	**Root P**	**Leaf N**	**Root N**	**Number of pods/plant**	**Number of seeds/plant**	**100-seed weight**	**Seed yield per plant**
Drought (D)	**1**	2.806*	0.226ns	0.111ns	0.005ns	37.82**	5681.4**	39.74ns	12.73**
Lines (L)	**1**	0.64ns	0.331ns	0.162ns	0.011ns	0.01ns	606.4ns	34.47ns	0.022ns
D × L	**1**	0.81ns	0.226ns	0.111ns	0.099ns	6.76ns	1113.9ns	133.7ns	0.228ns
Error	**12**	0.546	0.374	0.183	0.046	3.262	413.6	69.31	0.972

*, **, *** = significant at 0.05, 0.01, and 0.001 (probability) levels, respectively
ns = non-significant

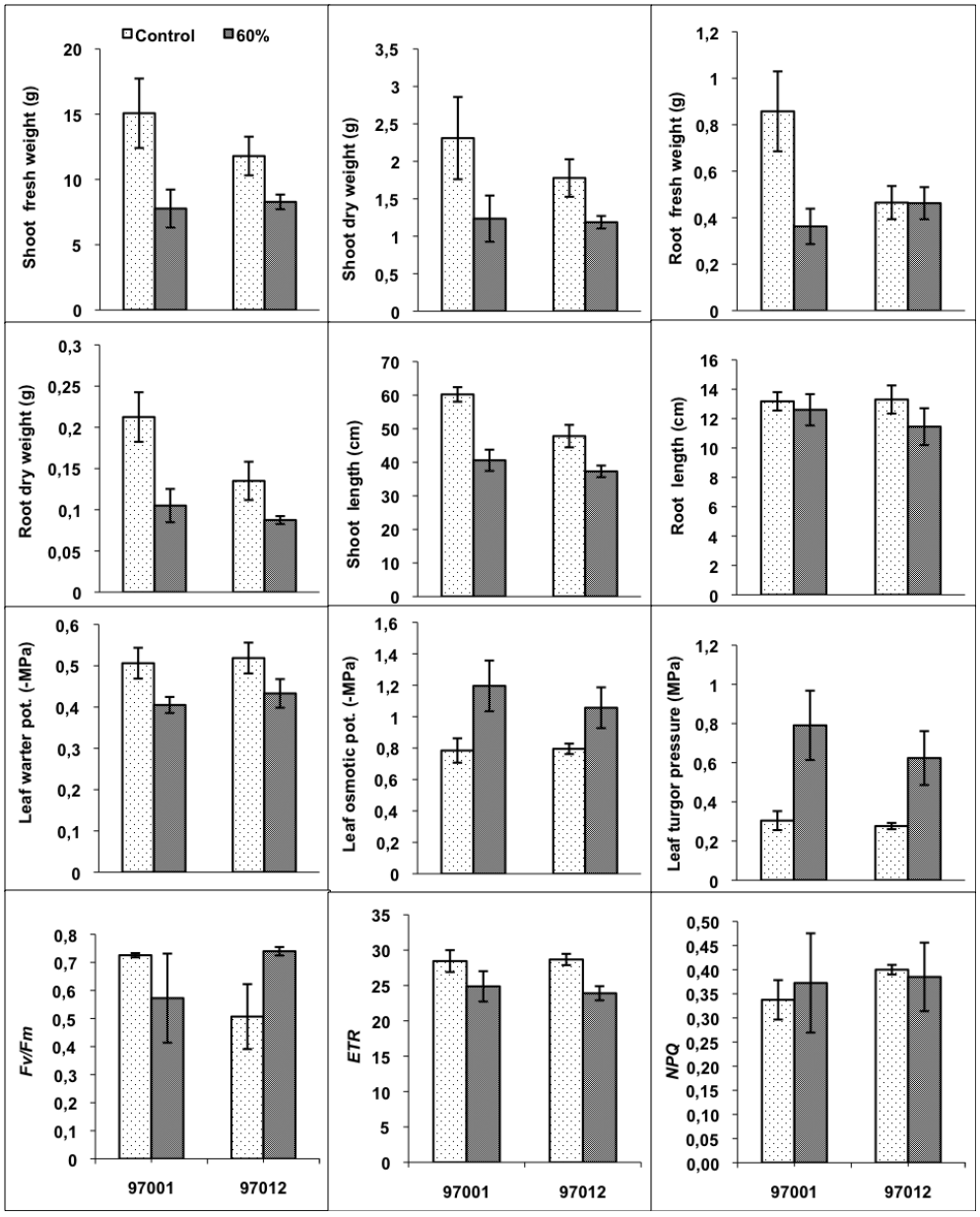

Fig. 1: Growth, water relations and chlorophyll fluorescence attributes of mungbean [*Vigna radiata* (L.) Wilczek] when 30 day old plants were subjected to drought stress (Mean ± S.E.; n = 4).

drought stress regimes (Tab. 1; Fig. 1 and 2). Water stress caused a slight decrease ($P \leq 0.05$) in electron transport ratio (*ETR*) of both mungbean lines. The behavior of the lines was uniform under both well-watered and water deficit conditions.

Water deficit conditions caused a significant decrease in net CO_2 assimilation rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration of the two mungbean lines (Tab. 1; Fig. 2). However, water use efficiency and *Ci*/*Ca* ratio were not altered by water deficit conditions (Tab. 1; Fig. 2). Both lines showed a uniform behavior with respect to all the above-mentioned gas exchange characteristics.

Drought stress caused a slight increase ($P \leq 0.05$) in leaf tocopherol contents in the two mungbean lines while it did not affect leaf ascorbic acid (Tab. 1; Fig. 2). Total phenolic concentration decreased significantly ($P \leq 0.01$) due to drought stress. Both mungbean lines showed a uniform behavior in all the earlier mentioned attributes under well watered and water deficit conditions. Imposition of water deficit conditions showed a non-significant effect on leaf MDA content and the variation between the two mungbean lines with respect to this attribute was also non-significant (Tab. 1; Fig. 3).

Activities of superoxide dismutase and peroxidase, and amount of total soluble proteins did not change due to water deficit conditions (Tab. 1; Fig. 3). Both mungbean lines did not show any variation under both well watered and water deficit conditions. The activity of catalase markedly ($P \leq 0.05$) increased under water deficit conditions. The lines also varied prominently as line 97001 showed more catalase activity than that of mungbean line 97012.

Water deficit conditions did not alter leaf K^+, N and root K^+, P and N contents in two mungbean lines, while it caused a significant decrease in leaf Ca^{2+} and increase in leaf P and root Ca^{2+} in both mungbean lines under well watered and drought stress conditions (Tab. 1; Fig. 3). Both mungbean lines showed uniform response to water deficit conditions due to root and leaf mineral elements and they did not show prominent difference in various ion contents.

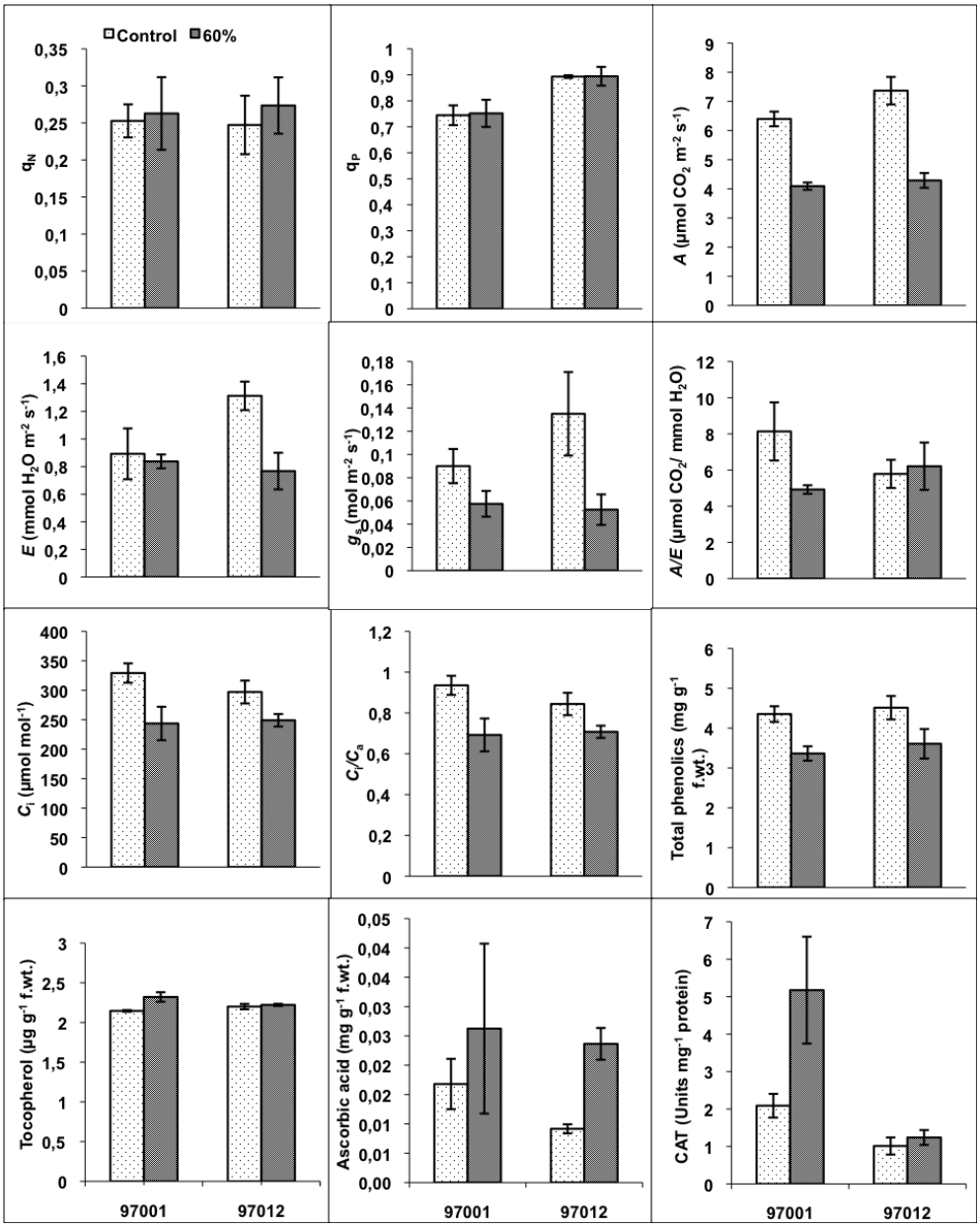

Fig. 2: Gas exchange characteristics and antioxidants of mungbean [*Vigna radiata* (L.) Wilczek] when 30 day old plants were subjected to drought stress (Mean ± S.E.; n = 4).

Number of pods per plant, number of seeds per plant and seed yield per plant of two mungbean lines decreased significantly ($P \leq 0.01$) due to imposition of drought stress (Tab. 1; Fig. 4). Water deficit conditions did not alter 100-seed weight prominently. Both mungbean lines did not show prominent difference with respect to yield attributes under various drought stress regimes.

Discussion

Crop growth and yield is adversely affected by drought stresss (Ashraf, 2010), which is ascribed to drought-induced impairment in a number of metabolic processes involved in regulation of growth and development (Chaves et al., 2009). Drought-induced growth reduction has been reported extensively in many crops like mungbean (Zare et al., 2012), chicory (Asghari et al., 2009), barley (Fateh et al., 2012), wheat (Shahbaz et al., 2011b) etc. In the present study, change in growth was appraised in terms of change in plant height, which decreased markedly under drought stress conditions. These results are in agreement with some previous studies on mungbean (Uddin et al., 2013), chickpea (Shamsi, 2010), and *Ocimum basilicum* (Alishah et al., 2006). Such reduction in plant height under water deficit conditions was ascribed to reduced cell division, expansion and elongation (Hussain et al., 2008).

The effect of drought stress on root growth is the matter of controversy in view of a number of earlier published reports. In the present study, the root length showed a significant decrease under drought stress which does not conform to some earlier published reports on mungbean (Ranawake et al., 2011) as well as on *Catharanthus roseus* (Jaleel et al., 2008). However, our results are in accordance with the findings of Terzi and Kadioglu (2006) for *Ctenanthe setosa* in which a significant decrease in root length was observed.

Water deficit conditions alter water relation parameters in most

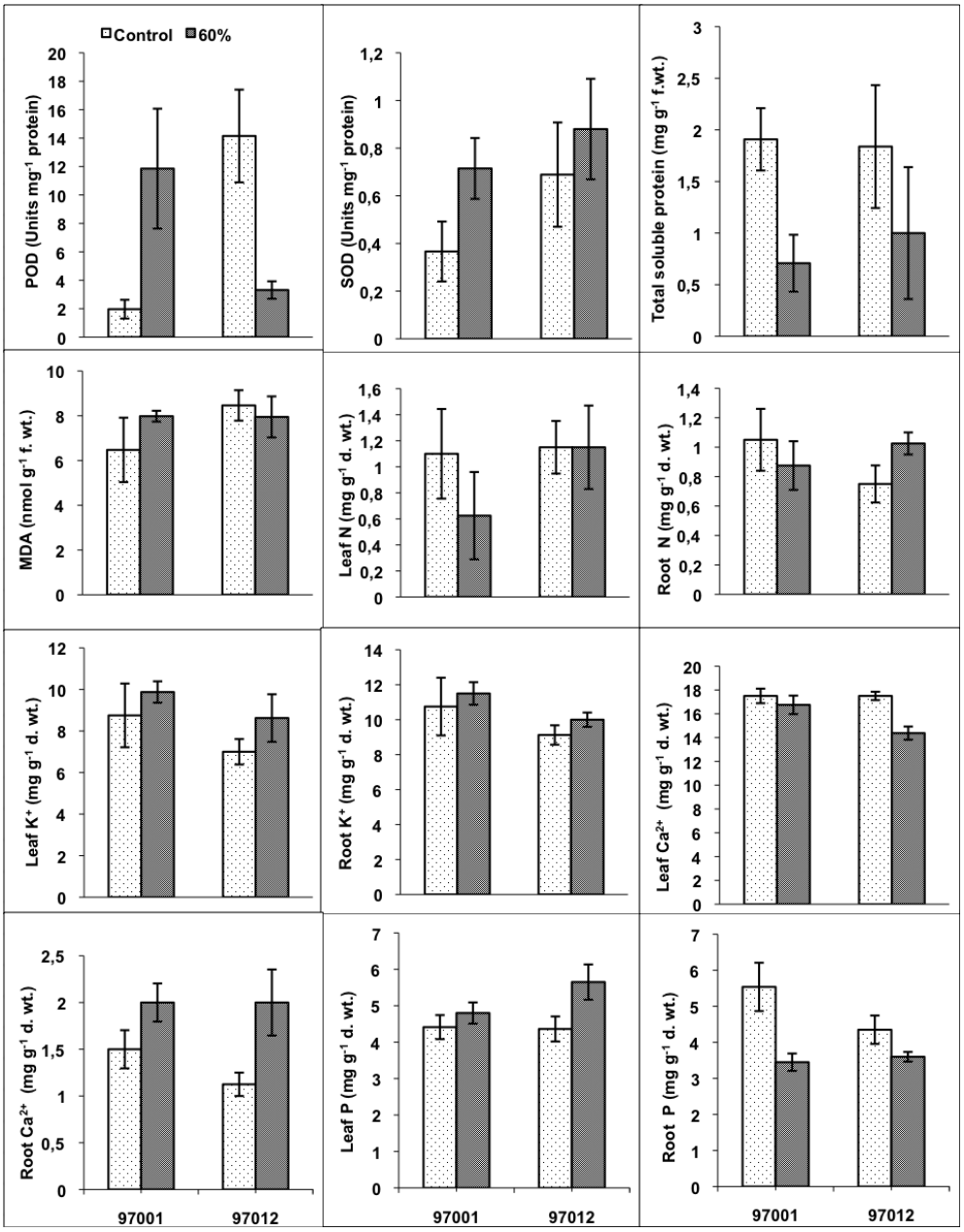

Fig. 3: Activities of antioxidants and mineral nutrients of mungbean [*Vigna radiata* (L.) Wilczek] when 30 day old plants were subjected to drought stress (Mean ± S.E.; n = 4).

plants. For example, imposition of water deficit stress reduced the water potential and osmotic potential of plants of melon (KUSVURAN, 2012), wild jujube (MARAGHNI et al., 2011), and common bean (GULER et al., 2011; ZALVET et al., 2005). But in the present study, leaf water potential and osmotic potential did not change significantly by imposition of drought stress while leaf turgor potential increased significantly in mungbean plants due to drought stress.

Drought stress caused a marked reduction in net CO_2 assimilation rate (A) which is in agreement with many previous studies on different crops, e.g. wheat (KAMRAN et al., 2009; SHAHBAZ et al., 2011b), sunflower (VANAJA et al., 2011), kidney bean (MIYASHITA et al., 2005), grapevine (ZOSFI et al., 2008), and chickpea (RAHBARIAN et al., 2011). Stress-induced reduction in photosynthetic capacity is believed to be due to a number of factors including reduced leaf area, damage to photosynthetic machinery, initiation of leaf senescence before maturity, lipid peroxidation in chloroplast and changes in pigment and protein composition (ANJUM et al., 2011). Reduction in A might also be due to reduction in Rubisco carboxylation activity and regeneration of RuBP (LAWLOR and CORNIC, 2002). Furthermore, low leaf water contents also leads to impaired metabolic system leading to less production of photo-assimilates (LAWLOR and CORNIC, 2002) under water deficit conditions. In the present study, drought stress slightly reduced the rate of transpiration (E) and stomatal conductance (g_s), but did not affect water use efficiency (A/E) in mungbean plants. These results with respect to E and g_s are in agreement with many previous studies on different crops e.g. barley (FATEH et al., 2012), forest plants (KEENAN et al., 2010), chickpea (MAFAKHERI et al., 2010), and rice (HALDER and BURRAGE, 2004) while contradictory with respect to A/E. Drought stress also caused a significant reduction in internal CO_2 concentration (C_i) and C_i/C_a ratio in mungbean plants as has been earlier observed in soybean (ANJUM et al., 2011), forest plants (KEENAN et al., 2010) and wheat

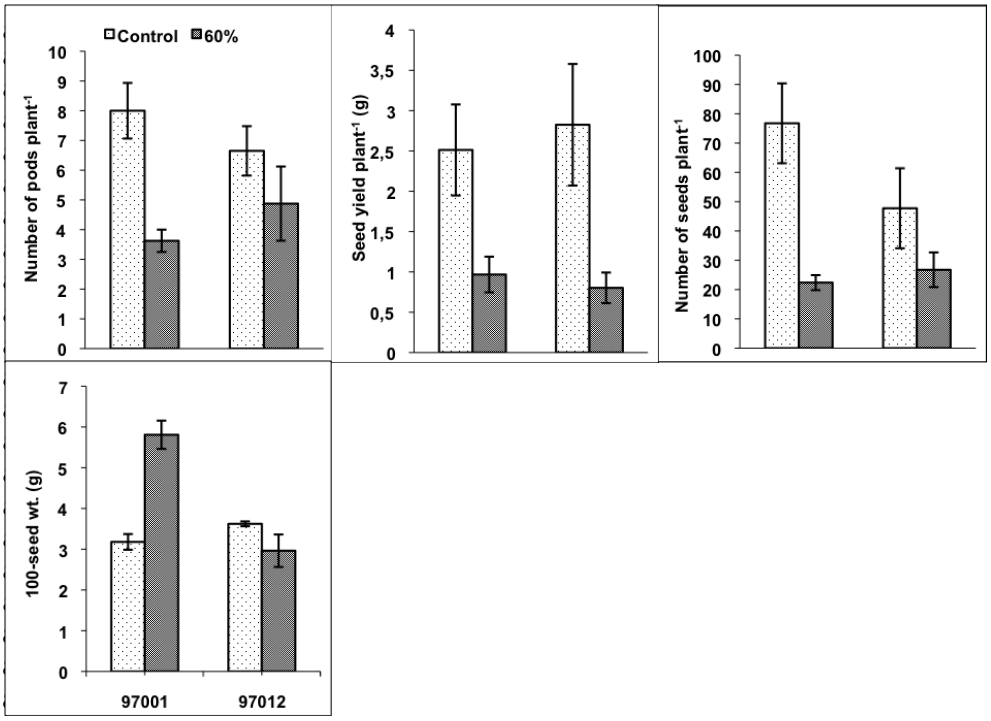

Fig. 4: Leaf and root N and yield attributes of mungbean [*Vigna radiata* (L.) Wilczek] when 30 day old plants were subjected to drought stress (Mean ± S.E.; n = 4).

(BOGALE et al., 2011), but in contrast, these results do not agree with those for chickpea (MAFAKHERI et al., 2010) and rice (HALDER and BURRAGE, 2004). Furthermore, no significant relations were found between water deficit conditions and chlorophyll fluorescence attributes. These results are analogous to what has been reported in chickpea (KHAMSSI et al., 2010) and olive (PETRIDIS et al., 2012). This might be due to low close relation of leaf water potential with chlorophyll fluorescence transients (JEFFERIES, 1992) and leaf water potential might have not affected these attributes. Decrease in *A* may also cause by the photodamage of PSII, but in the present study, drought stress did not affect the efficiency of PSII showing that light did not cause damage to PSII in mungbean under drought stress conditions (BAKER and HORTON, 1987). Decrease in *A* is also related to stomatal conductance as both are reduced under drought stress conditions, but these stomatal effects did not affect the efficiency of PSII (*Fv*/*Fm*) (FLEXAS and MEDRANO, 2002). Some coastal plants also maintained their *Fv*/*Fm* value even under drought stress conditions (DEMATTOS et al., 1997). In our study, drought stress did not cause any severe damage to PSII, and increased photorespiration might also be a factor causing protection to PSII from drought-induced adverse effects (FLEXAS and MEDRANO, 2002).

The activity of enzymatic anti-oxidants, most specifically of superoxide dismutase (SOD), did not alter due to drought stress in mungbean plants which is analogous to the findings of TERZI and KADIOGLU (2006) in *Ctenanthe setosa* and VARGA et al. (2012) in wheat. Peroxidase (POD) and catalase (CAT) in mungbean plants also remained unchanged under water deficit conditions which were in accordance with the findings of GAMBLE and BURKE (1984) who reported a non-significant change in the activity of CAT in wheat under water deficit conditions. One of the possible reasons is that CAT has low affinity for ROS particularly for H_2O_2 as compared to ascorbate peroxidase (MITTLER, 2002). Furthermore, MDA and ascorbic acid (AsA) contents in mungbean plants also did not change under drought regimes. In our study, damage to membrane in the form of lipid peroxidation as shown by MDA contents is not severe (non-significant due to drought stress) and this might be a main reason for the non-significant effect of drought stress on the activities of antioxidant enzymes. In addition, activities of antioxidant enzymes depend on a number of factors like plant age, drought stress duration, tolerance level of a particular species (DECARVALHO, 2008). Drawing correlation between induction of antioxidants and degree of drought tolerance among species of a same genus or even cultivars of a same species has been found not so easy (LOGGINI et al., 1999). These results are not in agreement with those reported for *Withania somnifera* (JALEEL et al., 2009) and tomato fruit (GHORBANLI et al., 2012) under drought stress conditions but tocopherol was increased by the application of water stress, similar to that reported in canna cultivars (ZHANG et al., 2013). Increase in tocopherol under water deficit conditions might be due to active expression of genes which are responsible for the synthesis of tocopherols (ZHU, 2002; ZONG et al., 2009). TERZI and KADIOGLU (2006) observed an increase in MDA content during early phase of drought while a decrease in later phase in *Ctenanthe setosa*. Leaf phenolics in the present study decreased by the imposition of stress which is contrary to that found in maize (HURA et al., 2008) and olive (PETRIDIS et al., 2012), while in contrast, an increase in phenolic contents was reported in horsegram (BHARDWAJ and YADAV, 2012).

Water deficit conditions are believed to generally reduce nutrient uptake in plants (BALIGAR et al., 2001) and plants with small or no reduction in nutrient uptake are considered drought resistant. However, great variation has been observed in nutrient uptake in even genotypes of a same species under drought stress conditions (SHAHBAZ et al., 2011a, b). In the present study, drought stress had a non-significant effect on leaf and root N and K^+ except P which decreased in mungbean plant roots under drought stress. Similar results have been observed in wheat (SHAHBAZ et al., 2011b), while in contrast, higher K^+ accumulation has been reported in drought stressed common bean (ZADEHBAGHERI et al., 2012) and okra (KUSVURAN et al., 2011) plants. A non-significant influence of drought stress was also observed on shoot and root K^+ for *Cenchrus ciliarus* and *Cyanodon*

dactylon (AKRAM et al., 2008).

In conclusion, water deficit conditions significantly reduced all growth attributes, net CO_2 assimilation rate, internal CO_2 concentration, C_i/C_a ratio, root P, total soluble proteins, phenolics and seed yield, but they markedly increased turgor potential, and tocopherol contents.

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