PSII Photochemistry and Antioxidant Responses of a Chickpea Variety Exposed to Drought

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The effect of drought on the chickpea variety ILC 3279 was investigated at the vegetative stage. After 20 days from sowing, the plants subjected to drought stress for 3, 5 and 7 days imposed by withholding water were permitted to recover by rewatering for 2 days after 3, 5 and 7 days of drought. Shoot elongation, leaf production, fresh and dry biomass reduced while MDA and proline accumulation increased with extended duration of stress. The plants stressed for 3 days exhibited a rapid drop in their relative and absolute water contents. The quantum efficiency of PSII open centres in the dark-adapted and light-saturated state, excitation energy trapping of PSII and electron transport rate decreased significantly from the 5th day to the end of the drought treatments. Plants drought-stressed for 7 days brought about a marked increase in non-photochemical energy dissipation and a marked decline in photochemical quenching. After rewatering all chlorophyll *a* fluorescence characteristics except for F_M completely recovered and reached the control values. Under 5 and 7 days of drought, the anthocyanin content increased gradually while the total chlorophyll content of leaves declined compared to the controls. The total carotenoid content remained unchanged during the experiments. The antioxidant enzyme response to drought treatments was quite variable. The total SOD activity upregulated with increasing duration of stress. On the other hand, the total APX activity was significantly higher only on the 7th day while the total POD activity increased from the 5th day. Differences in the total GR activity of treated groups were not statistically significant compared to their controls throughout the treatments. The present results indicate that the chickpea variety ILC 3279 withstands severe drought with its upregulated protective mechanisms at the vegetative stage.

Key words: Chickpea, Drought, Photosynthetic and Antioxidative Response

Introduction

Drought is the major cause of yield reduction in crop plants, since water is a major limiting factor for plant growth and development (Riccardi *et al.,* 2004) mainly in arid and semiarid regions. However, the response of agricultural crops to drought stress has not yet been extensively studied (Wan and Li, 2006). The damaging effect of drought depends not only on its severity but also on the developmental stage in which it occurs (Jongdee *et al*., 2002). It is well known that drought stress brings about numerous metabolic, biochemical and physiological changes in plants like growth (Ashraf and Iram, 2005; Benjamin and Nielsen, 2006; Kashiwagi *et al*., 2006), water status (Khanna-Chopra and Selote, 2007; Martínez *et al*., 2007), membrane stability (Bai *et al*., 2006; Tan *et al*., 2006), pigment content and photosynthetic activity (Ekmekçi *et al*., 2005; Miyashita *et al*., 2005).

The first response of all plants to acute water deficit is the closure of their stomata to prevent the transpirational water loss. This has been attributed to the decrease in both photosynthetic rate and internal CO_2 concentration (Reddy *et al*., 2004). As CO_2 availability is reduced in the chloroplasts, photosynthesis and photosynthetic capacity are progressively decreased under drought (Lawlor, 1995). It was shown that drought stress affects both photosystems, I and II (PSI and PSII), located in the thylakoid membanes. However, PSII is more sensitive to dehydration than PSI (He *et al*., 1995). PSII is the most vulnerable part of the photosynthetic apparatus and is believed to play a key role in the response of leaf photosynthesis to environmental stresses (Anderson and Barber, 1996). It was demonstrated that drought stress induces a loss of the D1 and D2 proteins of PSII (He *et al*., 1995).

Light energy harnessed by chlorophyll cannot be dissipated via photosynthesis under water-limiting conditions and can lead to an over-reduction of the photosynthetic electron chain and eventu-

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ally may result in the formation of oxygen-free radicals known as "reactive oxygen species (ROS)" including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl free radical ($OH^\bullet$), and singlet oxygen ($^1O_2$) (Asada, 1999). Drought stress invariably leads to oxidative stress in the plant cell due to higher leakage of electrons towards O_2 during photosynthetic and respiratory processes leading to an enhancement in the ROS generation form in the electron transport systems of chloroplasts and mitochondria (Asada, 1999; Khanna-Chopra and Selote, 2007). ROS are highly toxic and can directly attack membrane lipids, inactivate metabolic enzymes and damage nucleic acids leading to cell death (Mittler, 2002). During optimal conditions, plant cells are protected against the detrimental effects of ROS by both enzymatic and non-enzymatic antioxidant detoxification mechanisms (Van Breusegem *et al*., 1998), whereas during drought conditions the production of ROS exceeds the capacity of the antioxidative systems to remove them (Chaves *et al*., 2003). Antioxidant enzymes, which play an important role in scavenging ROS to response drought stress, include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidases (POD) and glutathione reductase (GR) (Srivalli *et al*., 2003; Tan *et al*., 2006; Khanna-Chopra and Selote, 2007). Plants can also prevent the absorption of excess light by the production of sun-screen pigments (carotenoids and anthocyanins) in order to protect themselves from the damaging effect of photoinhibion and ROS (Sherwin and Farrant, 1998; Chaves *et al*. 2003).

The decrease in the osmotic potential in response to water stress is a well-known strategy by which many plants adjust to drought conditions (Patakas and Noitsakis, 1999). Many solutes containing ions like K^+, Na^+, and Cl^- or organic solutes that include nitrogen-containing compounds, such as proline and other amino acids, polyamines and quaternary ammonium compounds like glycine betaine, may be used in osmotic adjustment (Tamura *et al*., 2003). The accumulation of proline is a commonly observed metabolic response of higher plants to drought (Alves and Setter, 2004). In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing subcellular structures (*e.g.* membranes and proteins), scavenging free radicals, and buffering the cellular redox potential under stress conditions (Ashraf and Foolad, 2007).

Drought is a very important stress in chickpea (*Cicer arietinum* L.) (Singh, 1997) which is the fourth most important food legume with a total annual global production of 9.1 million Mt from 11.2 million ha (FAOSTAT, 2006). The seeds of chickpea are a major source of plant-based dietary protein for humans (Gan *et al*., 2006). Besides being an important source of human and animal food, the crop also plays an important role in the maintenance of soil fertility, particularly in dry, rainfed areas (Saxena, 1990). Since chickpea is grown mostly as a rainfed and post-rainy season crop, drought stress during vegetative and/or reproductive growth stages is one of the most limiting factors of chickpea growth (Güneş *et al.,* 2006). Vegetative and/or reproductive growth and productivity of chickpea is also influenced adversely, with progressive global climate change and increasing shortage of water resources (Leport *et al*., 2006). However, most studies with effects of drought stress on chickpea have focused on terminal drought (Serraj *et al*., 2004; Kashiwagi *et al*., 2006) at the reproductive stage, and there is no publication available about the drought-induced biochemical and physiological changes in chickpea at the vegetative stage. Therefore, the aim of this study was to evaluate the effects of drought stress at the vegetative stage of chickpea by determining some physiological and biochemical parameters such as water status, lipid peroxidation and PSII photochemistry. Additionally, to better understand the defence mechanisms underlying the tolerance of chickpea to drought, the response of protective pigments, proline content and antioxidative enzymes were studied. Moreover, the recovery degree of drought-exposed plants after rewatering was examined by using the analysis of chlorophyll fluorescence which is known as a rapid and reliable indicator of stress in plants.

Materials and Methods

Plant material and stress treatments

The chickpea variety ILC 3279, described by Singh *et al*. (1992), was used in this study. Seeds of the variety were obtained from ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syrian Arab Republic). Seeds were surface-sterilized with 2% sodiumhypochlorite (NaOCl) solution for 20 min. Thereafter they were washed with and imbibed in distilled water for 12 h. After incubation, the seeds were planted

in PVC pots holding 1080 g air-dried soil with 30% water holding capacity. Some characters of soil were as follows: pH 8.3, EC 0.145 dS m^{-1}, total N 0.19%. Plants were grown under well-watered conditions, at a constant temperature regime of (25 ± 1) °C for a 16 h photoperiod at (40 ± 5)% humidity and at 250 μmol m^{-2} s^{-1} light intensity in a controlled growth room. Drought was initiated when plants were 20 d old. Plants were randomly divided into two groups, one of which served as the control group while the other was subjected to drought. Drought stress was imposed on plants for 3, 5 and 7 d by withholding water. The groups, which were exposed to drought for 7 d were rewatered for 2 d. Measurements were made at the end of each period, and subsequent rewatering.

Growth parameters

At the end of each period, the shoot lengths (the distance from soil surface to the node of newly emerging leaf) of chickpea seedlings were measured (mm $plant^{-1}$) and the number of leaves (imparipinnate compound leaves) was counted. Three plants for each group were taken randomly to determine fresh (FW) and dry weights (DW). Dry weights (g DW) of plants were measured by drying the fresh plants at 80 °C for 48 h.

Water status of leaves

The water status of the leaves was evaluated by calculating the percentage of relative water content (RWC). Five leaflets for each of six replicates were used in the RWC analysis at each drought period. The RWC was calculated using the standard formula RWC = [(FW – DW)/(HW – DW)] · 100, where DW, FW and HW stand for dry, fresh and hydrated weight, respectively, according to Farrant (2000). The absolute water content (AWC) was evaluated using the formula AWC = [(FW – DW)/FW] · 100.

Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence measurements were performed in a growth room at 24 °C using a portable, modulated fluorescence monitoring system (FMS-II-Hansatech, UK), on randomly selected leaves (sixth leaf) of the variety. Following 30 min dark adaptation, the minimum chlorophyll *a* fluorescence (F_o) was determined using a measuring beam of 0.2 μmol m^{-2} s^{-1} intensity. A saturation pulse (1 s white light of 7500 μmol m^{-2} s^{-1}) was used to obtain the maximum fluorescence (F_M) in the dark-adapted state. The quantum efficiency of PSII open centres in dark-adapted plants (F_V/F_M) was calculated from $(F_M - F_o)/F_M$. Light-induced changes in the chlorophyll *a* fluorescence following actinic illumination (300 μmol m^{-2} s^{-1}) were recorded prior to the measurement of F_o' (minimum chlorophyll *a* fluorescence in the light-saturated state) and F_M' (maximum fluorescence in the light-saturated stage). The quantum efficiency of PSII open centres in the light-adapted state, referred to as ΦPSII $[(F_M' - F_S)/F_M']$, was determined from F_M' and F_S (steady-stage fluorescence in the light-saturated stage) values and also the quantum efficiency of excitation energy trapping of PSII, (F_V'/F_M'), was calculated as done by Genty *et al.* (1989). The actinic light removed and minimum fluorescence in the light-adapted stage (F_o') was determined by illuminating the leaves with far-red light (7 μmol m^{-2} s^{-1}). The photochemical quenching $[qP = (F_M' - F_S)/(F_M' - F_o')]$, non-photochemical quenching $[NPQ = (F_M - F_M')/(F_M')]$, and electron transport rate (ETR) were also calculated according to Genty *et al.* (1989).

Pigment analysis

To determine the level of chlorophyll $(a+b)$ and total carotenoids $(x+c)$ of leaves, two leaflets for each of six replicates were used. The content of chlorophyll $(a+b)$ and carotenoids $(x+c)$ was calculated using adjusted extinction coefficients (Lichtenthaler, 1987). The anthocyanin content was calculated as done by Mancinelli *et al.* (1975).

Lipid peroxidation

Lipid peroxidation in the leaves (0.1 g fresh tissue) was measured in terms of the malondialdehyde (MDA) content as described by Ohkawa *et al.* (1979) with some modifications. The absorbance of the supernatant was recorded at 532 nm. Measurements were corrected for non-specific turbidity by substracting the absorbance at 600 nm. The content of MDA was determined by using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nm g FW^{-1}.

Proline content

The free proline content of control and treated plants was determined using the method of Bates

et al. (1973). Proline was extracted from leaf samples (20 mg DW) of each treatment according to Weimberg (1987) with minor modifications. The absorbance of the leaf samples was monitered at 520 nm. The proline content (μmol g DW^{-1}) was determined by calculations based on a proline standard curve.

Determination of antioxidant enzyme activities

For enzyme extracts and assays, fresh leaf samples (0.5 g) from each treatment were ground with liquid nitrogen and suspended in a specific buffer (with characteristic pH value) for each enzyme extraction. The protein contents in the leaf extracts were determined according to Bradford (1976).

Total SOD (EC 1.15.1.1) activity was assayed as described by Beyer and Fridovich (1987). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction. The activity was expressed in units per mg of protein.

Total APX (EC 1.11.1.1) activity was measured according to the method of Wang *et al.* (1991). The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of ascorbate (E = 2.8 mM^{-1} cm^{-1}) at 290 nm.

Total GR (EC 1. 6.4.2) activity was determined by following the decrease in the absorbance at 340 nm due to NADPH oxidation (E = 6.2 mM^{-1} cm^{-1}) (Rao *et al.,* 1995).

Guaiacol POD (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}) at 470 nm by H_2O_2 (Bergmeyer, 1974). One unit of peroxidase activity was defined as nmol H_2O_2 decomposed per min per mg of protein.

Data analysis

The experiments were performed in a randomized design. Differences among the treatments were tested using the SPSS statistical program. Statistical variance analysis was performed using ANOVA and compared with least significant differences (LSD) at a 5% level.

Results and Discussion

In this study the effects of drought on the chickpea variety ILC 3279 were investigated as a function of time. ILC 3279 has been classified as sensitive to terminal drought stress (ICARDA, 2000), but drought-induced limitation at its vegetative stage of growth has not been adverted yet. One of the physiological effects of drought on plants is the reduction in vegetative growth, in particular shoot growth (Mahajan and Tuteja, 2005). At vegetative stage, exposure to drought decreased dramatically the elongation of shoot as well as leaf production even the plants were subjected for 3 days compared to their controls (Table I). It has been demonstrated that water limitations cause a decline in shoot growth of legumes such as *Phaseolus vulgaris* and *Sesbania aculeata* (Ashraf and Iram, 2005). Slama *et al.* (2006) have reported that drought stress had reduced the plant growth by restricting leaf formation. In our study, the fresh

Table I. Effects of drought on some growth parameters of chickpea.

Genotype	Treatment	Shoot length [mm $plant^{-1}$]	Number of leaves $\left[\sqrt{\text{number plant}^{-1}}\right]$	Fresh biomass of shoot [g $plant^{-1}$]	Dry biomass of shoot [g $plant^{-1}$]
ILC 3279	0 day[a]	137.00[b] ± 2.60	2.851[b] ± 0.029	0.955[c] ± 0.083	0.143[c] ± 0.007
	3 d control	164.07 ± 2.80	3.201 ± 0.036	1.045 ± 0.008	0.150 ± 0.004
	3 d drought	142.27 ± 6.40	2.953 ± 0.036	0.571 ± 0.078	0.132 ± 0.011
	5 d control	165.40 ± 3.15	3.241 ± 0.039	1.044 ± 0.109	0.151 ± 0.001
	5 d drought	143.67 ± 6.01	2.986 ± 0.038	0.488 ± 0.070	0.132 ± 0.005
	7 d control	187.73 ± 5.27	3.491 ± 0.035	1.039 ± 0.086	0.181 ± 0.003
	7 d drought	149.07 ± 6.07	3.063 ± 0.031	0.363 ± 0.005	0.158 ± 0.002
LSD 5%		14.13	0.10	0.29	0.02

[a] 0 day, 20-day-old chickpea plant (predrought).
[b] Each value represents the mean of three replicates for 5 plants each (n = 15) and its standard error (± SE).
[c] Each value represents the mean of three replicates (n = 3) and its standard error (± SE).

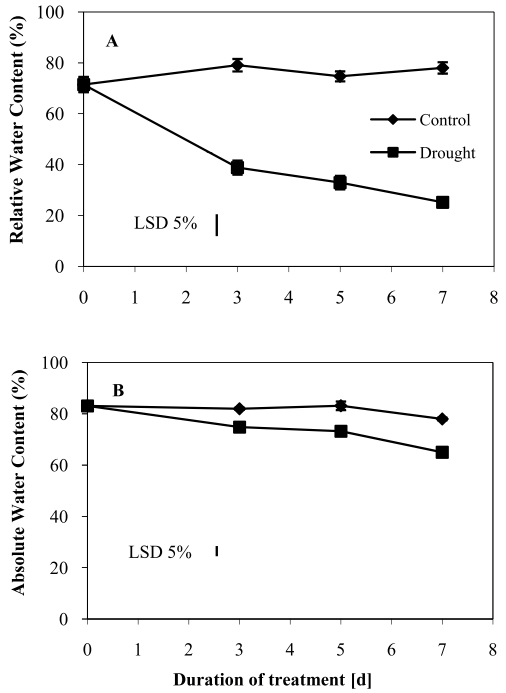
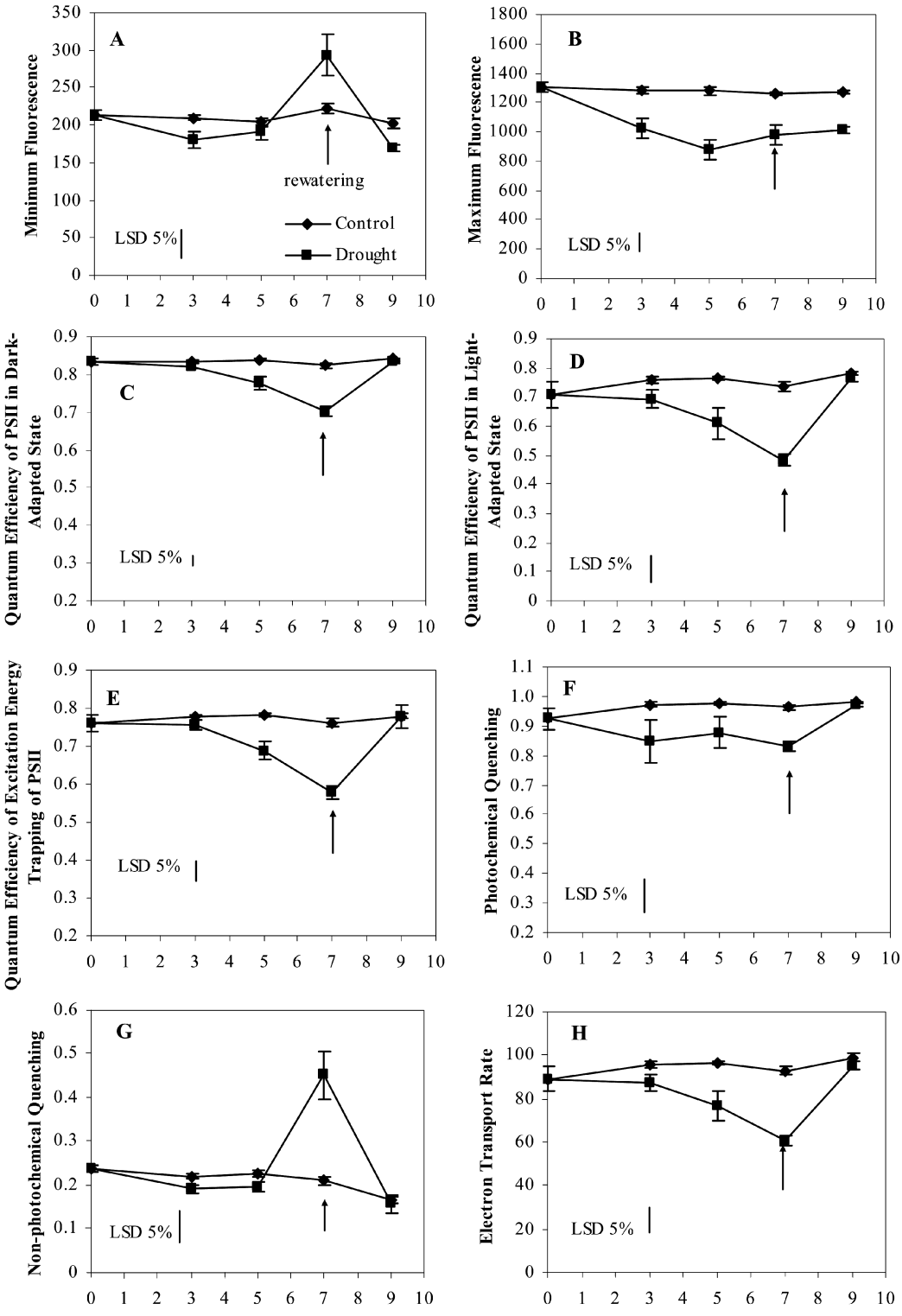


Fig. 1. Effect of drought on (A) relative and (B) absolute water content of chickpea. The error bars represent the standard error (± SE) for six replicates.

biomass of shoot decreased significantly on the 3rd day of drought, and then it continued to decrease slightly during the treatments whereas the dry biomass exhibited a significant decline on the 7th day (Table I).

Many investigations have shown that when plants are subjected to drought, leaves exhibit remarkable reductions in the RWC (Türkan *et al.*, 2005; Slama *et al.*, 2006; Galmés *et al.*, 2007; Khanna-Chopra and Selote, 2007). Our study results showed that the RWC decreased sharply on the 3rd day of treatments and thereafter it continued to decrease by approx. 68% at the end of stress duration compared to well-watered control groups. Moreover, the AWC decreased from 78% in 7-day-control plants to 65% in 7 days drought-treated chickpea plants (Figs. 1A and B). Kaiser (1987) reported that when the RWC was below 30%, plants experience the severe drought based on its photoinhibitory effects which are related to an irreversible decrease in plant photosynthetic capacity. However, it should be noted that, rewatering after such a serious stress condition, drought-treated chickpea plants exhibited similar fluorescence values like those found in corresponding control groups (Fig. 1A and Fig. 2).

Measurement of chlorophyll fluorescence from attached leaves proved to be a reliable, non-intrusive method for monitoring the photosynthetic performance and for judging the physiological status of the plant (Strasser *et al.*, 2000). It was reported that the PSII activity is more sensitive to a variety of stresses than the PSI activity, and even more so in drought-sensitive cultivars (Van Rensburg and Krüger, 1993). In the present study, all fluorescence parameters in leaves, except for F_M, recovered to their control values at the end of rewatering. Although there was no significant change in F_o under well-watered and drought stress conditions on the 3rd and the 5th day of treatments, drought caused a significant increase in F_o on the 7th day (Fig. 2A). On the other hand, all drought treatments resulted in a significant reduction in F_M of drought-treated plants compared to control groups (Fig. 2B). In control plants, the maximal photochemical efficiency of PSII, estimated by the fluorescence ratio F_V/F_M of dark-adapted leaves was approx. 0.82–0.84 (Fig. 2C). The F_V/F_M ratio remained constant under 3 days of drought conditions, suggesting that photoinhibition did not occur until the drought period extended to 5 or 7 days. On the 5th day of treatments the F_V/F_M ratio was reduced significantly under drought conditions mostly due to a decline in F_M which represents the reduction degree of the PSII acceptor side (Georgieva and Lichtenthaler, 1999). In addition to that, at the end of the drought period, the F_V/F_M ratio was affected by both decreasing F_M and increasing F_o, reflecting the state of the antennae chlorophyll being a measure for the initial distribution of energy to PSII and the effectiveness of excitation capture in PSII (Georgieva and Lichtenthaler, 1999). The decline in F_V/F_M after severe water stress was recently reported (Souza *et al.,* 2004; Miyashita *et al.,* 2005) and represents the accumulation of photodamaged PSII centres (Rosenqvist and van Kooten, 2003). Nevertheless, it was remarkable that complete recovery of F_V/F_M occurred upon rewatering which indicated that the photochemical activity may be mostly up-regulated. Similar to the F_V/F_M ratio, the F_V'/F_M' value decreased from day 5 to day 7 and recovered after rewatering. It has been shown in many studies that an increase in the thermal dissipation in the PSII antennae competes with the excitation energy transfer from


A
Minimum Fluorescence
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D
Quantum Efficiency of PSII in Light-Adapted State
LSD 5%
E
Quantum Efficiency of Excitation Energy Trapping of PSII
LSD 5%
F
Photochemical Quenching
LSD 5%
G
Non-photochemical Quenching
LSD 5%
H
Electron Transport Rate
LSD 5%
Duration of treatment [d]

the PSII antennae to PSII reaction centres in F_V'/F_M', thus resulting in a decrease in the efficiency of excitation energy capture by open PSII reaction centres (Lu and Zhang, 2000). In the present study, drought treatments had a significant impairment on ΦPSII as well as the other photosynthetic parameters (Fig. 2D). The reduced ΦPSII was a result of the decrease in the efficiency of excitation energy trapping of PSII reaction centres (Fig. 2E). Drought stress caused a significant decrease in F_V'/F_M' on the 5th day and also F_V'/F_M' was approx. 24% lower in 7 days treated plants than in the control groups. The overall efficiency of PSII is the product of both F_V'/F_M' and *qP* (which is determined by the redox state of Q_A, the first stable electron acceptor of PSII) (Genty *et al.,* 1989), and reductions of ΦPSII are related to significant reductions of F_V'/F_M' (Colom and Vazzana, 2003) or *qP* (Sinsawat *et al*., 2004). Drought stress resulted in a slight decline in *qP* at the end of the treatments (Fig. 2F). Therefore in chickpea, the low ΦPSII of drought-stressed plants was the consequence of a lower F_V'/F_M' more than a lower *qP*. On the contrary, Pieters and El Souki (2005) showed that F_V'/F_M' in water-stressed plants was less important than *qP* in determining the decrease in ΦPSII. Under drought stress conditions, the light which is in excess of what can be used in photosynthesis increases, resulting in photoprotection and/or photoinhibition (Galmés *et al*., 2007). The *NPQ* is an indicator of the fraction of the absorbed light energy that is dissipated thermally in PSII antennae (Lima *et al.,* 2002). It increased sharply on the 7th day of treatments at which drought stress became more severe (Fig. 2G). That may contribute to avoid photoinhibitory damage and to the fast recovery of photosynthetic activity following rewatering as also reported by Correia *et al*. (2006). Drought treatments brought about a marked decline in *ETR* from the 5th day (Fig. 2H). With increasing of the dehydration period to 7 days, the values of *ETR* decreased by 34.6% compared to their control groups. Depressed *ETR* may caused in part by processes that affect excess light energy dissipation.

In our experiment, drought affected not only the efficiency of the photochemical apparatus but also the integrity of the leaf chlorophyll content. Previous studies showed that drought stress may result in a decrease in the chlorophyll content (Fu and Huang, 2001; Colom and Vazzana, 2003). Leaves of chickpea showed a decrease in the chlorophyll content on the 5th day; the same time change in the maximum photochemical efficiency was observed (Fig. 3A and Fig. 2C). Chlorophyll loss is a negative consequence of stress that the photosynthetic apparatus must resynthesize *de novo* upon rewatering. On the other hand, it has also been considered as an adaptive feature which reduces the possibility of further damage to the photosynthetic machinery by the formation of ROS under an excess of excitation energy (Kranner *et al*., 2002; Jung, 2004). Carotenoids act as light-harvesting pigments; they can protect chlorophyll and membrane destruction by quenching triplet chlorophyll and removing oxygen from the excited chlorophyll-oxygen complex (Young, 1991). In the present study, in contrast to decreaeses in the total chlorophyll content, the carotenoid content remained stable throughout the treatments (Fig. 3B). On the other hand, the anthocyanin content of leaves sampled on the 5th and 7th day was approx. 3.5- and 4.5-fold higher, respectively, compared to corresponding controls (Fig. 3C). Increased anthocyanin contents are thought to mask chlorophyll and/or act as a filter for preventing high light absorption by leaves and thus minimize photoinhibition (Farrant, 2000). Therefore anthocyanin accumulation in drought-stressed leaves confirms a possible protective role of anthocyanins as sun-screens and ROS scavengers in stressed plants (Merzlyak and Chivkunova, 2000).

It was reported that, when plants were subjected to drought, oxidative stress resulted in lipid peroxidation, the level of which evaluated by determining the MDA content indicates possible damage to biological membranes (Bai *et al.,* 2006). Under drought stress, the MDA content of treated plants increased depending on the drought intensity, and at the end of a 7-day-drought, plants exhibited a

Fig. 2. Chlorophyll fluorescence responses of chickpea to imposed drought and recovery: (A) minimum fluorescence, F_o; (B) maximum fluorescence, F_M; (C) quantum efficiency of PSII in the dark-adapted state, F_V/F_M; (D) quantum efficiency of PSII in the light-adapted state, ΦPSII; (E) quantum efficiency of excitation energy trapping of PSII in the light-adapted state, F_V'/F_M'; (F) photochemical quenching, *qP*; (G) non-photochemical quenching, *NPQ*; (H) electron transport rate, *ETR*. The error bars represent the standard error (± SE) for six replicates.

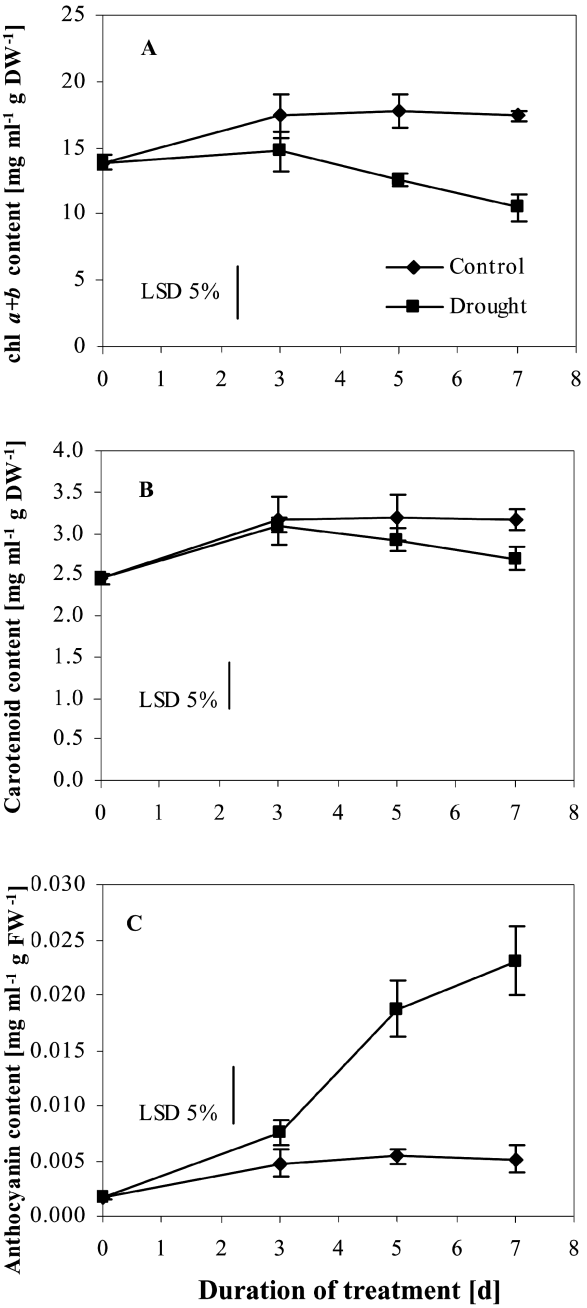

Fig. 3. Effect of drought on (A) total chlorophyll, (B) carotenoid and (C) anthocyanin contents of chickpea leaves. The error bars represent the standard error (± SE) for six replicates.

drastic MDA accumulation (3.8-fold of the well-watered plants) (Fig. 4). In the present study, all drought treatments resulted in enhanced MDA

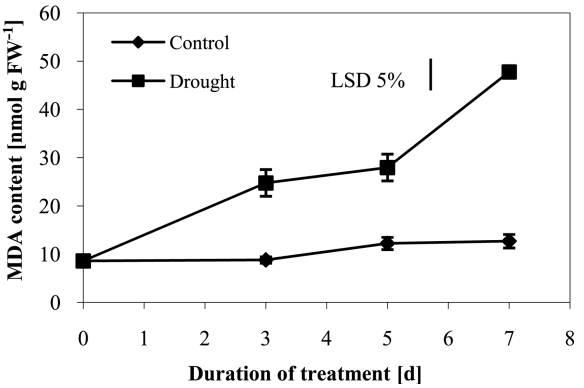

Fig. 4. Content of malondialdehyde (MDA) of chickpea leaves subjected to drought. The error bars represent the standard error (± SE) for eight replicates.

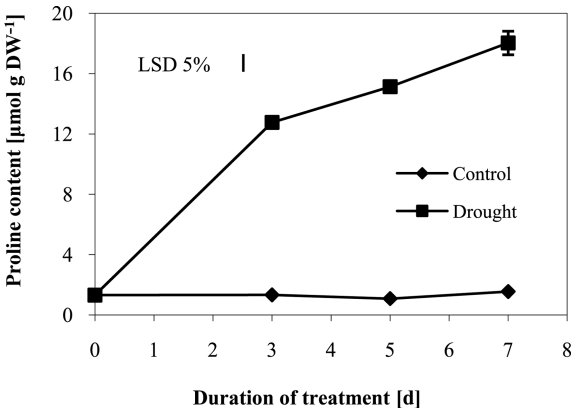

Fig. 5. Leaf proline content in chickpea exposed to drought. The error bars represent the standard error (± SE) for three replicates.

contents reflecting the oxidative injury to the membrane integrality (Fig. 4).

Plants have several physiological and biochemical strategies such as antioxidative defence and osmotic adjustment in order to prevent damaging effects of oxidative stress induced by drought (Tan *et al.*, 2006). Osmotic adjustment is known to be the main component of the physiological machinery, by which plants respond to soil water deficits (Zhu, 2003). Organic solutes, such as proline, are involved in the osmotic adjustment and accumulate to high levels in the cytosol with increasing drought to stabilize membranes and maintain the protein conformation (Reddy *et al.*, 2004). Additionally, they have a major role in protecting the cells by scavenging ROS (Pinhero *et al.*, 2001). In

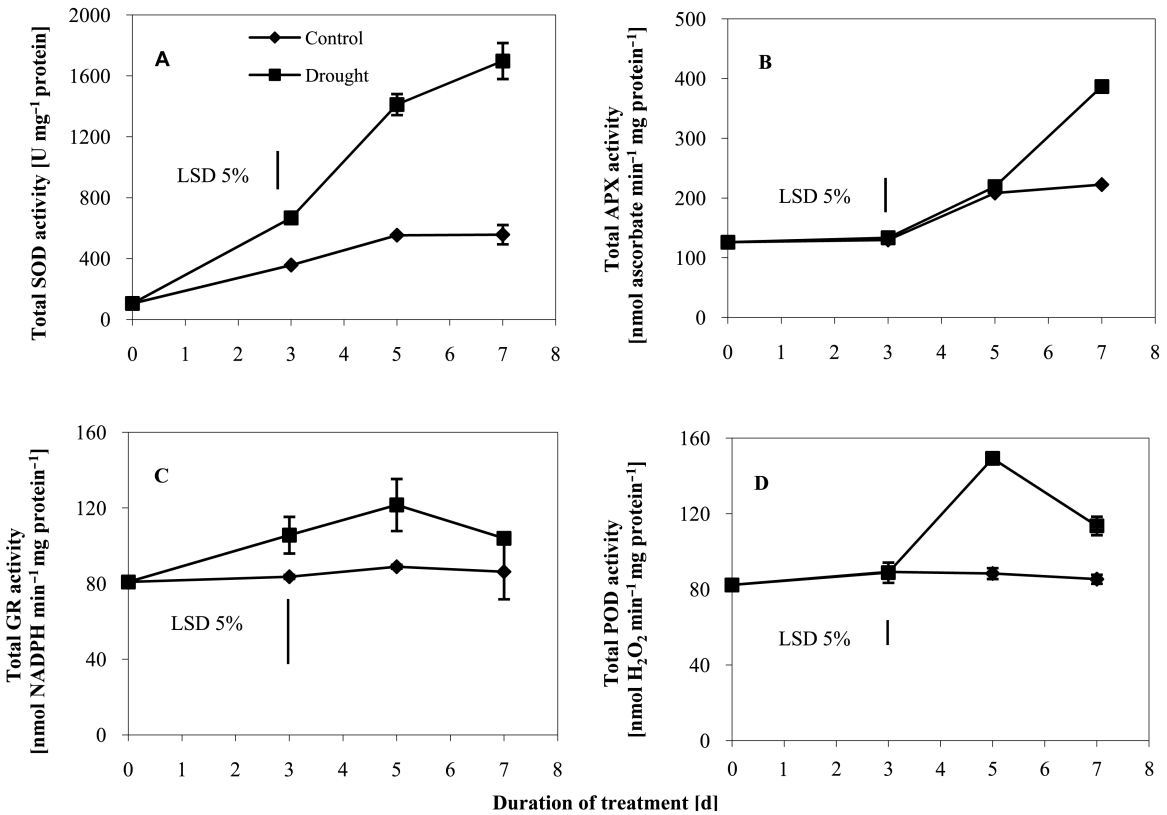

Fig. 6. Changes in antioxidant enzyme activities of chickpea under drought conditions: (A) total SOD activity; (B) total APX activity; (C) total GR activity; (D) total POD activity. The error bars represent the standard error (± SE) for three replicates.

this study, the free proline content of the chickpea variety increased sharply from the 3rd day of treatments when the RWC dropped to nearly 39% (Fig. 1A and Fig. 5). Moreover, the free proline level increased to 11.7-fold when stress duration was extended to 7 days. Similar results were reported by Tan *et al.* (2006) and Boominathan *et al.* (2004). The responses of antioxidant enzyme activities such as SOD, APX, GR and POD under drought stress were extremely variable in chickpea plants (Fig. 6). In plants growing under well-watered conditions, a remarkable age-dependent increase in the total SOD activity was observed from the beginning until the 5th day of treatments and then the activity remained stable (Fig. 6A). The total SOD activity of 3, 5 and 7 days stressed plants was determined as 1.9-, 2.6- and 3.1-fold of their control groups, respectively. Elevated activity of SOD under drought stress is indicative of an increase of $O_2^{-\bullet}$ production (Asada and Takahashi, 1987). Therefore, the total SOD activity in the leaves of stressed chickpea plants may play a key role in the conversion of the superoxide anion to H_2O_2. It was reflected that drought-tolerant species exhibit more SOD activity along with the other antioxidative enzymes than sensitive ones (Lima *et al.*, 2002; Türkan *et al.*, 2005). APX is the first enzyme of the AsA-GSH cycle functioning by reducing of cellular H_2O_2 to water (Asada, 1999). The total APX activity increased significantly when drought stress became more severe (7th day of stress period) (Fig. 6B). An increasing SOD activity without co-increasing of H_2O_2 detoxification systems could not be enough for protection against oxidative stress (Pritcher *et al.*, 1991). Our results showed that drought stress had no effect on the GR activity in leaves of stressed plants because the functioning of the cycle may not be achieved

efficiently (Fig. 6C). This enzyme, which participates in the removal of H_2O_2 via recycling of the GSH (reduced form of glutathione) pool, is the last and the rate-limiting enzyme of the AsA-GSH cycle (Asada, 1999). However, it may be suggested for ILC 3279 that the activities of SOD, APX and POD as well as anthocyanin and proline were enough for the protection from oxidative damage induced by drought stress as demonstrated by its entire recovery after 2 days of rewatering. Hence, the GR activity may not be involved in the protection mechanisms against drought of this variety. POD is another scavenging enzyme which is involved in H_2O_2 detoxification mechanisms to maintain cell membrane integrity (Tan *et al.*, 2006). Also the total POD activity in the leaves of stressed plants reflects the changed mechanical properties of cell walls (Sreenivasula *et al.,* 1999). The total POD activity of 5 days treated plants was higher than of their control groups (68.9%) (Fig. 6D). With increase of the stress duration, the total POD activity declined remarkably compared to the 5th day of treatments, but it still remained significantly higher than in well-watered controls. Our results were in agreement with other studies on increases in the POD activity (Fu and Huang, 2001; Tan *et al.*, 2006).

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