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DROUGHT STRESS EFFECTS ON PHOTOSYNTHESIS, CHLOROPHYLL FLUORESCENCE AND WATER RELATIONS IN TOLERANT AND SUSCEPTIBLE CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES

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In order to evaluate morphological and physiological traits related to drought tolerance and to determine the best criteria for screening and identification of drought-tolerant genotypes, we grew two tolerant genotypes (MCC392, MCC877) and two sensitive genotypes (MCC68, MCC448) of chickpea under drought stress (25% field capacity) and control (100% field capacity) conditions and assessed the effect of drought stress on growth, water relations, photosynthesis, chlorophyll fluorescence and chlorophyll content in the seedling, early flowering and podding stages. Drought stress significantly decreased shoot dry weight, CO_2 assimilation rate (A), transpiration rate (E), and PSII photochemical efficiency (F_v/F_m) in all genotypes. In the seedling and podding stages, PSII photochemical efficiency was higher in tolerant genotypes than in sensitive genotypes under drought stress. Water use efficiency (WUE) and CO_2 assimilation rate were also higher in tolerant than in sensitive genotypes in all investigated stages under drought stress. Our results indicated that water use efficiency, A and F_v/F_m can be useful markers in studies of tolerance to drought stress and in screening adapted cultivars of chickpea under drought stress.

Key words: Chlorophyll fluorescence, chickpea (*Cicer arietinum* L.), drought stress, photosynthesis.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important food legume crop which is grown in semi-arid regions. Generally, legumes are highly sensitive to water deficit stress (Labidi et al., 2009). Water deficit affects many morphological features and physiological processes associated with plant growth and development (Toker and Cagirgan, 1998). These changes include reduction of water content (RWC), diminished leaf water potential (Ψ_w) and turgor loss, closure of stomata and a decrease of cell enlargement and plant growth. Drought stress reduces plant growth by affecting photosynthesis, respiration, the membrane stability index (MSI) and nutrient metabolism (Jaleel et al., 2008a). The morphological and physiological changes in response to drought stress can be used to help identify resistant genotypes or produce new varieties of crops for better productivity under drought stress (Nam et al., 2001). The reactions of plants to drought stress depend on the intensity and duration of stress as well as the plant species and its stage of growth (Parameshwarappa and Salimath, 2008).

In drought stress conditions, plants close their stomata to avoid further water loss. Decreasing internal CO_2 concentration (C_i) and inhibition of ribulose-1, 5-bisphosphate carboxylase/oxygenase enzyme activity and ATP synthesis lead to a decrease of net photosynthetic rate under drought stress (Dulai et al., 2006). Reduced inhibition of photosynthesis under drought stress is of great importance for drought tolerance (Zlatev and Yordanov, 2004).

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The effect of drought stress on CO_2 assimilation rate (A), transpiration rate (E) and water use efficiency (WUE) has been investigated in many crops such as *Zea mays* (Ashraf et al., 2007), *Brassica napus* L. (Kauser et al., 2006) and mungbean genotypes (Ahmed et al., 2002).

Another plant response to drought stress is change in photosynthetic pigment content. Photosynthetic pigments play important roles in harvesting light. The content of both chlorophyll *a* and *b* changes under drought stress (Farooq et al., 2009). The carotenoids play fundamental roles and help plants to resist drought stress (Jaleel et al., 2009). Drought stress inhibits Chl a/b synthesis and decreases the content of Chl *a/b* binding proteins, leading to reduction of the light-harvesting pigment protein associated with photosystem II (Sayed, 2003). The effects of drought stress on chlorophyll and carotenoid content have been investigated in cotton (Mssacci, 2008) and *Catharanthus roseus* (Jaleel et al., 2008a).

Drought stress affects photosystem efficiency (F_v/F_m) and decreases the electron transport rate (ETR) and the effective quantum yield of photosystem II photochemistry (Y) (Ahmed et al., 2002). The F_v/F_m ratio is a parameter which allows detection of any damage to PSII and possible photoinhibition (Ahmed et al., 2002). Changes in the proportion of photochemical and energy-dependent quenching lead to alteration of fluorescence kinetics under drought stress (Zlatev and Yordanov, 2004). Chlorophyll fluorescence emitted from the chloroplast thylakoid membrane is often used as a very sensitive intrinsic indicator of the photosynthetic reaction in photosystem II (Ahmed et al., 2002). Analysis of chlorophyll fluorescence and measurement of the F_v/F_m ratio can be useful in determining damage to light reaction systems in photosynthetic mechanisms under drought stress.

The effects of drought stress on MSI, RWC and leaf water potential have also been investigated in many studies (Siddique et al., 2000; Jinmin and Huang, 2001; Terzi and Kadioglu, 2006; Bayoumi et al., 2008). It is believed that leaf water potential and RWC are reliable parameters for quantifying the plant drought stress response (Siddique et al., 2000; Bayoumi et al., 2008).

In this study we measured the early responses of certain parameters associated with photosynthesis and the involvement of various factors in photosynthetic damage in chickpea plants under drought stress. We assessed the effects of drought stress on leaf water potential, relative water content and membrane stability in sensitive and resistant chickpea genotypes to find a fast and easy technique for screening chickpea genotypes for drought tolerance.

MATERIALS AND METHODS

PLANT MATERIALS

Seeds of two tolerant genotypes (MCC392, MCC877) and two sensitive genotypes (MCC68, MCC448) were grown in pots containing 3 kg soil mixture composed of sand and farmyard manure (2:3) under drought stress (25% field capacity) and control conditions (100% field capacity) at the Research Center for Plant Science, Ferdowsi University of Mashhad, Iran. Three seeds were planted in each pot in a growth chamber. They were kept under a 12.5 h photoperiod (21°C day/8°C night) for the first month and under a 13 h photoperiod (27°C day/12°C night) the second month, similar to normal field situations in the chickpea growing region. Morphological and physiological indices were measured in the seedling, early flowering and podding stages in order to find reproducible, fast and easy techniques for screening chickpea genotypes for drought tolerance.

PHYSIOLOGICAL MEASUREMENTS

Gas exchange measurement

Photosynthetic gas exchange was measured from non-detached young and fully expanded leaves using a portable infrared gas analyzer (IRGA, LCA4, ADC Bio. Scientific Ltd., Herfordshire, UK): leaf surface area 1 cm^2, ambient CO_2 concentration 370 μmol mol^{-1}, and PPFD 200 μmol $m^{-2}s^{-1}$. The leaf internal CO_2 concentration (C_i), CO_2 assimilation rate (A), and transpiration rate (E) were recorded between 09.00 and 11.00 a.m. Water use efficiency (WUE) was calculated from the A/E ratio (Piper et al., 2007).

Chlorophyll fluorescence

Photosystem photochemical efficiency (F_v/F_m) was measured using a portable chlorophyll fluorometer (OS5-FL modulated chlorophyll fluorometer, ADC Bio Scientific Ltd. Hoddesdon, Hert, EN11 0DB England). Minimal fluorescence (F_o) was determined by applying weak modulated light (0.4 μmol $m^{-2}s^{-1}$) and maximal fluorescence (F_m) was induced by a short pulse (0.8 s) of saturating light (8000 μmol $m^{-2}s^{-1}$). Measurements were made from the same leaf used for gas exchange determination, after 20 min dark adaptation (Maxwell et al., 2000). All physiological measurements used four or more plants from each treatment under drought stress and control conditions.

Chlorophyll content

Fresh leaves (0.1 g) were extracted with 15 ml 80% acetone and centrifuged at 5000×g for 10 min. The absorbance of the supernatant was read at

TABLE 1. Total chlorophyll content (Total Chl) (mg g^{-1} FW), internal CO_2 concentration (C_i) (vpm), CO_2 assimilation rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$) and leaf water potential (MPa) in the seedling stage of chickpea genotypes in drought and control conditions

Genotype	Treatment	Total Chl	C_i	A	E	Ψw
MCC392	control	17.7a	817.0a	24.4a	3.9bcd	-1.2a
MCC68	control	22.1a	775.0a	17.9a	7.4a	-0.99ab
MCC877	control	14.7a	862.9a	25.2a	6.2ab	-0.91b
MCC448	control	22.7a	880.0a	18.9a	6.2ab	-0.95ab
MCC392	drought	14.5a	868.7a	18.2a	2.7cd	-1.01ab
MCC68	drought	14.5a	882.3a	15.2a	6.2ab	-0.88b
MCC877	drought	16.4a	817.4a	18.3a	2.3d	-1.08ab
MCC448	drought	22.1a	800.4a	14.4a	2.6cd	-0.90b

Values with the same letter within column do not differ significantly ($p<0.05$).

663, 647 and 470 nm and calculated for chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content according to Arnon (1949). The chlorophyll stability index (CSI) was determined according to Sairam et al. (1997) and calculated as follows:

CSI = (total chlorophyll under stress/total chlorophyll under control) × 100

Leaf water potential (Ψ_w)

Leaf water potential (Ψ_w) of control and stressed plants was measured using a pressure chamber (ARIMAD 3000, MRC), from the terminal leaflet of the uppermost fully expanded leaf of each plant (Gindaba et al., 2004).

Relative water content (RWC)

Relative water content was determined according to Barr and Weatherley (1962). Fresh weight of the young fully expanded leaf was determined within 2 h after excision. Turgid weight was obtained after soaking the leaf for 16 to 18 h in distilled water. After soaking, the leaves were quickly and carefully blotted dry with tissue paper prior to determination of turgid weight. Shoot dry weight was obtained after drying the leaf sample for 72 h at 70°C. Relative water content was calculated from the following equation:

RWC = [(fresh weight – dry weight)/(turgid weight – dry weight)] × 100

Membrane stability index (MSI)

Leaf samples (0.1 g) of leaf material were taken in 10 mL double-distilled water in glass vials and kept at 40°C for 10 min. Initial conductivity (C_1) was recorded with a conductivity meter after bringing the sample to 25°C. The samples were kept at 100°C for 30 min and cooled to 25°C, and final conductivity (C_2) was recorded according to Premachandra et al. (1990) as modified by Sairam (1994). The membrane stability index (MSI) was calculated as

MSI = [1– (C_1/C_2)] × 100

STATISTICAL ANALYSIS

The study was conducted as a factorial experiment based on a completely random design with four replicates. The data were analyzed by ANOVA and the significance of differences between treatment means was checked with Duncan's multiple range test ($p<0.05$).

RESULTS

SHOOT DRY WEIGHT

Drought stress decreased shoot dry weight in all genotypes in the three investigated stages (Fig. 3b), but the effects of drought stress on shoot dry weight were significant only in the podding stage. There were no significant differences between genotypes in either drought or control conditions in the seedling and early flowering stages (Fig. 3b). In the podding stage, however, shoot dry weight was higher in one tolerant genotype (MCC392) than in one sensitive genotype (MCC68) in both drought and control conditions (Fig. 3b). Drought stress decreased shoot dry weight in the podding stage more than in other stages. In the podding stage, genotype MCC877 had lower shoot dry weight than the other genotypes under drought stress (Fig. 3b).

TABLE 2. Mean of total chlorophyll content (Total Chl) (mg g^{-1} FW), internal CO_2 concentration (C_i) (vpm), CO_2 assimilation rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$) and leaf water potential (MPa) in the early flowering stage of chickpea genotypes in drought and control conditions

Genotype	Treatment	Total Chl	C_i	A	E	ψw
MCC392	control	3.7a	589.5ab	14.9b	1.5b	-0.72c
MCC68	control	4.0a	606.7ab	13.0b	2.3a	-0.85bc
MCC877	control	1.8b	634.1a	25.9a	2.7a	-0.77c
MCC448	control	3.9a	617.6b	15.5b	1.0bc	-0.69c
MCC392	drought	1.5b	533.0ab	11.1ab	0.67cd	-0.87bc
MCC68	drought	1.8b	537.3ab	6.4b	0.41d	-0.92ab
MCC877	drought	0.8b	612.4ab	16.2ab	0.55cd	-0.99a
MCC448	drought	1.6b	524.7b	10.5ab	0.54cd	-0.91a

Values with the same letter within column do not differ significantly ($p<0.05$).

TABLE 3. Total chlorophyll content (Total Chl) (mg g^{-1} FW), internal CO_2 concentration (C_i) (vpm), CO_2 assimilation rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$) and leaf water potential (MPa) in the podding stage of chickpea genotypes in drought and control conditions

Genotype	Treatment	Total Chl	C_i	A	E	ψw
MCC392	control	7.9ab	450.1ab	16.7a	3.1a	-0.67b
MCC68	control	5.7b	439.2ab	10.95ab	5.9a	-0.80ab
MCC877	control	10.6ab	464.3ab	15.7ab	2.9a	-0.64b
MCC448	control	11.3ab	580.6a	12.87ab	5.8a	-0.60b
MCC392	drought	10.6ab	440.9ab	14.61ab	1.3b	-0.74ab
MCC68	drought	8.6ab	515.3ab	7.52ab	1.3b	-0.90a
MCC877	drought	12.4ab	445.3ab	14.5ab	0.5b	-0.70ab
MCC448	drought	13.5a	420.6b	6.15b	1.3b	-0.75ab

Values with the same letter within column do not differ significantly ($p<0.05$).

GAS EXCHANGE

Leaf internal CO_2 concentration (C_i)

Under both drought stress and control conditions, in the seedling stage there were no significant differences in leaf internal CO_2 concentration between genotypes (Tab. 1).

In the early flowering stage, C_i decreased in all genotypes versus the levels in control plants, but these differences were not significant (Tab. 2). Genotype MCC877 (tolerant) had significantly higher C_i than genotype MCC448 (sensitive) in control conditions. C_i was highest in genotype MCC877 in both control and drought-stressed plants.

In the podding stage, C_i decreased in MCC392, MCC877 and MCC448 but increased in MCC68 under drought stress as compared to the control (Tab. 3). The lowest C_i belonged to genotype MCC448 under drought stress (Tab. 3).

CO_2 assimilation rate (A)

In all investigated stages the CO_2 assimilation rate decreased in all genotypes under drought stress as compared to the control (Tabs. 1–3). In the seedling stage, A was highest in the tolerant genotypes (MCC877, MCC392) in both drought and control conditions.

In the early flowering stage, A was highest in MCC392 and lowest in MCC68 in both drought-stressed and control plants (Tab. 2).

In the podding stage, A had less reduction in MCC392 and MCC877 genotypes under drought stress, but it showed more reduction in MCC68 and MCC448 genotypes under drought stress as compared to control conditions (Tab. 3). In the podding stage, A fell less in MCC392 and MCC877 under drought stress versus the control than it did in MCC68 and MCC448 (Tab. 3). In both the early flowering and the podding stages, A was higher in the tolerant (MCC392, MCC877) than in the sensitive (MCC68, MCC448) genotypes under drought stress.

Transpiration rate (E)

In all three investigated stages, the transpiration rate decreased in all genotypes under drought

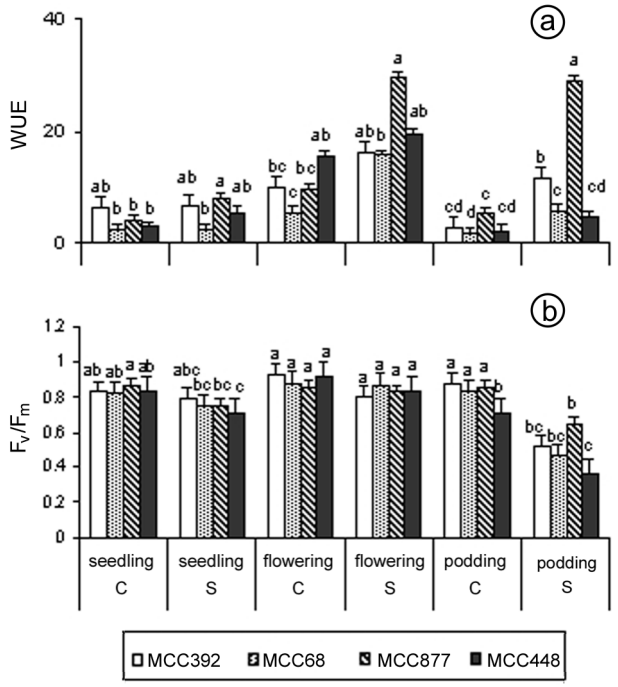

Fig. 1. Effects of drought stress on water use efficiency (WUE) (**a**) and PSII photochemical efficiency (F_v/F_m) (**b**) in the seedling, early flowering and podding stages in chickpea genotypes. C – control (100% FC); S – drought stress (25% FC). Means of four replicates. Values with different letters differ significantly ($p<0.05$).

stress versus the control (Tabs. 1–3). In the seedling stage, E was highest in MCC68 and lowest in MCC392 in control plants. Sensitive genotypes (MCC68, MCC448) had higher E than tolerant genotypes (MCC392, MCC877) under drought stress (Tab. 1).

In the early flowering stage, MCC392 had significantly lower E than MCC68, and MCC877 had significantly lower E than MCC448 in control plants. It decreased by 82% in MCC68, by 80% in MCC877, by 55% in MCC392 and by 46% in MCC448 in drought-stressed plants. In the podding stage, E significantly decreased in all genotypes under drought stress, but there were no significant differences between genotypes in either drought or control conditions. The decrease was greatest in MCC877 (92%) under drought stress.

WATER USE EFFICIENCY (WUE)

Water use efficiency significantly increased under drought stress in all three investigated stages. The tolerant genotypes (MCC392, MCC877) had higher WUE than the sensitive genotypes (MCC68, MCC448) under drought stress in all stages (Fig. 1a). WUE was highest in MCC877 genotype under drought stress in all investigated stages (Fig. 1a).

Water use efficiency significantly increased from the seedling to the early flowering stage and then decreased in the podding stage.

CHLOROPHYLL FLUORESCENCE

PSII photochemical efficiency (F_v/F_m ratio)

The F_v/F_m ratio decreased in all genotypes and in all three stages under drought stress (Fig. 1b). In the seedling stage the F_v/F_m ratio was highest in MCC877 (tolerant) and lowest in MCC68 (sensitive) in control plants; under drought stress it was highest in MCC392 (tolerant) and lowest in MCC448 (sensitive) (Fig. 1b). Under drought stress, in this stage the decrease in the F_v/F_m ratio versus the control was significant in MCC877 and MCC448 ($p<0.01$) (Fig. 1b).

In the early flowering stage, MCC68 had the highest and MCC392 the lowest F_v/F_m ratio under drought stress (Fig. 1b), but there were no significant differences between genotypes in either drought stress or control conditions (Fig. 1b).

In the podding stage, drought stress significantly decreased the F_v/F_m ratio in all genotypes (Fig. 1b). The F_v/F_m ratio was highest in MCC877 under drought stress and in MCC392 in the control. The F_v/F_m ratio was significantly higher in the tolerant genotypes (MCC392, MCC877) than in the sensitive genotypes (MCC68, MCC448) in both seedling and podding stages under drought stress (Fig. 1b).

Chlorophyll content

In the seedling stage under drought stress, total chlorophyll content decreased in MCC392 (by 18%), MCC448 (3%) and MCC68 (30%), and increased in MCC877 (12%) (Tab. 1); carotenoid content decreased in MCC392, MCC448 and MCC68, and increased in MCC877 (Fig. 2b).

MCC448 had the highest chlorophyll *a* and *b* and carotenoid content under drought stress in all stages (Fig. 2a–c). In the seedling stage, the chlorophyll *a*/*b* ratio in all genotypes was significantly lower under drought stress than in control conditions (Fig. 2d).

In the early flowering and podding stages, chlorophyll *a* and *b* content significantly increased in all genotypes under drought stress (Fig. 2a, c). In the early flowering stage, chlorophyll *b* and carotenoid content in MCC877 was significantly lower than in the other genotypes in control conditions (Fig. 2b, c). In MCC392, MCC68 and MCC877 the chlorophyll *a*/*b* ratio in drought conditions was significantly higher than in control conditions (Fig. 2d).

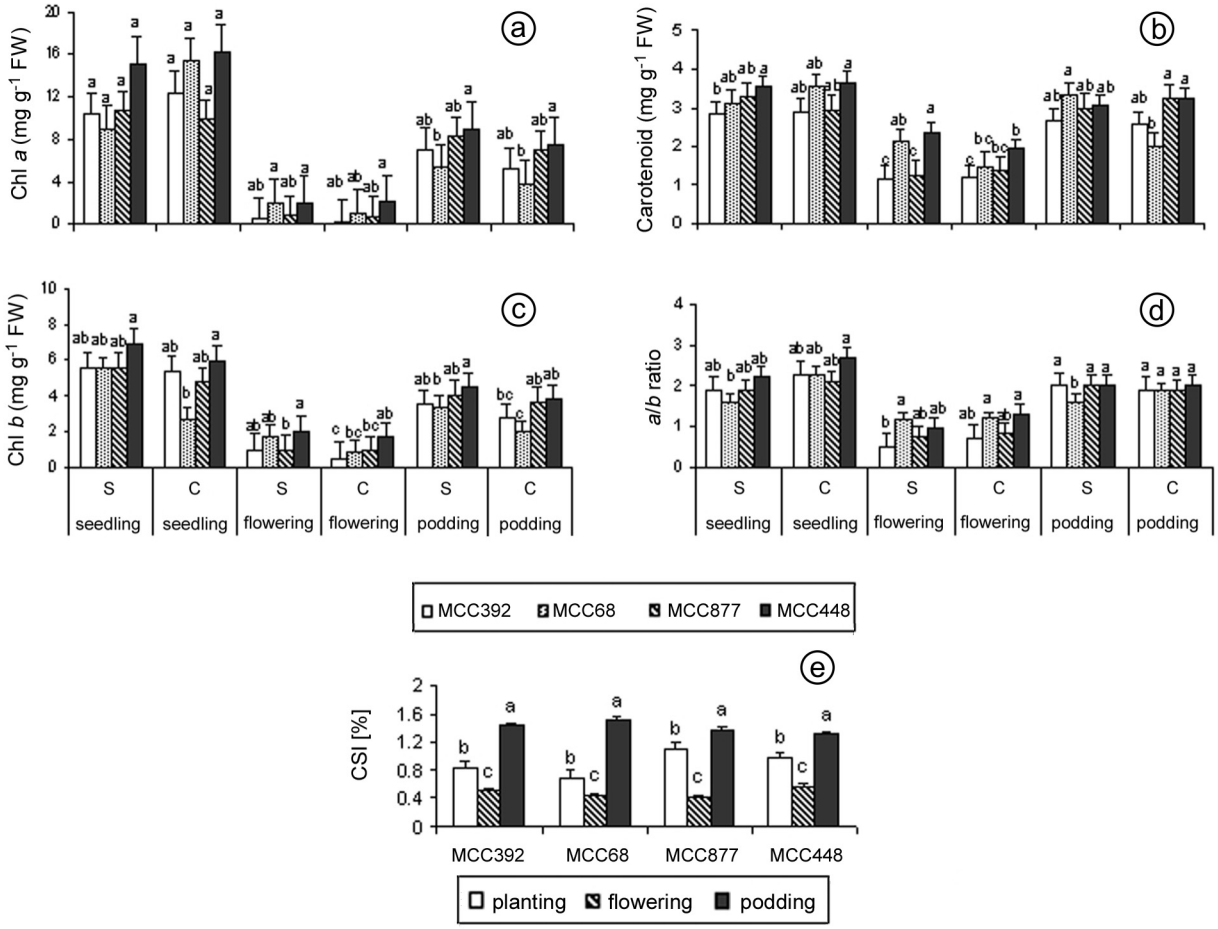

Fig. 2. Effects of drought stress on chlorophyll *a* (**a**), carotenoid content (**b**), chlorophyll *b* (**c**) *a*/*b* ratio (**d**) and chlorophyll stability index (CSI) (**e**) in the seedling, early flowering and podding stages of chickpea genotypes. C – control (100% FC); S – drought stress (25% FC). Means of four replicates. Values with different letters differ significantly ($p<0.05$).

In the podding stage, carotenoid content decreased in MCC877 and increased in MCC448 and MCC68 (Fig. 2b), so the chlorophyll *a*/*b* ratio was highest in MCC392 and lowest in MCC68 under drought stress (Fig. 2d)..

Chlorophyll content and carotenoid content were highest in seedlings and lowest during early flowering.

The three investigated stages differed significantly in the chlorophyll stability index (CSI). It was highest in the podding stage and lowest during early flowering (Fig. 2e).

There were significant positive correlations between chlorophyll *a* and chlorophyll *b* in all stages: seedling ($r^2=0.60$), early flowering ($r^2=0.76$), and podding ($r^2=0.23$), and also between total chlorophyll and carotenoids ($r^2=0.74$, 0.95 and 0.94 respectively) ($p<0.01$).

LEAF WATER POTENTIAL

In the seedling stage, MCC392, MCC68 and MCC448 had higher and MCC877 had lower leaf water potential under drought stress than in control conditions (Tab. 1). Drought stress significantly affected Ψ_w in the seedling and flowering stages. The tolerant genotypes had significantly lower Ψ_w than the sensitive genotypes under drought stress ($p<0.05$) (Tab. 1).

In the early flowering and podding stages, drought stress decreased Ψ_w versus the control ($p<0.05$) (Tabs. 2, 3). In early flowering, MCC392 had the highest and MCC877 the lowest Ψ_w under drought stress (Tab. 2). In the early flowering and podding stages, one sensitive genotype (MCC68) had lower Ψ_w than one tolerant genotype (MCC392), but MCC877 and MCC448 did not differ significantly under drought stress, nor in control conditions (Tabs. 2, 3).

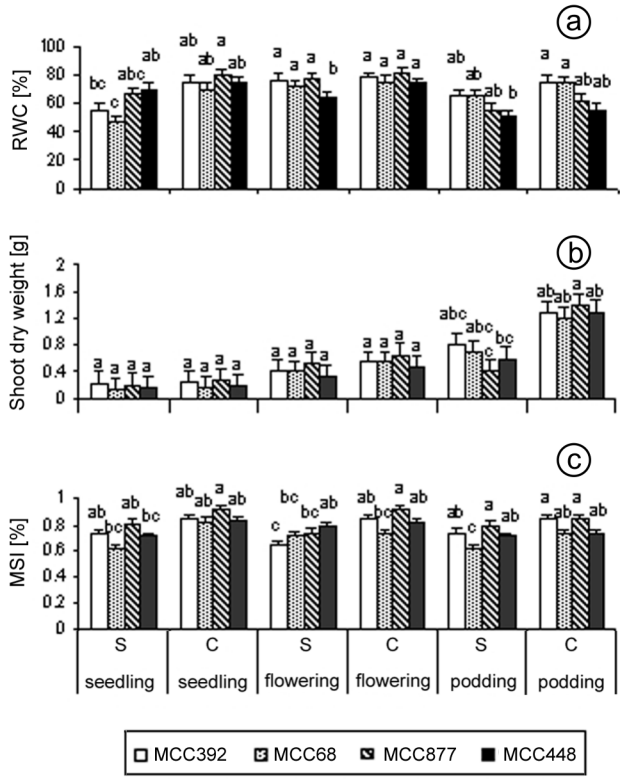

Fig. 3. Effects of drought stress on relative water content (RWC) (**a**), shoot dry weight (**b**) and membrane stability index (MSI) (**c**) in the seedling, early flowering and podding stages of chickpea genotypes. C – control (100% FC); S – drought stress (25% FC). Means of four replicates. Values with different letters differ significantly ($p<0.05$).

RELATIVE WATER CONTENT (RWC)

Drought stress decreased the relative water content of all genotypes in all three stages (Fig. 3a).

In the seedling stage there were no significant differences in RWC between genotypes in control conditions. MCC68 (sensitive) had significantly lower RWC than MCC448 (sensitive) under drought stress (Fig. 3a). It was lowest in MCC68 under drought stress (Fig. 3a). In the early flowering and podding stages, RWC was higher in the tolerant genotypes (MCC392, MCC877) than in one sensitive genotype (MCC448) under drought stress. RWC was highest during early flowering in all genotypes under drought stress (Fig. 3a).

MEMBRANE STABILITY INDEX (MSI)

In the seedling and early flowering stages, membrane stability significantly decreased versus the control in all genotypes under drought stress (Fig. 3). In the seedling stage, MSI was highest in MCC877 (tolerant) and lowest in MCC68 (sensitive) under drought stress (Fig. 3c). In control conditions, MSI was highest in MCC877 (Fig. 3c). In the early flowering stage, MSI was significantly higher in MCC877 than in the sensitive genotypes (MCC68, MCC448) in control plants (Fig. 3c), but under drought stress MCC448 had the highest and MCC392 (tolerant) the lowest MSI.

In the podding stage, MSI decreased in MCC392 and MCC68 under drought stress, but did not significantly differ between MCC877 and MCC448 (Fig. 3c). MSI in tolerant MCC392 and MCC877 was higher than in sensitive MCC68 and MCC448 in both drought and control conditions.

DISCUSSION

Drought stress alters many physiological and metabolic processes in plants (Gunes et al., 2006). Shoot dry weight is one of the earliest plant responses to drought. In this study, drought stress decreased shoot dry weight in all three investigated stages. The tolerant genotypes (MCC392, MCC877) had higher shoot dry weight than the sensitive ones (MCC68, MCC448) under drought stress. Higher shoot dry weight in tolerant genotypes under drought stress may be related to greater root growth, which helps in uptake of water and nutrients, boosting growth under drought stress. Reduction of shoot dry weight under drought stress has been reported in *Zea mays* L. (Ashraf et al., 2007), *Beta vulgaris* L. (Hussein et al., 2008) and *Cicer arietinum* L. (Gunes, et al., 2006).

In this work we found that drought stress decreased the CO_2 assimilation rate, relative water content, leaf water potential and membrane stability in all investigated stages. The tolerant genotypes (MCC392, MCC877) had higher values for relative water content, the membrane stability index, CO_2 assimilation rate and water use efficiency than the sensitive genotypes (MCC68, MCC448) under drought stress in all investigated stages. These results are in agreement with Piper et al.'s (2007) findings in *Nothofagus dombeyi* and *Nothofagus nitida*; they reported that the greater drought tolerance of *N. dombeyi* versus *N. nitida* was associated with higher water use efficiency and photosynthesis under drought stress. Nageswara et al. (2008) considered water use efficiency to be an important trait for selection of drought-tolerant varieties. In mungbean plants, Ahmed et al (2002) also found that drought stress decreased the CO_2 assimilation rate and leaf water potential.

Of the three investigated stages, the CO_2 assimilation rate in drought-stressed plants was highest in the seedling stage. Zlatve et al (2004) suggested that decreasing CO_2 assimilation under drought stress

may be related to restriction of CO_2 diffusion into the leaf, and also inhibition of biochemical processes such as ATP synthase and Rubisco activity.

Our results showed significant positive correlations between the membrane stability index and relative water content in all genotypes in the seedling (r^2=0.184) and flowering (r^2=0.12) stages ($p<0.01$); genotypes that could maintain higher relative water content had higher membrane stability and higher tolerance to drought stress.

Decreasing membrane stability under drought stress has been reported in wheat varieties (Simova-Stoilova et al., 2008).

We found that relative water content decreased under drought stress in all investigated genotypes, but with no significant differences between tolerant and sensitive genotypes.

Leaf water potential differed significantly between the growth stages, and was highest in the podding stage. The sensitive MCC68 genotype had lower Ψ_w than the tolerant MCC392 in the flowering and podding stages.

Most studies have shown decreased relative water content and leaf water potential in response to drought stress (Siddique et al., 2000; Jinmin and Huang, 2001; Terzi and Kadioglu, 2006; Bayomi et al., 2008).

Genotypic variation of leaf water potential may be attributed to differences in the ability to absorb more water from the soil and the ability to reduce water loss through stomata (Siddique et al., 2000). It may also be due to differences in the ability of genotypes to maintain tissue turgor and hence physiological activities (Terzi and Kadioglu, 2006). At the cell level, plants attempt to decrease the damaging effects of stress by altering their metabolism to cope with stress. Genotypes that maintain higher relative water content under drought stress are believed to be more tolerant and give higher yield than others (Bayomi et al., 2008). It has been observed generally that genotypes with higher leaf water potential and relative water content have a higher photosynthetic rate under drought stress (Siddique et al., 2000). We found that leaf water potential correlated positively with the photosynthetic rate in the podding stage (r^2=0.047, $p<0.05$), but there were no significant correlations between the photosynthetic rate and relative water content in any of the investigated stages.

There were significant positive correlations between the membrane stability index and the transpiration rate in all stages: seedling (r^2=0.073) early flowering (r^2=0.129) and podding (r^2=0.075), and also between the membrane stability index and shoot dry weight (r^2=0.144, 0.052 and 0.091 respectively) ($p<0.05$). There was also a significant positive correlation between the transpiration rate and shoot dry weight in the early flowering (r^2=0.24) and podding (r^2=0.28) stages ($p<0.05$).

The tolerant genotypes had higher values for shoot dry weight, the membrane stability index and photosynthetic rate than the sensitive genotypes.

Drought stress also had effects on the F_v/F_m ratio. The drought-sensitive MCC68 chickpea genotype had lower F_v/F_m than the two drought-tolerant genotypes in all three stages. The F_v/F_m ratio can be used to detect damage to photosystem II and possible photoinhibition (Ahmed et al., 2002). Several studies have demonstrated damage to the PSII oxygen-evolving complex and the PSII reaction centers, and, in turn, degradation of D_1 protein under drought stress (Lu and Zhang, 1998; Maxwell and Johnson, 2000; Galle et al., 2002). Photoinhibition is represented by decreasing F_v/F_m ratio, effective quantum yield of photosystem II photochemistry and electron transport rate (Piper et al., 2007). Decreases of the F_v/F_m ratio and electron transport rate may be the result of Calvin cycle disturbances that delay redoxidation of Q_A^- and induce photosystem II down-regulation or damage thylakoid membrane electron transport (Galle et al., 2002).

The plants in our drought-stress treatment showed a marked decrease of the F_v/F_m ratio. Zlatve and Yordanov (2004) found lower F_v/F_m in bean genotypes under drought stress, suggesting chronic photoinhibition due to photoinactivation of photosystem II centers, possibly attributable to D_1 protein damage. Piper et al. (2007) reported that the F_v/F_m ratio declined in *Nothofagus* species under drought stress and was higher in tolerant than in sensitive genotypes. Similar effects on these chlorophyll fluorescence parameters have been observed in different species, among them *Brassica napus* L. (Kauser et al., 2006) and Aegilops species (Dulai et al., 2006).

We studied the correlations between photosynthesis and the F_v/F_m ratio in chickpea. There were no significant correlations between the CO_2 assimilation rate and the F_v/F_m ratio, but the transpiration rate correlated positively with the F_v/F_m ratio in the seedling (r^2=0.09) and podding (r^2=0.259) stages ($p<0.05$).

Drought stress can also alter the tissue concentrations of chlorophylls and carotenoids (Hussein et al., 2008). In our drought-stress treatment, the MCC448 genotype had the highest chlorophyll and carotenoid content. Increased chlorophyll and carotenoid content under drought stress may be related to a decrease in leaf area. It can be a defensive response to reduce the harmful effects of drought stress (Farooq et al., 2009). The chlorophyll stability index was highest in the podding stage and lowest during early flowering. The higher chlorophyll stability index in the podding stage showed increasing chlorophyll content under drought stress. Reduction of chlorophyll content has been reported in drought-stressed cotton (Mssacci, 2008) and *Catharanthus roseus* (Jaleel et al, 2008b). Chlorophylls decreased significantly under higher water deficit in sunflower

plants (Kiani et al., 2008) and in *Vaccinium myrtillus* (Tahkokorpi et al., 2007).

In our experimental treatments the tolerant genotypes showed significantly higher values for water use efficiency, the F_v/F_m ratio and the CO_2 assimilation rate under drought stress, indicating their superior ability to osmoregulate and ensure survival. These parameters can serve as useful markers for screening chickpea genotypes and identifying drought-tolerant genotypes.

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