

Differential Responses of Pigments, Lipid Per-oxidation, Organic Solutes, Catalase and Per-oxidase Activity in the Leaves of Two *Vicia faba* L. Cultivars to Drought

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ABSTRACT

A preliminary experiment on germination of five (*Vicia faba* L.) cultivars (Giza 40, Giza 67, Giza 102, Giza 103 and Giza 667) in different levels of polyethylene glycol-induced water stress indicated that cv Giza 40 was the most drought-tolerant cultivar, while cv Giza 667 was the most sensitive one. Seeds of cv Giza 40 and Giza 667 were selected and the effect of low soil water content (40% field capacity) on growth, photosynthetic pigment content, relative water content (RWC), lipid per-oxidation, membrane stability index (MSI), organic compounds accumulation as well as catalase (CAT) and per-oxidase (POX) activity in the leaves of 21 day old plants was studied. With respect to dry weight (DW), drought caused more decrease in cv Giza 667 than in cv Giza 40, indicating more tolerance of cv Giza 40 under low soil water content. Drought decreased chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid and Chl a/ b, carotenoid/ Chl a + b ratios in the leaves of cv Giza 667. On the other hand, in cv Giza 40 a significant increase in these pigment criteria under drought stress was proved. Drought decreased RWC, MSI and increased lipid per-oxidation level, catalase (CAT) and peroxidase (POX) activity in both the cultivars. The decline in RWC and MSI and the increase in lipid per-oxidation level in response to drought stress were higher in cv Giza 667 than cv Giza 40. CAT and POX activity was higher in case of Giza 40 than in Giza 667 under both control and drought conditions. Drought induced accumulation of soluble sugars, soluble proteins, free amino acids and proline in both the cultivars. However, this accumulation was lower in cv Giza 667 than in the more tolerant cv Giza 40. These results indicate that cv Giza 40 showed a better protection against drought-induced oxidative stress by higher (CAT) and (POX) activity and osmolyte concentration than cv Giza 667.

Key Words: Catalase; Drought; Lipid peroxidation; Peroxidase; *Vicia faba* L.

INTRODUCTION

Drought is one of the important environmental factors, which induces significant alterations in plant physiology and biochemistry. Some plants have a set of physiological adaptations that allow them to tolerate water stress conditions. The degree of adaptations to the decrease of water potential caused by drought may vary considerably among species (Savé *et al.*, 1995) and also within a species (Parker & Pallardy, 1985). The most common symptom of water stress injury is the decrease in seed germination (Schmidhalter & Oertli, 1991) and the inhibition of growth, which reflected in a reduction in the dry matter yield (Schmidhalter & Oertli, 1991; El-Tayeb & Hassanien, 2000; Le Thiec & Manninen, 2003). The inhibition of plant growth under water stress conditions is associated with altered water relations (Torrecillas *et al.*, 1995; Dichio *et al.*, 2003). Drought was found to decrease the relative water content of plant leaves (Sánchez-Blanco *et al.*, 2002). Total chlorophyll, carotenoids content and Chl a/ b ratio decline under water deficit conditions. The rate of decline in drought-sensitive cultivar is much faster than in more drought-resistant cultivar (Shaddad & El-Tayeb, 1990).

However, Sestak and Vaclavik (1965) reported that the chlorophyll content may increase under the conditions of water stress. It was found that drought influence carbohydrate and nitrogen metabolism. Some drought-stressed plants accumulate the carbohydrates in soluble form, which is related to the osmoregulation metabolism (Zrenner & Stitt, 1991). Drought was found to induce the accumulation of soluble proteins and free amino acids including proline in various plants (Vyas *et al.*, 1985; Girousse *et al.*, 1996; Ain-Lahout *et al.*, 2001). Proline accumulation varied with the degree of plant resistance to drought (Levy, 1983). Therefore, proline could be used for the evaluation of the tolerance or sensitivity of plants to stress (Patel & Vora, 1984). In addition, Claussen (2005) reported that proline is a reliable indicator of the environmental stress on plants and establish stress thresholds for fruit yield and product quality of tomato plants.

Drought induces generation of reactive oxygen species, causing lipid per-oxidation, and consequently membrane injury, protein degradation, enzyme inactivation and disruption of DNA strands (Imlay & Linn, 1988; Becana *et al.*, 1998). Removing of the toxic oxygen radicals

rapidly is of prime importance in any defense mechanism. Plants protect cells and sub-cellular systems from the cytotoxic effects of these active oxygen radicals with both non-enzymatic and enzymatic antioxidant system such as carotenoids, ascorbic acid, α -tocopherol, per-oxidase (POX) and catalase (CAT) (Munné-Bosch & Alegre, 2000; Sairam & Srivastava, 2001; Fu & Huang, 2001). There are many reports underline the intimate relationship between antioxidant enzyme activities and increased tolerance to environmental stress (Sairam *et al.*, 2002; Bor *et al.*, 2003; Reddy *et al.*, 2004; Türkan *et al.*, 2005).

The present work was conducted to detect, which cultivars of *Vicia faba* could germinate and sustain growth under water stress conditions and to detect, which physiological mechanisms underlying differential tolerance of two cultivars of *Vicia faba* to drought. The changes of growth, photosynthetic pigments, relative water content (RWC), membrane stability index (MSI), lipid per-oxidation in the leaves of cv Giza 40 (relatively drought tolerant) and cv Giza 667 (relatively drought sensitive) were studied. POX and CAT activity and the content of soluble sugars, soluble proteins, total free amino acids and free proline in the two cultivars were also followed.

MATERIAL AND METHODS

The seeds of five *Vicia faba* cultivars (Giza 40, Giza 67, Giza 102, Giza 102 and Giza 667) were obtained from agricultural research center and the faculty of agriculture, Assuit University, Egypt. A preliminary experiment was carried out to study the effect of water stress on the seed germination of the different cultivars to detect the more tolerant and the more sensitive one to water stress. The water stress levels [0.0 (control), - 0.05, - 0.1, - 0.2, - 0.4 and - 0.6 MPa] were achieved using polyethylene glycol (PEG, 6000 MW) in 1/ 10 Hoagland solution (Hoagland & Arnon, 1950). The seeds in the control group were germinated in 1/ 10 Hoagland solution as substrate. The seeds of all genotypes were sterilized in 5% Clorox solution (sodium hypochlorite) for 5 min followed by a 5 min dip in 70% ethanol. The seeds were washed three times with sterilized distilled water and left to germinate in Petri dishes in the dark at 25°C (20 seeds per dish). Each dish was sealed with parafilm strip to avoid evaporation. Three replicates were prepared for each treatment.

Seeds of cv Giza 40 (relatively drought tolerant) and cv Giza 667 (relatively drought sensitive) were selected and sown into plastic pots containing 2 kg air-dried soil (sand/ clay 1:1 v/ v) per pot. The pots were watered with ½ strength Hoagland solution and then adjusted to the desired soil water content [90% (control) and 40% (drought) of the maximum field capacity]. Twenty seeds were used per pot. Three pots were assigned to each treatment. The plants were irrigated every other day with distilled water to the desired moisture level. The plants left to grow under controlled conditions (light/ dark regime of 12/ 12 h at 22/ 16°C, light

intensity of 3040 lux) for three weeks. At the end of experimental period the leaves were harvested. Half of the samples were rapidly dried in an oven at 80°C to constant weight and then ground to powder, which was used for further analysis. The second half used immediately for pigment extraction, estimation of relative water content (RWC) and membrane stability index (MSI) of leaves, the remaining samples were frozen in liquid nitrogen and stored at 20°C for biochemical analysis.

Chlorophyll a, b and carotenoids were determined according to Metzner *et al.* (1965). Leaf material (0.1 g each) of young fully expanded leaves of three plants from each replicate, were used for pigment extraction. The pigments extract was measured against a blank of pure 85% acetone at wave-lengths of 452.5, 644 and 663 nm. Leaf relative water content (RWC) was estimated according to the method of Whetterley (1950). Leaf material was weighed (0.5 g) to determine fresh weight and placed in double-distilled water for 4 h and then turgid weight was recorded. Finally, the samples were dried in an oven at 65°C for 48 h and the dry weights were recorded. RWC was calculated as:

$$\text{RWC} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100.$$

The membrane stability index (MSI) was determined according to Sairam *et al.* (2002). Leaf samples (0.1 g each) were cut into discs of uniform size and placed in 10 ml of double-distilled water in two sets. One set was kept at 40°C for 30 min and its conductivity recorded (C_1) using conductivity meter. The second set was kept in a boiling water bath (100°C) for 15 min and its conductivity also recorded (C_2). The membrane stability index (MSI) was calculated as:

$$\text{MSI} = [1 - (C_1 / C_2)] \times 100.$$

The level of lipid per-oxidation in leaf tissue was measured by determination of malondialdehyde (MDA), a breakdown product of lipid per-oxidation. MDA content was determined with thiobarbituric acid reaction. Briefly, 0.25 g of frozen sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10,000 x g for 5 min. To 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA were added. The mixture was heated at 95°C for 15 min and then immediately cooled. The developed colour was extracted with 2 ml n-butanol and the absorbance was measured at 532 nm. The value for the non-specific absorption at 600 nm was subtracted. The level of lipid per-oxidation was expressed as nmole of MDA formed using an extinction coefficient of 155 m mol L⁻¹ cm⁻¹ (Zahor *et al.*, 1994).

For the assays of CAT and POX enzymes, leaves (200 mg) was homogenized in an ice-cooled mortar, ground in 1 ml of 100 mM K-phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 10 min under cooling and the supernatant was used for enzyme assay. The activities of CAT and POX were determined according to Chance and Maehly (1955). CAT activity was determined by measuring the

decomposition of H_2O_2 and the decline in absorbance at 240 nm was followed for 3 min. The 3-ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 , and 0.1 ml of enzyme extract, which started the reaction. The activity of POX assayed by measuring the oxidation of guaiacol and the increase in absorbance at 470 nm was recorded for 3 min. The reaction mixture contained 50 μ l of 20 mM guaiacol, 2.8 ml of 10 mM phosphate buffer (pH 7.0), and 0.1 ml enzyme extract. The reaction was started with 20 μ l of 40 mM H_2O_2 . The activity was defined as $\Delta OD/\text{min}/\text{mg FW}$.

The water-soluble sugars quantified by the anthrone sulfuric acid method (Fales, 1951). Samples (100 mg each) of dried leaf powder were heated in water bath at 100 C for 2 h in 10 ml distilled H_2O . The solution was cooled and transferred to a 50 ml measuring flask after filtration through centered glass funnel, then completed to a known volume by distilled water. Then 4.5 ml of anthrone reagent was added to 0.5 ml of the prepared solution, the soluble sugars content was calculated as mg g^{-1} dry weight of the plant leaves. To estimate soluble proteins, leaves powder samples (100 mg each) were boiled in 10 ml s distilled water for two h. After cooling, the water extract was centrifuged and the supernatant was decanted and completed to a definite volume using distilled water, then the soluble proteins were determined according to Lowry *et al.* (1951). Free amino acids were extracted from leaf tissues and determined according to the method of Moore and Stein (1948) and free proline was determined according to Bates *et al.* (1973).

The data were statistically analysed by one-way analysis of variance using PC stat (PC program). The least significant difference (LSD) was used to test the difference between the treatments.

RESULTS AND DISCUSSION

Drought, through its osmotic effect in natural and agricultural habitats, induce various responses such as germination and growth inhibition and synthesis of some non-toxic compounds to increase the osmotic potential of the cell and thus allow metabolic processes to continue the enhancement of antioxidant enzymes activities.

In this study, although the germination percentage decreased progressively in the tested (*Vicia faba* L.) cultivars as the level of PEG-induced water stress increased, Giza 40 was the more tolerant cultivar and Giza 667 was the more sensitive one (Table I). The inhibitory effect of water stress on seed germination has been reported by other authors using various plants (Schmidhalter & Oertli, 1991; El-Tayeb & Hassanien, 2000; Schütz *et al.*, 2002). In agreement with this, Bayuelo-Jimenez (2002) who found that, the germination capacity was higher in *Phaseolus filiformis* (more drought resistance) than in *Phaseolus vulgaris* (more drought-sensitive) under water stress.

The effect of low soil water content exhibited that cv

Table I. Final germination percentage of five *Vicia faba* cultivars as affected by different levels of PEG-induced water stress. Each value is the mean of three replicates

Osmotic Potential [MPa]	Vicia faba cultivars				
	Giza 40	Giza 67	Giza 102	Giza 103	Giza 667
-0.00 (Control)	100	100	100	100	100
-0.05	100	100	100	100	100
-0.10	96.67	96.67	91.67**	93.33**	93.33**
-0.20	80**	78.33**	76.67**	80**	73.67**
-0.40	61.67**	50**	46.67**	48.33**	40**
-0.60	36.67**	21.67**	18.33**	23.33**	11.67**
LSD _{5%}	5.05	4.68	3.82	3.31	4.27
LSD _{1%}	7.01	6.49	5.30	4.59	5.93

* Significant and ** Highly significant differences at the $P < 0.05$ and $P < 0.01$ levels

Giza 40 was also more tolerant than cv Giza 667 with regard to growth. Drought treatment (40% field water capacity) caused a decrease in leaves dry mass of both the cultivars. These results are in agreement with those obtained in previous works (Shaddad & El-Tayeb, 1990; Kirnak *et al.*, 2001). cv Giza 40 showed leaves dry mass higher than cv Giza 667 (Table II). Loggini *et al.* (1999) working with two wheat cultivars found that drought caused a more pronounced inhibition in growth in the more drought-sensitive cv Adamello compared with the relatively drought-tolerant cv Ofanto. In addition, Türkan *et al.* (2005) reported a superior performance of *Phaseolus acutifolius* (more drought-tolerant) to that of *Phaseolus vulgaris* (more drought-sensitive) in terms of biomass production under water stress. In general, drought resulted in a significant decrease in the RWC in the leaves of both the cultivars. However, under drought stress cv Giza 40 exhibited higher RWC than cv Giza 667 (Table II). In accordance with this, previous works reported that drought-tolerant genotypes exhibited high RWC compared to the drought-sensitive genotypes in several species, including wheat (Sairam & Srivastava, 2001), moth bean (Garg *et al.*, 2001), barley (Kocheva & Georgiev, 2003) and *Phaseolus* (Türkan *et al.*, 2005).

It is well known that drought enhanced free radicals production, which induced lipid per-oxidation of biomembranes reflecting the stress-induced damage in tissues. The content of MDA is often used as an indicator of the extent of lipid per-oxidation resulting from oxidative stress (Smirnoff, 1993). In the present work, MDA content significantly increased in leaves of the two *V. faba* cultivars in response to drought stress. However, cv Giza 40 shows lower MDA accumulation than cv Giza 667 under drought stress (Fig. 2). In addition, the results of membrane stability index (MSI) showed that, the decrease in MSI under drought stress was lower in case of cv Giza 40 than that of Giza 667 (Fig. 1). In consistence with this, Sairam and Srivastava (2001) who reported that the drought-tolerant genotypes of wheat showed lower lipid per-oxidation level and higher MSI value than the susceptible ones. In addition, Türkan *et al.* (2005) found that MDA content was lower in the leaves of drought-tolerant *Phaseolus acutifolius* Gray

Table II. Effect of soil moisture content on leaves dry weight (g plant⁻¹), relative water content (RWC) and the content of Chlorophyll a, b and carotenoids (mg g⁻¹ FW) of 21 day old *Vicia faba* cv Giza 40 and *Vicia faba* cv Giza 667 plants. Each value is the mean of three replicates (3 plants each)

Cultivar	Field capacity %	Dry wt.	RWC%	Chl a	Chl b	Caroten-oids	Total	Chl (a+b)	Chl a/b	Carot./ Chl (a+b)
Giza 40	90% (Control)	1.156	87.157	0.243	0.397	0.037	0.676	0.639	0.610	0.057
	40%	0.739**	69.865**	0.322**	0.437**	0.088**	0.848**	0.760**	0.740**	0.117**
	LSD _{5%}	0.106	5.901	0.021	0.011	0.0074	0.0173	0.012	0.0179	0.01
	LSD _{1%}	0.176	8.940	0.034	0.019	0.0122	0.0286	0.021	0.0297	0.017
Giza 667	90% (Control)	0.943	88.312	0.322	0.427	0.095	0.844	0.749	0.752	0.126
	40%	0.473**	62.769**	0.277**	0.403*	0.064**	0.744**	0.680	0.686**	0.094**
	LSD _{5%}	0.058	5.691	0.0091	0.014	0.0103	0.036	0.072	0.0136	0.01
	LSD _{1%}	0.096	8.622	0.015	0.024	0.017	0.059	0.119	0.0227	0.017

* Significant and ** Highly significant differences at the P < 0.05 and P < 0.01 levels

than that in drought-sensitive *P. vulgaris* L. The lower MDA increases and the lower MSI decreases in drought stressed cv Giza 40 suggesting a better protection from the oxidative damage. The better protection in cv Giza 40 seems to result from the high CAT and POX activity while the highest accumulation of MDA and the lowest MSI in leaves of cv Giza 667 could be attributed to low CAT and POX activity. These results are in agreement with the result of Sairam *et al.* (2002) and Bor *et al.* (2003) who found a correlation between increased antioxidant enzymes activities and decreased lipid per-oxidation in drought tolerant wheat, *Triticum aestivum* cv Kharchia 65 and wild beet, *Beta maritima*, respectively under salt stress.

Drought, like other environmental stresses such as high and low temperatures and salinity also induces oxidative stress. To be able to endure oxidative damage under un-favorable conditions, plants possess both non-enzymatic antioxidants such as carotenoid, flavanoids, α -tocopherol, ascorbic acid and glutathione and enzymatic antioxidants such as CAT and POX (Smirnoff, 1995; Munné-Bosch & Alegre, 2000). These enzymatic and non-enzymatic antioxidants are reported to increase under various environmental stresses (Yu & Rengel, 1999; Sgherri *et al.*, 2000; Acar *et al.*, 2001). Comparatively higher activity has been reported in tolerant cultivars than in sensitive ones (Sairam *et al.*, 2002; Reddy *et al.*, 2004), indicating that higher antioxidant enzymes activity have a role in imparting tolerance to these cultivars against environmental stress. In the light of such background, the higher CAT and POX activities in cv Giza 40 under drought stress indicates its relative tolerance to drought while cv Giza 667 was inferior on this count.

Catalase (CAT) and per-oxidase (POX) play an essential role in scavenging the H₂O₂ toxicity. Under drought, CAT activity increased significantly in the leaves of both the cultivars. Drought induced significant increase in POX activity while no significant change in case of cv Giza 40 was observed (Fig. 3 & 4). However, the level of CAT and POX activity was higher in the tolerant cv Giza 40 than the sensitive cv Giza 667 under control conditions. These results clearly indicate the drought-tolerant cv Giza 40 has higher CAT and POX activity, suggesting higher capacity to decompose H₂O₂ under drought stress. These results are in a

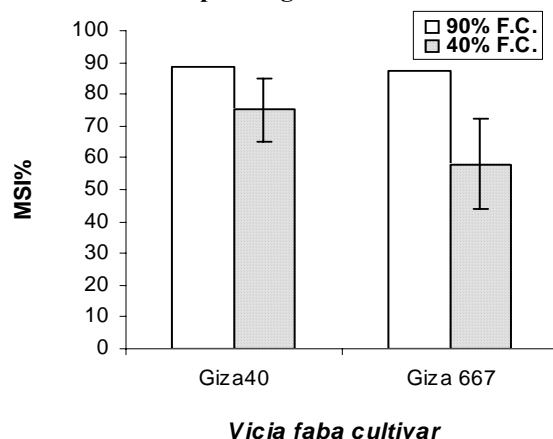
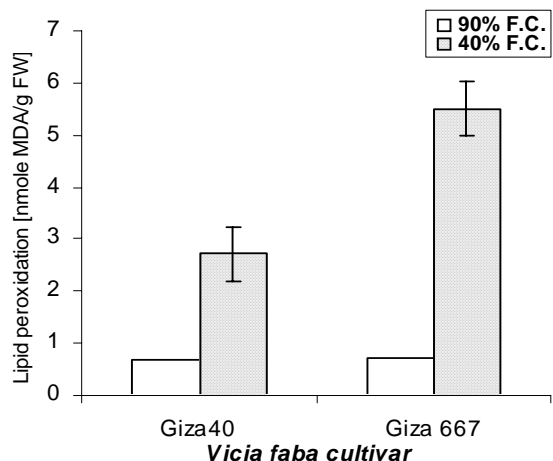
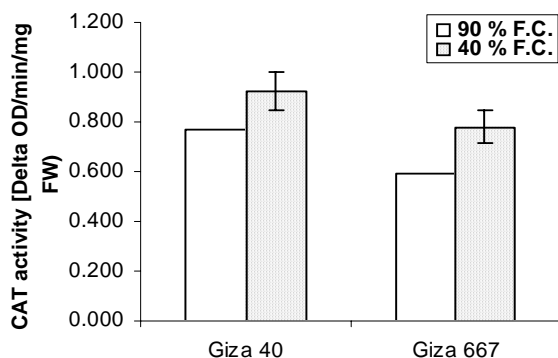
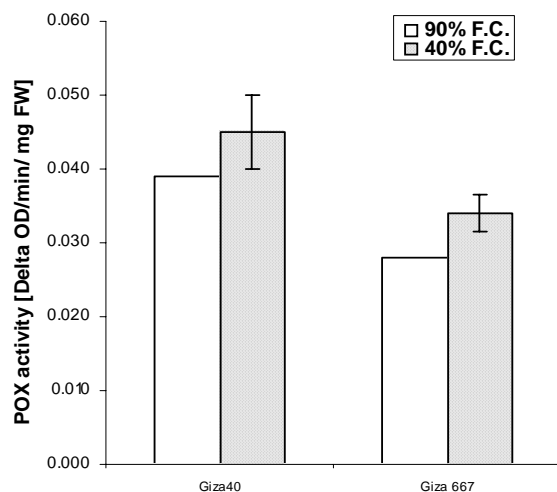
Fig. 1. Effect of soil moisture content on the membrane stability index (MSI%) in the leaves of 21 day old *Vicia faba* cv Giza 40 and *Vicia faba* cv Giza 667 plants. The data is the mean of three replicates (3 plants each). Vertical lines indicate the LSD at P< 0.05 for the corresponding control.**Fig. 2.** Effect of soil moisture content on the lipid peroxidation level (nmol MDA/g FW) in the leaves of 21 day old *Vicia faba* cv Giza 40 and *Vicia faba* cv Giza 667 plants. The data is the mean of three replicates. Vertical lines indicate the LSD at P< 0.05 for the corresponding control

Fig. 3. Effect of soil moisture content on the catalase (CAT) activity (Δ OD/min/mg FW) in the leaves of 21 day old *Vicia faba* cv Giza 40 and *Vicia faba* cv Giza 667 plants. The data is the mean of three replicates. Vertical lines indicate the LSD at $P < 0.05$ for the corresponding control



V. faba cultivar

Fig. 4. Effect of soil moisture content on the peroxidase (POX) activity (Δ OD/min/mg FW) in the leaves of 21 day old *Vicia faba* cv Giza 40 and *Vicia faba* cv Giza 667 plants. The data is the mean of three replicates. Vertical lines indicate the LSD at $P < 0.05$ for the corresponding control



V. faba cultivar

good agreement with the result of Reddy *et al.* (2004) and Türkan *et al.* (2005) who found higher constitutive level of CAT and POX activity in drought tolerant mulberry, cvs S - 13 and BC 2 - 59 and *Phaseolus acutifolius*, respectively.

The contents of Chl a, Chl b and Car pigments and total chlorophyll (a + b), chl a/ b and Carot/ chl a + b ratios decreased in the leaves of the more drought-sensitive cv Giza 667 under drought conditions (Table II). These results are in accordance with those obtained by other workers (Loggini *et al.*, 1999; Barathi *et al.*, 2001; Fu & Huang,

2001; & Egert *et al.*, 2002). Photoinhibition and photodestruction of pigments may contribute to such alterations. On the other hand, in case of cv Giza 40 the pigment content increased in the stressed-plants. Sestak and Vaclavik (1965) pointed out that chlorophyll content may increase under conditions of water deficit. In addition, Garcia-Valenzuela *et al.* (2005) reported that, the chlorophyll cell line ('TADH-XO'), from highly water stress tolerant grass *Bouteloua gracilis*, developed substantially higher amounts of chlorophyll (a & b) when subjected to PEG-induced water stress. In the present work, the drought-induced increase in chlorophyll a and b in cv Giza 40 was associated with a significant increase in carotenoids content and Carot/ Chl a + b ratio, which indicate the capacity to protect the photosynthetic apparatus. Loggini *et al.* (1999) reported that carotenoids are involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet oxygen (1O_2), which is produced by the excited triplet state of chlorophyll.

The results also showed that in cv Giza 667 (more drought-sensitive) Chl a/ b ratio decreased significantly under drought conditions (Table II), indicating that drought affected the light-harvesting antenna size. However, in cv Giza 40 (more drought-tolerant) Chl a/ b ratio increased with drought treatment. The change in the Chl a/ b ratio is used as an indicator for relative photosystem stoichiometry (P fannschmidt *et al.*, 1999). Under control conditions, Chl a/ b ratio was comparatively higher in cv Giza 667 than in cv Giza 40, suggesting that the light-harvesting antenna size was smaller in line Giza 667 than in Giza 40. Is the change in light-harvesting antenna size useful as an indicator in drought tolerance between both plant species and genotypes? This question needs further studies.

Accumulation of soluble sugars, free amino acids, including proline under stress protect the cell by balancing the osmotic strength of cytosol with that of the vacuole and external environments (Hellebust, 1976; Greenway & Munns, 1980; Gadallah, 1999). Beside to their role as cytosolic osmotica, these solutes may interact with cellular macromolecules such as enzymes and stabilize the structure of such macromolecules (Rhodes, 1987; Smirnov & Cumbe, 1989; Jain *et al.*, 2001). In the present study, higher accumulation of osmolyte in drought-tolerant cv Giza 40 than drought-sensitive cv Giza 667 was associated with higher accumulation of soluble proteins (Table III). A direct consequence to higher osmolyte concentration in drought-tolerant cv Giza 40 is the comparatively higher water retaining capacity, as reflected by RWC (Table II) and more efficient antioxidant enzymes activity. In a good agreement with this, Garg *et al.* (2001) found higher contents of soluble sugars, free amino acids and proline in drought-tolerant genotype CZM-32-E than the sensitive genotypes of moth bean. Türkan *et al.* (2005) also found a higher proline content in drought-tolerant *Phaseolus acutifolius* under PEG-induced water deficit. Consistently, Sairam *et al.* (2002) observed higher soluble sugars and

Table III. Effect of soil moisture content on the content of soluble sugars, soluble proteins, free amino acids and proline (mg g⁻¹ DW) in the leaves of two *Vicia faba* cultivars (21 day old). Each value is the mean of three replicates

Cultivar	Field capacity %	Soluble sugars	Soluble proteins	Total amino acids	Proline
Giza 40	90% (Control)	16.27	86.675	31.065	5.15
	40%	32.21**	116.65**	33.015**	7.89**
	LSD _{5%}	2.12	9.20	0.578	0.453
	LSD _{1%}	3.52	15.26	0.958	0.751
Giza 667	90% (Control)	11.32	65.965	30.18	4.95
	40%	14.71	111.73**	30.48	6.84*
	LSD _{5%}	7.95	7.14	NS	0.792
	LSD _{1%}	13.19	11.84	NS	1.248

* Significant and ** Highly significant differences at the $P < 0.05$ and $P < 0.01$ levels

proline content in tolerant wheat genotype (Kharchia 65) under sat stress.

In conclusion, based on the data of dry mass, RWC and the photosynthetic active pigments, it is clear that *V. faba* cv Giza 40 is more tolerant to drought stress than *V. faba* cv Giza 667. It is possible that better resistance to drought of cv Giza 40 was related to its ability to maintain higher levels of activity of antioxidant enzymes, resulting in lower H₂O₂ production, lipid per-oxidation and higher membrane stability. Higher osmolyte concentration (soluble sugars and free amino acids especially proline) involved in osmoregulation, which reflected in higher RWC and stabilization of essential enzyme proteins such as CAT and POX resulting in higher activity in cv Giza 40 under drought stress. Finally, selection of *Vicia faba* cultivars with genetic trait like antioxidants and osmolyte accumulation might be useful in assessing the adaptive responses of *Vicia faba* to drought.

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