*Journal of Integrative Plant Biology* 2006, **48** (11): 1318–1329

# Differential Sensitivity of *Macrocarpa* and *Microcarpa* Types of Chickpea (*Cicer arietinum* L.) to Water Stress: Association of Contrasting Stress Response with Oxidative Injury

**Harsh Nayyar[1*], Smita Singh[2], Satwinder Kaur[1], Sanjeev Kumar[1] and Hari D. Upadhyaya[3]**

(1. *Department of Botany*, *Panjab University*, Chandigarh 160 014, India;
2. *Department of Plant Biology*, *University of New Hampshire*, Durham, New Hampshire 03824, USA;
3. *International Crop Research Institute for Semi-Arid Tropics*, Hyderabad, India)

## Abstract

**Chickpea (*Cicer arietinum* L.) is particularly sensitive to water stress at its reproductive phase and, under conditions of water stress, will abort flowers and pods, thus reducing yield potential. There are two types of chickpea: (i) *Macrocarpa* ("Kabuli"), which has large, rams head-shaped, light brown seeds; and (ii) *Microcarpa* ("Desi"), which has small, angular and dark-brown seeds. Relatively speaking, "Kabuli" has been reported to be more sensitive to water stress than "Desi". The underlying mechanisms associated with contrasting sensitivity to water stress at the metabolic level are not well understood. We hypothesized that one of the reasons for contrasting water stress sensitivity in the two types of chickpea may be a variation in oxidative injury. In the present study, plants of both types were water stressed at the reproductive stage for 14 d. As a result of the stress, the "Kabuli" type exhibited an 80% reduction in seed yield over control compared with a 64% reduction observed for the "Desi" type. The decrease in leaf water potential ($\Psi_w$) was faster in the "Kabuli" compared with the "Desi" type. At the end of the water stress period, $\Psi_w$ was reduced to –2.9 and –3.1 MPa in the "Desi" and "Kabuli" types, respectively, without any significant difference between them. On the last day of stress, "Kabuli" experienced 20% more membrane injury than "Desi". The chlorophyll content and photosynthetic rate were significantly greater in "Desi" compared with "Kabuli". The malondialdehyde and $H_2O_2$ content were markedly higher at the end of the water stress in "Kabuli" compared with "Desi", indicating greater oxidative stress in the former. Levels of anti-oxidants, such as ascorbic acid and glutathione, were significantly higher in "Desi" than "Kabuli". Superoxide dismutase and catalase activity did not differ significantly between the two types of chickpea, whereas on the 10th day, the activities of ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase were higher in "Desi". These findings indicate that the greater stress tolerance in the "Desi" type may be ascribed to its superior ability to maintain better water status, which results in less oxidative damage. In addition, laboratory studies conducted by subjecting both types of chickpea to similar levels of polyethylene glycol-induced water stress and to 10 μmol/L abscisic acid indicated a greater capacity of the "Desi" type to deal with oxidative stress than the "Kabuli" type.**

**Key words:** "Desi"; "Kabuli"; anti-oxidants; chickpea; drought; oxidative stress.

**Nayyar H, Smita S, Kaur S, Kumar S, Upadhyaya HD** (2006). Differential sensitivity of *Macrocarpa* and *Microcarpa* types of chickpea (*Cicer arietinum* L.) to water stress: Association of contrasting stress response with oxidative injury. *J Integr Plant Biol* **48**(11), 1318–1329.

doi: 10.1111/j.1672-9072.2006.00350.x; available online at www.blackwell-synergy.com, www.jipb.net

Received 12 Dec. 2005 Accepted 19 Jun. 2006

Publication of this paper is supported by the National Natural Science Foundation of China (30424813) and Science Publication Foundation of the Chinese Academy of Sciences.

*Author for correspondence. E-mail: <nayarbot@pu.ac.in> and <harshnayyar@hotmail.com>.

Chickpea is the one of the most important grain legume crops in the world and ranks first in the Mediterranean basin and South Asia (Singh and Ocampo 1997). There are two types of chickpea, namely *Macrocarpa* and *Microcarpa*, which are also known as "Desi" and "Kabuli", respectively. The "Desi" type has small, angular and dark-brown seeds, whereas the "Kabuli" type has large, rams head-shaped, light brown seeds and there is a slight variation in the seed composition between the two types (Malhotra et al. 1982).

Water stress is one of the major abiotic stresses that limits chickpea yields, especially at the reproductive stage of the plants (Leport et al. 1999). "Desi" and "Kabuli" show differential sensitivity to water deficits; under such conditions, the former has been reported to perform better in terms of yield than the latter (Leport et al. 1999; Behboudian et al. 2001). It has been observed by Davies et al. (2000) that even though both types showed almost similar values of leaf water potential ($\Psi_w$) and photosynthesis under conditions of water stress, the yield of the "Desi" type was greater than that of the "Kabuli" type, which was attributed to the superior ability of the former to remobilize the assimilates to developing seeds during the post-stress period. The metabolic reasons governing the differential sensitivity of the two types of chickpea to water stress are not known and investigating these mechanisms would provide vital clues about the varied responses.

Water stress causes diverse effects at various organizational levels (Chaves et al. 2003), which may differ depending upon the stress sensitivity of the different species or genotypes within a species (Nayyar and Walia 2004; Türkan et al. 2005). Water deficit stress induces oxidative stress because of inhibition of photosynthetic activity owing to an imbalance between light capture and its utilization (Noctor and Foyer 1998). Changes in the photochemistry of chloroplasts in the leaves of water-stressed plants result in dissipation of excess light energy, thus generating active oxygen species ($O_2^{\cdot-}$, $^1O_2$, $H_2O_2$, and $OH^-$), which are potentially dangerous to cell constituents (Peltzer et al. 2002). Active oxygen species (AOS) attack the most sensitive biological macromolecules and membranes to impair their function (Johnson et al. 2003). Mechanisms of AOS detoxification exist in all plants and include activation of enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase, glutathione reductase (GR), monodehydroascorbate reductase, dehydroreductase) as well as non-enzymatic (flavones, anthocyanins, carotenoids, ascorbic acid (ASC), and glutathione) anti-oxidants (Doulis et al. 1997; Lei et al. 2005). The degrees to which the activity of enzymatic anti-oxidants and the levels of non-enzymatic anti-oxidants are elevated under drought stress varies considerably among several plant species (Zhang and Kirkham 1995; Nayyar and Gupta 2005) and can even vary between two cultivars of the same species (Bartoli et al. 1999). In general, in earlier studies, the capacity to detoxify AOS was related to water stress tolerance (Selote and Khanna-Chopra 2004; Türkan et al. 2005) and stress tolerant genotypes have been found to possess a superior ability to detoxify AOS compared with stress-sensitive genotypes (Aroca et al. 2001).

A perusal of the literature reveals that information on the differential sensitivity of "Desi" and "Kabuli" chickpeas to water stress, especially at the metabolic level, is lacking. Hence, in the present study, we assessed the status of oxidative stress in the two chickpea types and it was hypothesized that the varied responses of these two chickpea types to water stress may arise as a result of differences in the levels of oxidative stress in the plants and their ability to deal with it.

## Results

### Yield traits

Total vegetative dry mass (Table 1) was reduced to a slightly higher extent in "Desi" compared with "Kabuli" (36% and 28% over control, respectively). The "Desi" type exhibited a 30% reduction in seed growth rate as a result of water stress compared with a 46% decrease in the "Kabuli" type. In stressed plants, the duration of seed filling was decreased by 5.3 and 8.2 d in "Desi" and "Kabuli", respectively. Compared with control, the average seed weight of stressed "Desi" and "Kabuli" plants decreased by 46% and 54%, respectively, and average seed size was reduced by 25% and 46%, respectively. Consequently, seed weight per plant decreased to a larger extent in the "Kabuli" type (80% over control) compared with the "Desi" type (64% over control). Seed number per 100 pods was reduced by 50% as a result of water stress in "Kabuli" compared with just a 24% decrease in "Desi". Consequently, the harvest index decreased significantly in the "Kabuli" type compared with the "Desi" type, suggesting a much higher sensitivity of the former to water stress. The stressed "Kabuli" plants lost 53% of pods compared with a loss of 32% of pods by stressed "Desi" plants; double-seeded pods exhibited a proportionally greater loss.

### Leaf water potential and stress injury

Leaf water potential (Figure 1) started to decrease on the 6th day of stress in "Kabuli" plants, whereas "Desi" only showed an appreciable change in $\Psi_w$ on the 8th day. The extent of the decrease in water potential was greater in "Kabuli" compared with "Desi". Eventually, $\Psi_w$ declined to –2.9 and –3.1 MPa in "Desi" and "Kabuli", respectively, at the end of the water stress period and, at this stage, the differences between the two genotypes were not significant. Stress injury (as electrolyte leakage) appeared on the 6th day (Figure 1) in "Kabuli" (26%)

**Table 1.** Effects of water stress during seed filling on yield traits of control and stressed "Desi" and "Kabuli" chickpea plants

| Parameter | "Desi" | | "Kabuli" | |
|---|---|---|---|---|
| | Control | Stressed | Control | Stressed |
| Vegetative dry matter (g/plant) including pod wall | 6.1 ± 1.1b | 3.9 ± 1.2c (36) | 9.6 ± 1.2a | 6.9 ± 1.3b (28) |
| Seed growth rate (mg/seed per day) | 8.2 ± 0.68b | 5.7 ± 0.74c (20) | 9.8 ± 0.57a | 5.3 ± 0.61c (46) |
| Seed fill duration (d) | 18.9 ± 1.4a | 13.6 ± 1.3b (28) | 20.2 ± 1.3a | 12.0 ± 1.2c (40) |
| Average seed weight (mg) | 168 ± 2.6b | 90.2 ± 3.4d (46) | 231 ± 2.5a | 106 ± 6.4c (54) |
| Average seed size (mm) | 6.3 ± 0.6b | 4.7 ± 0.8c (25) | 8.3 ± 1.3a | 4.4 ± 1.6c (46) |
| Seed weight per plant (g) | 5.1 ± 0.6b | 1.8 ± 0.4c (64) | 6.1 ± 0.8a | 1.2 ± 0.7c (80) |
| Total weight per plant (g) | 12.9 ± 1.3b | 6.8 ± 1.4d (47) | 15.8 ± 1.2a | 8.7 ± 1.3c (45%) |
| Harvest index | 0.39 ± 0.11a | 0.26 ± 0.13b | 0.38 ± 0.10a | 0.13 ± 0.11c |
| Seed number per 100 pods | 121 ± 3.1a | 92 ± 2.6c (24) | 102 ± 3.1b | 51 ± 4.1d (50) |
| Pods per plant | 27.2 ± 2.1a | 18.6 ± 2.6b (32) | 18.9 ± 2.7b | 8.8 ± 3.1d (53) |
| Single-seeded pods per plant | 21.8 ± 2.2a | 13.6 ± 2.4c (37) | 15.6 ± 1.6b | 8.3 ± 1.8d (46) |
| Double-seeded pods per plant | 5.1 ± 1.1a | 2.9 ± 0.8b (43) | 2.8 ± 0.7b | 1.2 ± 0.2c (57) |
| Infertile pods per plant | 0.3 ± 0.1b | 2.1 ± 0.3a | 0.5 ± 0.2b | 2.3 ± 0.2a |

Data are the mean ± *SEM*. Values within a row followed by same letter are not significantly different from one another ($P > 0.05$). Values in parenthesis indicate the percentage decrease over control due to stress.

and on the 8th day (22%) in "Desi", which paralleled the decrease in leaf $\Psi_w$ in these genotypes. On the last day of water stress, "Kabuli" experienced 20% stress injury than "Desi". The chlorophyll (Chl) content (Figure 1) decreased significantly on the 8th day in "Kabuli" compared with "Desi" (1.9 *vs* 2.1 mg/g fresh weight (FW), respectively). At the end of the stress, "Desi" had a significantly higher more Chl content than "Kabuli" (1.7 *vs* 1.0 mg/g FW, respectively). The net photosynthetic rate (Pn) started to decline rapidly (Figure 1) due to stress in both genotypes and reached 8 and 12 μmol/$m^2$ per s on the 6th day in "Kabuli" and "Desi" plants, respectively. Finally, the Pn in "Desi" was significantly greater than that in "Kabuli" (5.3 *vs* 2.4 μmol/$m^2$ per s, respectively).

### Oxidative damage and anti-oxidants

Malondialdehyde (MDA), a product of lipid peroxidation and an indicator of membrane damage, was significantly increased on the 6th day in "Kabuli" compared with "Desi" (289 *vs* 177 μmol/g dry weight (DW), respectively; Figure 2). Eventually, at the end of the water stress period, the MDA content in "Kabuli" was significantly greater than that in "Desi" (890 *vs* 700 μmol/g DW, respectively). The $H_2O_2$ content (Figure 2) was increased significantly on the 4th day of stress in both types and continued to increase throughout the stress period. The "Kabuli" type had a higher $H_2O_2$ content than the "Desi" type during the water stress period and $H_2O_2$ levels in the plants were determined to be 9.2 and 7.9 μmol/g DW, respectively, on last day of the water stress.

Ascorbic acid (ASC) and reduced glutathione (GSH) were elevated significantly (Figure 2) in both types on the 4th day of stress compared with controls. Peak levels of ASC and GSH were reached on the 8th day of stress in "Kabuli" (2 315 nmol ASC/g DW; 755 nmol GSH/g DW) and on the 10th day of stress in "Desi" (2 741 nmol ASC/g DW; 893 nmol GSH/g DW) and the differences between the two types were statistically significant. Thereafter, ASC and GSH levels started to decline and reached below control values on last day of stress, with the decrease in "Kabuli" being faster and greater compared with that in "Desi". Superoxide dismutase activity increased markedly on 4th day of stress in both types (Figure 3) and remained elevated until the 10th day (0.56 and 0.53 units/s per g FW in "Desi" and "Kabuli", respectively), when it started to decrease. No significant difference was found between the two chickpea types in terms of SOD activity. Catalase activity was also increased (Figure 3) up to the 10th day of stress (4.6 and 4.9 μmol $H_2O_2$ reduced/s per g FW in "Desi" and "Kabuli", respectively), declining thereafter in both genotypes. Catalase activity did not vary significantly between the two genotypes during the stress period.

Ascorbate peroxidase activity increased (Figure 4) to almost similar levels in both types of chickpea until the 6th day; thereafter, a noticeable increase was found in APX activity on the 10th day in "Desi" compared with "Kabuli" (0.80 *vs* 0.64 μmol ascorbate oxidized/s per g FW, respectively ). Ascorbate peroxidase activity began to decline subsequently in both genotypes and, at the end of the water stress period, the "Desi" type had significantly higher APX activity than the "Kabuli" type (0.41 *vs* 0.23 μmol ascorbate oxidized/s per g FW, respectively). At their peak on the 10th day of stress, the activities of dehydroascorbate reductase (DHAR) and GR were determined to be 1.3 and 1.9 μmol/min per g FW, respectively, in "Desi"

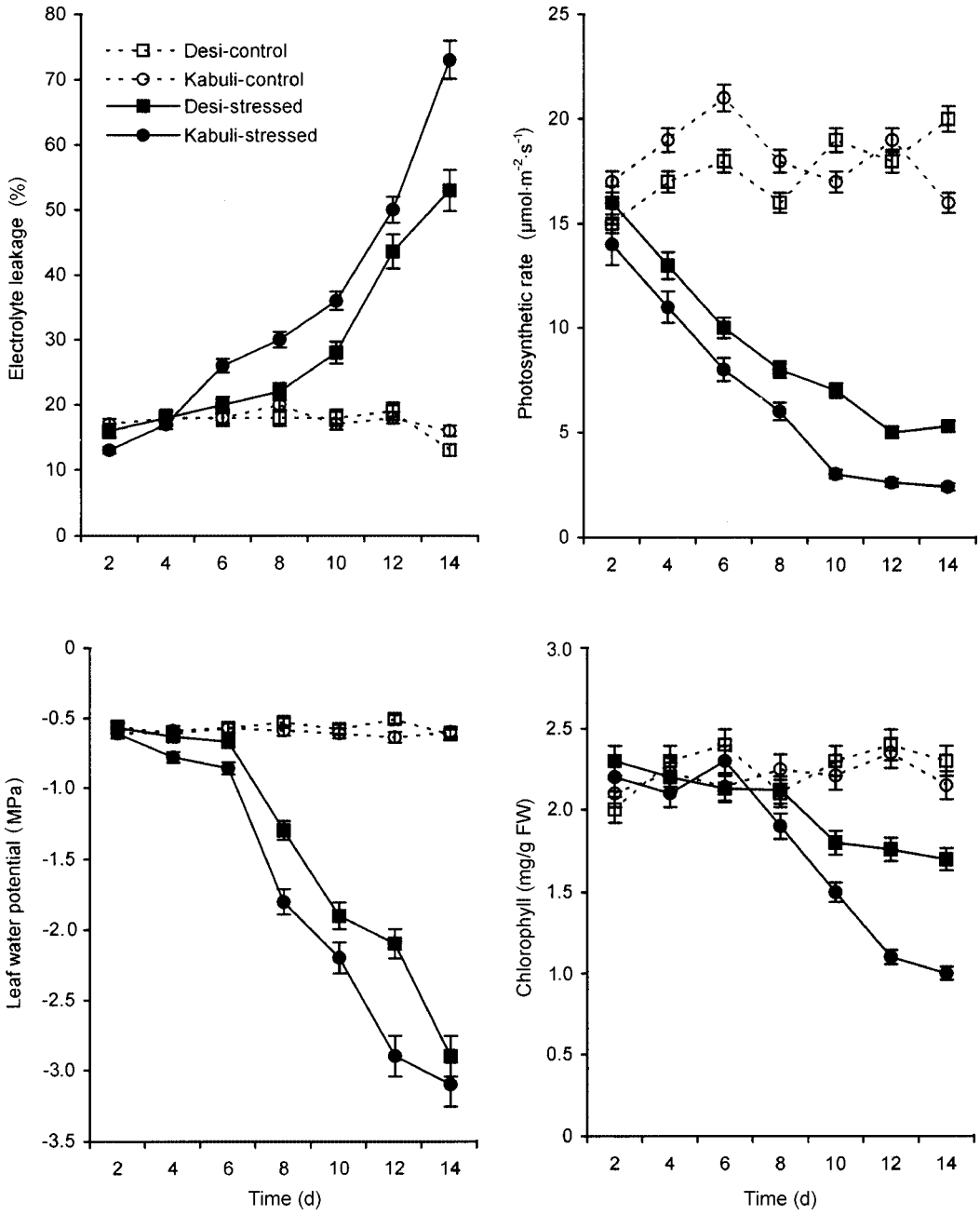


**Figure 1.** Effect of water stress (14 d during the reproductive stage of plants) on electrolyte leakage, leaf water potential ($\Psi_w$), photosynthetic rate, and chlorophyll content in "Desi" and "Kabuli" types.

Data are the mean ± *SEM*. Differences between the treatments are significant at $P < 0.05$.

compared with 0.72 and 0.98 μmol/min per g FW, respectively, in "Kabuli" (Figure 4). A decrease in enzyme activity was noted thereafter, with "Desi" maintaining higher activities of both DHAR and glutathione reductase (GR) than "Kabuli".

### Oxidative response at similar levels of water stress under controlled conditions

In order to validate the oxidative response of the two types of

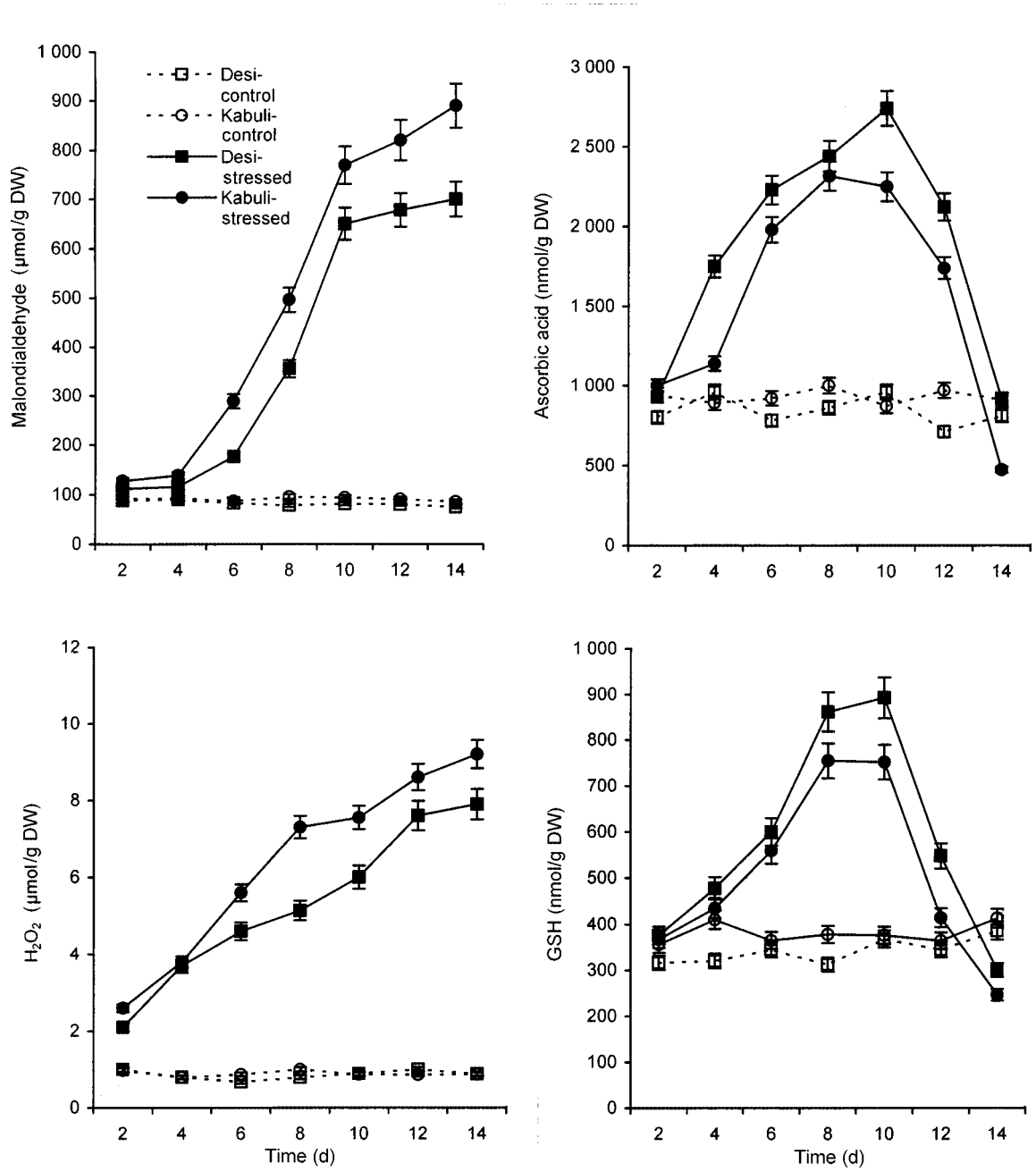


**Figure 2.** Effect of water stress (14 d during the reproductive stage of plants) on malondialdehyde, hydrogen peroxide ($H_2O_2$), ascorbic acid, and glutathione (GSH) in "Desi" and "Kabuli" types.

Data are the mean ± *SEM*. Differences between the treatments are significant at $P < 0.05$.

chickpea under field conditions, the genotypes were also evaluated under similar levels of water deficit stress by subjecting 15-day-old seedlings to polyethylene glycol-6000 (PEG) at –0.4, –0.8, and –1.2 MPa, corresponding to mild, moderate, and high stress, respectively, or 10 μmol/L abscisic acid (ABA; Table 2).

Observations indicated that, under conditions of mild stress, both chickpea types did not differ appreciably from each other in terms of growth, membrane damage, and oxidative stress (as MDA and $H_2O_2$), as well as anti-oxidants, but differences became apparent at moderate and high levels of stress. Oxidative damage in terms of MDA and $H_2O_2$ was significantly higher

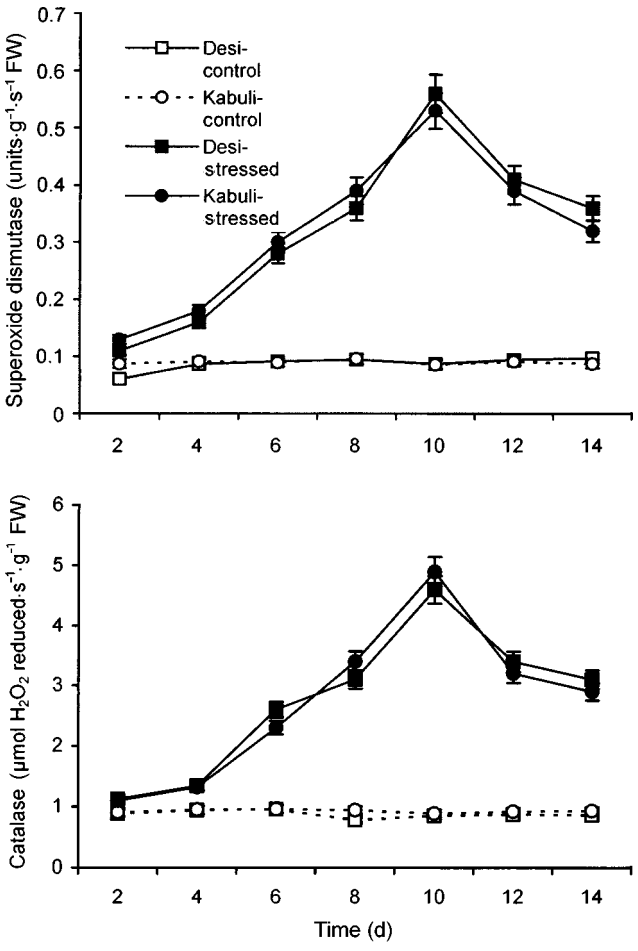


**Figure 3.** Effect of water stress (14 days during the reproductive stage of plants) on superoxide dismutase and catalase activity in "Desi" and "Kabuli" types.

Data are the mean ± *SEM*. Differences between the treatments are significant at $P < 0.05$.

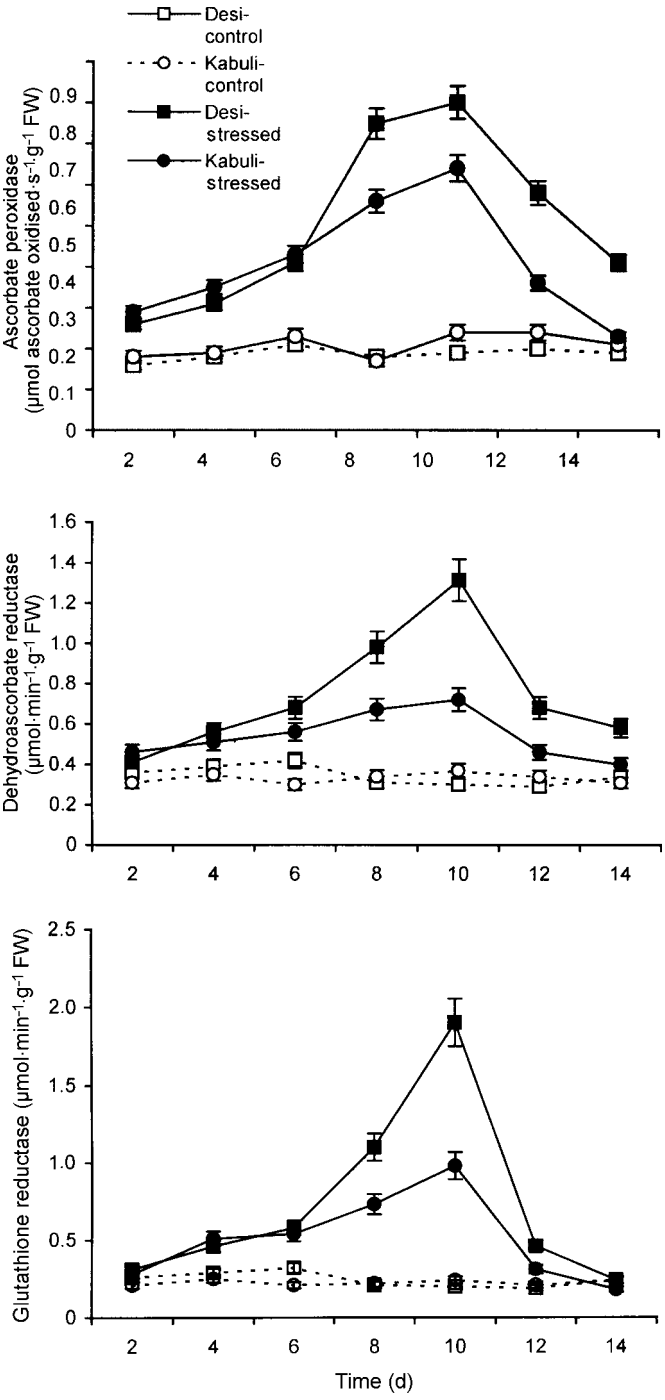


**Figure 4.** Effect of water stress (14 d during the reproductive stage of plants) on ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase in "Desi" and "Kabuli" types.

Data are the mean ± *SEM*. Differences between the treatments are significant at $P < 0.05$.

and anti-oxidants levels were considerably lower at these stress intensities in "Kabuli" compared with "Desi". Shoot growth exhibited greater inhibition in "Kabuli".

Because ABA is known to evoke an oxidative response (Jiang and Zhang 2001), the 15-day-old seedlings were treated with 10 μmol/L ABA for 24 h to compare the oxidative response of two types of chickpea. Observations indicated a variation in the expression of the oxidative response in both types of chickpea. "Kabuli" showed a greater elevation in MDA and $H_2O_2$ than "Desi" in the presence of ABA. The SOD and CAT activity did not vary appreciably between the two types of chickpea, whereas APX, DHAR, and GR activity was significantly greater in "Desi". The growth of the shoots was inhibited to a greater extent in "Kabuli".

**Table 2.** Response of "Desi" and "Kabuli" genotypes to varying degrees of water deficit stress induced by PEG-6000 as well as to 10 μmol/L abscisic acid

| Parameter | "Desi" | | | | | "Kabuli" | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water deficit stress | | | | ABA | Water deficit stress | | | | ABA |
| | Control | –0.4 MPa | –0.8 MPa | –1.2 MPa | (10 μmol/L) | Control | –0.4 MPa | –0.8 MPa | –1.2 MPa | (10 μmol/L) |
| Electrolyte leakage (%) | 11.6±1.2 | 21.6±1.3 | 37.5±1.6 | 48.2±1.4 | 39.5±1.3 | 13.3±1.4 | 23.1±1.2 | 46.4±1.1 | 58.1±1.5 | 48.9±1.2 |
| MDA (μmol/g DW) | 80±2 | 95±3 | 160±3 | 290±3 | 178±4 | 89±2 | 102±3 | 267±3 | 420±2 | 210±3 |
| $H_2O_2$ (μmol/g DW) | 5.2±1.4 | 8.2±1.6 | 16.6±2.5 | 30±2 | 18.9±2.0 | 4.6±1.2 | 13.2±2.3 | 33.6±2.4 | 42±2 | 38.9±2.1 |
| ASC (nmol/g DW) | 4.6±2.2 | 11.2±2.6 | 19.6±2.1 | 12.2±1.4 | 17.8±1.6 | 5.3±1.2 | 11.9±1.6 | 14.6±1.3 | 3.2±1.1 | 11.4±1.4 |
| GSH (nmol/g DW) | 302±6 | 406±5 | 691±7 | 416±5 | 578±5 | 298±3 | 398±4 | 456±3 | 280±3 | 430±4 |
| SOD (units/g FW per s) | 0.08±0.02 | 0.15±0.04 | 0.42±0.08 | 0.32±0.06 | 0.49±0.06 | 0.09±0.02 | 0.12±0.02 | 0.26±0.04 | 0.13±0.06 | 0.32±0.03 |
| CAT (μmol $H_2O_2$ reduced/s per g FW) | 0.22±0.08 | 0.36±0.06 | 0.59±0.07 | 0.49±0.06 | 0.51±0.08 | 0.29±0.05 | 0.56±0.08 | 0.4±0.1 | 0.34±0.06 | 0.42±0.07 |
| APX (μmol ascorbate oxidized/s per g FW) | 0.09±0.04 | 0.19±0.05 | 0.29±0.05 | 0.21±0.04 | 0.28±0.06 | 0.11±0.03 | 0.15±0.03 | 0.19±0.04 | 0.08±0.02 | 0.18±0.05 |
| DHA (μmol/min per g FW) | 0.41±0.07 | 0.53±0.05 | 1.6±0.1 | 0.12±0.05 | 0.86±0.06 | 0.38±0.04 | 0.51±0.05 | 0.71±0.06 | 0.45±0.05 | 0.59±0.06 |
| GR (μmol/min per g FW) | 0.48±0.05 | 0.7±0.1 | 1.5±0.1 | 1.2x±0.04 | 1.2±0.1 | 0.42±0.05 | 0.74±0.06 | 0.9±0.1 | 0.57±0.05 | 0.64±0.06 |
| Shoot growth rate (cm/week) | 0.43±0.03 | 0.39±0.03 | 0.31±0.03 | 0.22±0.02 | 0.24±0.03 | 0.46±0.04 | 0.40±0.05 | 0.26±0.03 | 0.17±0.03 | 0.14±0.04 |

Values are the mean ± *SEM* of 15-day-old seedlings subjected to various treatments (see text for details).

MDA, malondialdehyde; ASC, ascorbic acid; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; DHA, dehydroascorbate; GR, glutathione reductase; DW, dry weight; FW, fresh weight.

## Discussion

The water-stressed "Kabuli" plants experienced a significantly greater loss in yield in terms of the number of seeds as well as pods, implying greater sensitivity of this genotype to drought conditions. In this context, the results of the present study match those reported by Leport et al. (1999), who also observed a greater inhibition of yield in "Kabuli" compared with "Desi" following water stress and attributed this to a less effective remobilization of the assimilates towards developing seeds under stress conditions in the former. The present observations on greater pod abortion in "Kabuli" compared with "Desi" are in accord with the results of Leport et al. (2006). We noticed that $\Psi_w$ declined at a faster rate in "Kabuli" compared with "Desi", suggesting a superior water-control mechanisms in "Desi". These observations are similar to those of Leport et al. (2006), who also noticed a greater reduction in $\Psi_w$ in "Kabuli". Nevertheless, the genotypes did not differ significantly in terms of $\Psi_w$ at the end of the stress period, which is in agreement with the findings of Leport et al. (1999). It is of note that, at matching values of $\Psi_w$, "Kabuli" showed relatively higher membrane damage than "Desi", as well as lower Chl and Pn, implying greater sensitivity of the former to water deficit. The larger inhibition in Pn in "Kabuli" compared with "Desi" could also explain the larger decrease in seed-filling rate and duration, and, hence, reduced seed yield, in the former. In addition, pod yield was less in "Kabuli", which can be ascribed to a decrease in pod production or an increase in pod abortion, which is in accordance with the observations of Leport et al. (2006).

Because water stress is known to induce oxidative damage (Reddy et al. 2004), we assessed the extent of oxidative damage in both types of chickpea to determine whether the differential sensitivity of the two chickpea types towards water stress was related to this type of damage. In the present study, we observed that although both types of chickpea showed symptoms of oxidative injury in stressed plants in terms of increased levels of $H_2O_2$ and MDA (a final product of the peroxidation of unsaturated fatty acids in phospholipids), proportionately "Kabuli" showed a markedly higher content of these two molecules suggesting greater oxidative damage. Malondialdehyde is often used as a measure of free radical damage to cell membranes under stress conditions (Halliwell

and Gutteridge 1989). Thus, greater membrane injury (as electrolyte leakage) observed in "Kabuli" can be ascribed to a higher reactive oxygen species content, which possibly is the result of greater water loss in "Kabuli". This is in agreement with previous findings in other plant species where higher membrane damage due to water stress was linked to larger increases in the peroxidation of membrane lipids, especially in stress-sensitive genotypes (Sairam and Srivastava 2000; Huang and Xu 2001). Hydrogen peroxide, produced through dismutation of superoxides, inhibits chloroplast sulfahydryl-containing enzymes by readily oxidizing their sulfahydryl groups, hence reducing the assimilation of photosynthetic carbon dioxide (Takeda et al. 1995). Stress-sensitive species have been reported to possess higher levels of $H_2O_2$, which have been attributed to its less effective removal (Quartacci et al. 1994). Higher levels of these oxidative species in "Kabuli" may be related to its relatively lower water content.

The decrease in the Chl content in stressed plants on the 8th day matched the decline in $\Psi_w$ in both types and may have occurred as a consequence of photo-oxidation mediated by oxy-radicals (Wise and Naylor 1997). The Chl content acts as an indicator of chloroplast stability and has been associated with stress tolerance in previous studies (Chaves et al. 2003). A drought-induced reduction in Chl content has been reported previously for several species, such as pea (Moran et al. 1994), wheat (Nayyar and Walia 2004) and chickpea (Nayyar and Chander 2004). A greater loss of Chl in "Kabuli" may have occurred as a result of higher oxidative damage. In an earlier study, Loggini et al. (1999) also reported a larger decrease in Chl, as well as carotenoids, during dehydration in a drought-sensitive wheat genotype compared with a drought-tolerant genotype and related this reduction to a higher intensity of oxidative stress in the sensitive genotype. In the present study, the decrease in Pn occurred before the loss of Chl became apparent, which may, perhaps, be related to stomatal (Lawlor and Comic 2002) and non-stomatal (Lawson et al. 2003) reasons. The reduction in Pn due to stomatal restrictions in water-stressed plants is said to be one of the major reasons for the induction of oxidative stress because a decrease in the $CO_2$ concentration in leaf mesophyll tissue results in a decrease in NADP but an accumulation of NADPH. Under such conditions, where NADP is limiting, oxygen acts as an alternative acceptor of electrons, resulting in the formation of superoxide radicals (Cadenas 1989). In the present study, we noted a significantly higher Pn in "Desi" compared with "Kabuli" during stress. Stress-tolerant species have been observed to maintain their Pn for a longer time during stress, possibly due to their ability to operate carbon fixation as well as their ability to reduce oxidative damage (Reddy et al. 2004). This may be attributed to a higher water content in "Desi". It was also noted that, even at comparable values of $\Psi_w$ (e.g. on the 14th day), the "Desi" type maintained a higher Pn, which was possibly due to less oxidative stress. This is in agreement with the findings of Selote and Khanna-Chopra (2004) in drought-stressed rice genotypes.

The findings on anti-oxidants revealed that SOD, which catalyses the dismutation of superoxide produced in the chloroplast, mitochondria, and cytosol to $H_2O_2$ (Lawson et al. 2003), did not vary between the two types, suggesting similar abilities of both types of chickpea to neutralize the superoxides. In this context, our observations are similar to those of Sairam et al. (2004), who reported no difference in SOD activity between tolerant and sensitive wheat genotypes under conditions of water stress. Conversely, in another study (Selote and Khanna-Chopra 2004), higher SOD activity was observed in water stress-sensitive genotypes of rice. Catalase, which converts hydrogen peroxide into water, also showed an almost similar profile in both the genotypes under stress conditions in the present study. These observations are different from previous reports on genotypes of other plant species that have different water stress sensitivity, where CAT activity was observed to be greater in stress-tolerant types than their counterparts (Sairam et al. 2004). Conversely, APX, which catalyses the conversion of $H_2O_2$ to water using ASC as a substrate, was found to be significantly higher in "Desi" than "Kabuli" in the present study, especially after the 8th day of stress, when CAT activity had started to decline. Ascorbate peroxidase is a major scavenger of $H_2O_2$ in plant cells and its activity has been reported to increase in response to various environmental stresses (Asada 1997). Ascorbate peroxidase activity may be triggered, and to a higher degree, in "Desi" probably as a result of the reduction in CAT activity in order to increase the cell's ability to scavenge $H_2O_2$ (Saruyama and Tanida 1995). Because CAT activity remained almost similar in both chickpea types in the present study, the lower $H_2O_2$ levels and, hence, less oxidative damage in "Desi" may possibly be due to greater APX activity. Ascorbate peroxidase activity has been reported to remain higher in water stress-tolerant rice genotypes compared with their sensitive counterparts (Zhang and Kirkham 1995). A greater reduction in APX activity in "Kabuli" ay have resulted in restrictions in the regeneration of ASC in this type of chickpea, as indicated by relatively higher inhibition of DHAR activity, which regenerates ASC from dehydroascorbate (DHA) using oxidized glutathione (GSSG). Ascorbic acid and GSH levels were comparatively lower in "Kabuli" than "Desi", suggesting a less efficient ascorbate–glutathione cycle in the former. The decrease in GSH levels in "Kabuli" appears to be correlated with reduced GR activity, which regenerates GSH using NADPH. Glutathione reductase is involved in the removal of $H_2O_2$ within chloroplasts by maintaining more favorable levels of GSH and GSSG. The increased GR activity may protect the chloroplastic components against oxidation by $H_2O_2$ and, hence, minimize potential inactivation of

SOD within chloroplasts (Foster and Hess 1980). Elevated levels of GR may also increase the ratio of NADP/NADPH, thereby ensuring the availability of NADP to accept electrons from the photosynthetic electron transport chain and, thus, reducing the generation of AOS. Previous studies on this strongly implicate the ASC-GSH cycle in governing the stress response of differentially sensitive genotypes (Zhang and Kirkham 1995) and it appears to have a deciding effect on the stress response in the present case too. The results of the present study also indicated that the extent of the reduction of DHAR and GR activities was much higher relative to APX in "Kabuli" than "Desi". This suggests an overriding role of DHAR and GR among the anti-oxidants observed here and that these enzymes may have a decisive role in governing the sensitivity of both types of chickpea to water stress. The levels of anti-oxidants decreased after the 10th day of stress in both genotypes, which may be related to an increase in stress intensity at this time. Previous observations have suggested that severe water deficit in plant tissue may impair $O_2^{\cdot}$ scavenging in the cell and favor accumulation of $O_2^{\cdot}$ (Castillo 1996). These observations indicate that the superior performance of "Desi" under conditions of water stress may be ascribed to its better ability to maintain higher water status, which also enables it to deal with oxidative stress in a more effective manner.

Our laboratory studies (Table 2), in which both genotypes were subjected to controlled conditions of water stress and ABA treatment, indicated that "Desi" experienced less oxidative injury and possessed a more efficient anti-oxidant system in terms of SOD, APX, DHAR, GR, ASC, and GSH than "Kabuli", which may increase the protection in this genotype against water stress. Consequently, damage to membranes and growth was relatively lesser in the "Desi" type of chickpea.

Thus, the results of the present study indicate that a greater stress tolerance in "Desi" may exist as a result of its greater ability to retain water, which may be assisted by its better capacity to deal with oxidative stress.

## Materials and Methods

### Raising of plants

"Desi" plants (cv. GPF2 ) and "Kabuli" plants (cv. L550) were raised in earthen pots (height 30 cm, diameter 25 cm, volume 14.72 L) with a mixture of air-dry soil, sand, and farm yard manure in a ratio of 2∶1∶1 (v/v) and recommended doses of fertilizers. The soil was loam with a pH of 7.1. Seeds were inoculated with *Rhizobium ciceri* at the recommended rate of 1.95 g/kg seeds. Four seeds were planted in each pot in November and, after emergence, plants were thinned to two per pot.

### Application of water stress

Plants were subjected to water stress at a stage when they had set seven to nine pods per plant by withholding water for 14 d. Water potential and photosynthesis of the leaves was measured in stressed and control plants between 10:00 and 11:00 hours using a pressure chamber and IRGA, respectively. Pods of branches at the upper three nodes were tagged for analysis of seed parameters.

### Stress injury

Stress injury was examined from leaf samples (uppermost leaves) using electrolyte leakage (*EL*) and Chl content as markers. For *EL*, leaves were washed with deionized water to remove surface-adhered electrolytes (Lutts et al. 1996). These were placed in closed vials containing 10 mL deionized water and incubated at 25 °C on a rotary shaker for 24 h. Subsequently, the electrical conductivity of the solution ($L_1$) was determined. Samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity ($L_2$) was obtained after equilibration at 25 °C. The EL was defined as follows:

$$EL\ (\%) = (L_1/L_2) \times 100$$

Chlorophyll was extracted with 80% acetone from samples of fresh leaves gathered from control and stressed plants. The extract was measured spectrophotometrically at 645 and 663 nm (Arnon 1949).

### Yield observations

Approximately 50 plants were selected in three replicates from control and stress treatments for observations of seed growth rate, seed weight, seed size, and pod yield.

### Oxidative damage

Lipid peroxidation was measured in terms of MDA content as described by Dhindsa and Plumb-Dhindsa (1981). A 1-mL aliquot of the supernatant of tissue extracts was mixed with 4 mL of 20% (v/v) trichloroacetic acid (TCA) containing 0.5% (v/v) thiobarbituric acid. The mixture was heated at 100 °C for 30 min, cooled quickly, and then centrifuged at 10 000*g* for 10 min. The absorbance of the supernatant was read at 532 and 600 nm. The concentration of MDA was calculated by means of an extinction coefficient of 155 L/mmol per cm (Heath and Packer 1968). The $H_2O_2$ content was measured using titanium reagent (Teranishi et al. 1974) as follows: 1 g titanium dioxide and 10 g potassium sulfate were mixed and digested with 150 mL concentrated sulfuric acid for 2 h on a hot plate. The digested mixture was cooled, diluted to make 15 mL with distilled water, and used as a titanium reagent. Sample preparation and $H_2O_2$

estimation were as described previously (Nayyar and Kaushal 2002).

### Anti-oxidants (enzymatic and non-enzymatic)

For enzyme extracts and assays, tissues were frozen and then ground in 4 mL solution containing 50 mmol/L phosphate buffer (pH 7.0), 1% (w/v) polyvinylpolypyrrolidone, and 0.2 mmol/L ascorbic acid. The homogenate was centrifuged at 15 000*g* for 30 min and the supernatant was collected and used for enzyme assays.

The activity of SOD was measured according to the method of Giannopolities and Ries (1977). The assay medium contained 50 mmol/L phosphate buffer (pH 7.8), 13 mmol/L methionine, 75 mmol/L *p*-nitro blue tetrazolium chloride (NBT), 2 mmol/L riboflavin, 0.1 mmol/L EDTA, and 5 mL enzyme extract. One unit of enzyme activity was determined as the amount of enzyme required to achieve 50% inhibition of the NBT reduction rate by monitoring absorbance at 560 nm.

The activity of CAT was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of $H_2O_2$ (Change and Maehly 1955). The reaction mixture contained 50 mmol/L phosphate buffer (pH 7.0) and 15 mmol/L $H_2O_2$. The activity of APX was measured as a decrease in absorbance at 290 nm for 1 min (Nakano and Asada 1981). The assay mixture consisted of 0.5 mmol/L ascorbic acid, 0.1 mmol/L $H_2O_2$, 0.1 mmol/L EDTA, 50 mmol/L sodium phosphate buffer (pH 7.0), and 0.15 mL enzyme extract.

Ascorbate peroxidase activity was measured according to the methods of Aono et al. (1995). Tissue was homogenized in 1 mL of 50 mmol/L phosphate buffer (pH 7.8) containing 5 mmol/L ascorbate, 5 mmol/L dithiothreitol, 5 mmol/L EDTA, 100 mmol/L NaCl, and 2 % (w/v) polyvinyl pyrrolidone (PVP). The homogenized material was centrifuged at 15 000*g* for 15 min at 4 °C. The reaction was initiated by the addition of $H_2O_2$ to a final concentration of 44 µmol/L as described by Nakano and Asada (1981). The reaction rate was monitored by a decrease in absorbance at 290 nm. The rate constant was calculated using an extinction coefficient of 2.7 L/mmol per cm.

Glutathione reductase activity was determined as described by Foyer and Halliwell (1976). The GSSG-dependent oxidation of NADPH was followed at 340 nm in a 1-mL reaction mixture containing 100 mmol/L sodium phosphate buffer (pH 7.8), 0.5 mmol/L GSSG, 50 µL extract, and 0.1 mmol/L NADPH.

Dehydroascorbate reductase was assayed by following the increase in absorbance at 265 m of a 1-mL reaction mixture containing 50 mmol/L potassium phosphate buffer (pH 7.0), 0.1 mmol/L EDTA, 2.5 mmol/L GSH, 0.2 mmol/L DHA, and 10 µL extract as described by Doulis et al. (1997).

Ascorbic acid was measured according to the method of Mukherjee and Choudhuri (1983). Tissue was extracted with 10 mL of 6% TCA. The extract was mixed with 2 mL of 2% dinitrophenylhydrazine (in acidic medium) followed by the addition of 1 drop of 10% thiourea (in 70% ethanol). The mixture was boiled for 15 min in a water bath and, after cooling to room temperature, 5 mL of 80% (v/v) $H_2SO_4$ was added to the mixture at 0 °C. Absorbance was recorded at 530 nm. The concentration of ASC was calculated from a standard curve plotted with known concentration of ASC.

For measurement of glutathione content, fresh tissue was homogenized in 2 mL of 2% metaphosphoric acid and centrifuged at 17 000*g* for 10 min. Aliquots of the supernatant were neutralized by the addition of 0.6 mL of 10% sodium citrate to 0.9 mL extract. A total volume of 1 mL for assay, containing 700 µL NADPH (0.3 mmol/L), 100 µL DTNB (6 mmol/L), 100 µL distilled water, and 100 µL extract, was prepared and stabilized at 25 °C for 3–4 min. Later. 10 µL GR was added and the absorbance was measured at 412 nm (Griffth et al. 1980).

### Laboratory experiments

Twenty-five seeds of both types of chickpea were surface sterilized with 0.1% mercuric chloride and then thoroughly washed three times. These seeds were raised in glass Petri dishes lined with double-layered germination paper moistened with distilled water. Seeds were grown at (25 ± 2) °C for 15 d in distilled water and, subsequently, in order to impose water stress, 15-day-old seedlings of both types of chickpea were transferred to another set of Petri dishes containing PEG at –0.4, –0.8, and –1.2 MPa, corresponding to mild, moderate, and high stress, respectively, or ABA (10 µmol/L). Leaves were analysed after 3 d for EL, MDA, $H_2O_2$ and anti-oxidants (as described above) in the water stress treatments and after 24 h in the case of ABA-treated seedlings.

Experiments were replicated three times, mean values were pooled, and standard errors (*SEM*) were calculated. Data are given as the mean ± *SEM*. All data were subjected to analysis of variance. Differences between mean values of the treatments were evaluated using least significant difference (LSD) at a 0.05 level of significance.

## References

**Aono M, Sajio H, Fujiyama K, Sugita M, Kondo N, Tanaka K** (1995). Decrease in activity of glutathione reductase enhances paraquat sensitivity in transgenic *Nicotiana tabacum. Plant Physiol* **107**, 645–648.

**Arnon D** (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidases in *Beta vulgaris*. *Plant Physiol* **24**, 1–15.

**Aroca R, Irigoyen JJ, Sánchez-Díaz M** (2001). Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. *Plant Sci* **161**, 719–726.

**Asada K** (1997). The role of ascorbate peroxidase and monodehydroascorbate reductase in $H_2O_2$ scavenging in plants. In: Scandalios JG, ed. *Oxidative Stress and the Molecular Biology of Antioxidant Defences.* Cold Spring Harbor Laboratory Press, NY. pp. 715–735.

**Bartoli CG, Simontacchi M, Tambussi E, Beltrano J, Montaldi E, Puntarulo S** (1999). Drought and watering-dependent oxidative stress: Effect on antioxidant content in *Triticum aestivum* L. leaves. *J Exp Bot* **50**, 375–383.

**Behboudian MH, Ma Q, Turner NC, Palta JA** (2001). Reactions of chickpea to water stress: Yield and seed composition. *J Sci Food Agr* **81**, 1288–1291.

**Cadenas E** (1989). Biochemistry of oxygen toxicity. *Annu Rev Biochem* **58**, 79–110.

**Castillo FJ** (1996). Antioxidant protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. *Oecologia* **107**, 469–477.

**Chance B, Maehly AC** (1955). Assay of catalase and peroxidase. *Methods Enzymol* **2**, 764–775.

**Chaves MM, Maroco JP, Pereira JS** (2003). Understanding plant responses to drought: From genes to the whole plant. *Funct Plant Biol* **30**, 239–264.

**Davies SL, Turner NC, Palta JA, Siddique JHM, Plummer JA** (2000). Remobilization of carbon and nitrogen supports seed filling in chickpea subjected to water deficit. *Aust J Agr Res* **51**, 855–866.

**Dhindsa RS, Plumb-Dhindsa P, Thorpe TA** (1981). Leaf senescence: Correlated with increased leaves of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot* **32**, 93–101.

**Doulis AG, Debian N, Kingston-Smith AH, Foyer C** (1997). Differential localization of antioxidants in maize leaves. *Plant Physiol* **114**, 1031–1037.

**Foster JG, Hess JL** (1980). Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere of enriched oxygen. *Plant Physiol* **66**, 482–487.

**Foyer CH, Halliwell B** (1976). The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta* **133**, 21–25.

**Giannopolities CN, Ries SK** (1977). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol* **59**, 309–314.

**Griffth OW** (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2 vinyl pyridine. *Anal Biochem* **106**, 207–212.

**Halliwell B, Gutteridge JMC** (1989). *Free Radicals in Biology and Medicine*, 2nd edn. Claredon Press, Oxford.

**Heath RL, Packer L** (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stochiometery of fatty acid peroxidation. *Arch Biochem Biophys* **125**, 189–198.

**Huang B, Liu X, Xu X** (2001). Supraoptimal soil temperatures induced oxidative stress in leaves of creeping bentgrass cultivars differing in heat tolerance. *Crop Sci* **41**, 430–435.

**Jiang M, Zhang J** (2001). Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol* **42**, 1265–1273.

**Johnson SM, Doherty SJ, Croy RRD** (2003). Biphasic superoxide generation in potato tubers. A self amplifying response to stress. *Plant Physiol* **13**, 1440–1449.

**Lawlor DW, Comic G** (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ* **25**, 275–294.

**Lawson T, Oxborough K, Morison JIL, Baker NR** (2003). The responses of guard and mesophyll cell photosynthesis to $CO_2$, $O_2$, light, and water stress in a range of species are similar. *J Exp Bot* **54**, 1743–1752.

**Lei YB, Song SQ, Fu JR** (2005). Possible involvement of antioxidant enzymes in the cross-tolerance of the germination/growth of wheat seeds to salinity and heat stress. *J Integr Plant Biol* **47**, 1211–1219.

**Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL et al.** (1999). Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *Eur J Agron* **11**, 279–291.

**Leport L, Turner NC, Davies SL, Siddique KHM** (2006). Variation in pod production and abortion among chickpea cultivars under terminal drought. *Eur J Agron* **24**, 236–246.

**Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F** (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol* **119**, 1091–1100.

**Lutts S, Kinet JM, Bouharmont J** (1996**)**. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann Bot* **78**, 389–398.

**Malhotra RS, Pundir RPS, Slinkard AE** (1982). Genetic resources of chickpea. In: Saxena MC, Singh KB, eds. *The Chickpea.* CAB International Cambrian News Ltd., Aberystwyth. pp. 67–81.

**Moran JF, Becana M, Iturbe-Ormaetyl I, Frechilla S, Klucas RV, Aparicio-Tejo P** (1994). Drought induces oxidative stress in pea plants. *Planta* **194**, 346–352.

**Mukherjee SP, Choudhuri MA** (1983). Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol Plant* **58**, 166–170.

**Nakano Y, Asada K** (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* **22**, 867–880.

**Nayyar H, Chander S** (2004). Protective effects of polyamines against oxidative stress induced by water and cold stress in chickpea. *J Agron Crop Sci* **190**, 355–365.

**Nayyar H, Gupta D** (2006). Differential sensitivity of $C_3$ and $C_4$ plants to water deficit stress: Association with oxidative stress and antioxidants. *Environ Exp Bot* **58**, 106–113.

**Nayyar H, Kaushal SK** (2002). Chilling induced oxidative stress in

germinating wheat grains as affected by water stress and calcium. *Biol Plant* **45**, 601–604.

**Nayyar H, Walia DP** (2004). Genotypic variation in response to water stress and abscisic acid induced accumulation of osmolytes in flag leaf and developing wheat grains. *J Agron Crop Sci* **190**, 39–45.

**Noctor G, Foyer CH** (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol Biol* **49**, 249–279.

**Peltzer D, Dreyer E, Polle A** (2002). Differential temperature dependencies of antioxidative enzymes in two contrasting species. *Plant Physiol Biochem* **40**, 141–150.

**Quartacci MF, Sgherri CLM, Pinzino C, Navari-Izzo F** (1994). Superoxide radical production in wheat plants differently sensitive to drought. *Proc R Soc Edinburgh* **102**, 291–294.

**Reddy AR, Chaitanya KV, Vivekanandan M** (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol*. **161**, 1189–1202.

**Sairam RK, Srivastava GC** (2000). Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. *Biol Plant* **43**, 381–386.

**Sairam RK, Rao KV, Srivastava GC** (2004). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci* **163**, 1037–1046.

**Saruyama H, Tanida M** (1995). Effect of chilling on activated oxygen-scavenging enzymes in low temperature-sensitive and -tolerant cultivars of rice (*Oryza sativa* L.). *Plant Sci* **109**, 105–113.

**Selote DS, Khanna-Chopra R** (2004). Drought-induced spikelet sterility is associated with an inefficient antioxidant defense in rice panicles. *Physiol Plant* **121**, 462–471.

**Singh KB, Ocampo B** (1997). Exploitation of wild *Cicer* species for yield improvement in chickpea. *Theor Appl Genet* **95**, 418–423.

**Takeda T, Yokota A, Shigeoka S** (1995). Resistance of photosynthesis to hydrogen peroxide in algae. *Plant Cell Physiol* **36**, 1089–1095.

**Teranishi Y, Tanaka A, Osumi M, Fukui S** (1974). Catalase activity of hydrocarbon utilising candida yeast. *Agr Biol Chem* **38**, 1213–1216.

**Türkan I, Bor M, Özdemir F, Koca H** (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci* **168**, 223–231.

**Wise RR, Naylor AW** (1997). Chilling-enhanced photo-oxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiol* **83**, 278–282.

**Zhang JX, Kirkham MB** (1995). Water relations of water-stressed, split-root $C_4$ (*Sorghum bicolor*; Poaceae) and $C_3$ (*Helianthus annuus*; Asteraceae) plants. *Am J Bot* **82**, 1220–1229.

(Managing editor: Ping He)