Water deficit-induced oxidative stress and the activation of antioxidant enzymes in white clover leaves

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Abstract

The objective of this study was to determine the development of the antioxidant enzymes induced by drought stress in white clover (*Trifolium repens* L.) leaves. Water stress was imposed during 28 d by decreasing the daily irrigation. Leaf water potential (Ψ_w) gradually decreased from -0.46 to -2.33 MPa. For the first 7 d, dry mass (DM), H_2O_2 and lipid peroxidation were not significantly affected by water deficit. From 14 d of treatment, water stress decreased dry mass and increased content of reactive oxygen species ($O_2^{\bullet-}$ and H_2O_2) and oxidative stress (malondialdehyde content). The ascorbate peroxidase (APOD) was activated most rapidly, already during the first week of water stress, but then its activity slowly decreased. Activation of superoxide dismutase (SOD) and catalase (CAT) by water deficit continued during the 14 d ($\Psi_w \geq$ -1.65 MPa) and then their activities remain on the similar level. The activity of guaiacol-peroxidase (GPOD) increased mostly under progressive water stress and was correlated with increase in lipid peroxidation and growth restriction.

Additional key words: ascorbate peroxidase, catalase, drought, leaf water potential, guaiacol peroxidase, superoxide dismutase, *Trifolium repens*.

Introduction

Reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide and hydroxyl radical, and the antioxidative enzymes that scavenge them take important part in plant responses to a wide range of environmental stresses including extreme temperatures, salinity, water deficit, heavy metals, and pathogens (Cakmak and Horst 1991, Smirnoff 1993, Caruso *et al*. 1999, Jung 2004, Cavalcanti *et al*. 2004). Superoxide dismutase (SOD; EC 1.15.1.1) dismutates $O_2^{\bullet-}$ into H_2O_2 and O_2, and H_2O_2 is eliminated by catalase (CAT; EC 1.11.1.6) and peroxidases (POD; EC 1.11.1.7) utilizing both enzymatic and non-enzymatic H_2O_2 degradation pathways (Peltzer *et al*. 2002).

Although several works have provided evidence for an effective protector role of POD-CAT-SOD systems against oxidative stress in diverse plant species (*e.g.* Vaidyanathan *et al*. 2003, Jung 2004), some physiological aspects remain unclear, for example it is questionable whether the increase in guaiacol-POD activity is related to elimination of H_2O_2 *per se*, or whether it promotes lignification which then lead to stunted plant growth (Chazen and Neumann 1994, Chen *et al*. 2002). Another point is that some reports have shown enhanced, stress-induced, CAT and/or SOD activity (Smirnoff 1993, Vaidyanathan *et al*. 2003, Sivritepe *et al.* 2008), whereas others record a down-regulation of activity (Foyer and Noctor 2000, Jiang and Huang 2001). Moreover, how the dynamic balance between the ROS and the scavenging enzymes might be disturbed in plants exposed to drought remains unclear.

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Abbreviations: APOD - ascorbate peroxidase; CAT - catalase; DM - dry mass; GPOD - guaiacol peroxidase; MDA - malondialdehyde; NBT - nitroblue tetrazolium; ROS - reactive oxygen species; RWC - relative water content; SOD - superoxide dismutase; TEMED - N,N,Ń,Ń-tetramethylethylenediamine; TBA - thiobarbituric acid; TCA - trichloroacetic acid; ε - coefficient of absorbance; Ψ_w - leaf water potential.
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In this study, we hypothesized that ROS generation and the activation of antioxidative enzymes are responsible for water stress tolerance or intolerance. To test this hypothesis, we investigated effect of decreased leaf water potential on ROS generation, lipid peroxidation and the activities of antioxidant enzymes (SOD, CAT, APOD and GPOD).

Material and methods

Plant culture and sampling: White clover (*Trifolium repens* L.) seedlings were transplanted into 3.0 dm^3 pots containing a mixture of sand and fritted clay. Plants were regularly watered to field capacity during first 2 weeks. Water-deficit stress was imposed by decreasing the volume of water supply per day from 50 (control) or 5 cm^3 of water per pot. Half the volume of the daily irrigation for each treatment was applied at 10:00 and the remaining half at 16:00 to minimize the differences between predawn and midday soil water potential (Ψ_{soil}) (Kim *et al*. 2004). Each treatment lasted for 28 d and leaf tissues were sampled at 7 d intervals and immediately frozen in liquid nitrogen. Freeze-dried samples were finely ground and stored under vacuum for further analysis.

Measurements of leaf water relations: Leaf water potential (Ψ_w) was evaluated according to the petiole xylem-pressure potential measured by the pressure chamber (*PMS Instrument*s, Corvallis, OR, USA). Relative water content (RWC) was determined gravimetrically as described previously (Kim *et al*. 2004). The measurements of leaf water status were carried out before dawn on the first or second fully expanded green leaf proximal to a stolon apex.

Determination of $O_2^{\bullet-}$ and H_2O_2 concentration and lipid peroxidation: The detection of $O_2^{\bullet-}$ was made by hydroxylamine oxidation (Wang and Luo 1990). A mixture of 0.5 cm^3 enzyme extract and 1 cm^3 of the prepared hydroxylation was incubated at 25 °C for 1 h, then reacted with 1 cm^3 of 17 mM *p*-aminobenzene sulfonic acid and 7 mM α-naphthylamine solution at 25 °C for 20 min. The absorbance was determined at 530 nm with a UV-visible spectrophotometer (*Shimadzu UV-1601*, Kyoto, Japan). $O_2^{\bullet-}$ content was obtained using a linear calibration curve of $NaNO_2$. For H_2O_2 determination, about 200 mg of dry mass was homogenized with 3 cm^3 of 50 mM phosphate buffer (pH 6.8) and then centrifuged at 6 000 *g* for 25 min. The resulting extract was mixed with 1 cm^3 of 0.1 % titanium chloride in 20 % (v/v) H_2SO_4 and centrifuged at 6 000 *g* for 15 min. The absorbance was immediately read at 410 nm. H_2O_2 concentration was calculated using the coefficient of absorbance 0.28 μM^{-1} cm^{-1} (Lin and Kao 2001). The lipid peroxidation level was determined in terms of malondialdehyde (MDA) content by the method of Cakmak and Horst (1991), with minor modification. Fresh tissue (500 mg) was homogenized in 5 cm^3 of 0.1 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 *g* for 5 min and the supernatant obtained was added to 1.5 cm^3 0.5 % (m/v) thiobarbituric acid (TBA) in 20 % TCA. The mixture was heated at 95 °C for 30 min, and the reaction stopped by cooling the tube in an ice water bath. The samples were centrifuged at 10 000 *g* for 15 min and the absorbance of the supernatant was measured at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of MDA was calculated using the absorption coefficient of 155 mM^{-1} cm^{-1}.

Measurement of antioxidant enzyme activities: For extraction of enzymes, fresh samples (0.5 g) were homogenized with 1.5 cm^3 of 100 mM potassium phosphate buffer solution (pH 7.0) containing 2 mM phenylmethylsulfonyl fluoride (PMSF), and centrifuged at 14 000 *g* at 4 °C for 20 min. Protein concentration was determined using the method of Bradford (1976). The activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) (Giannopolities and Ries 1977). One unit of enzyme activity was defined as the amount of enzyme required to inhibit 50 % of the NBT photoreduction in comparison with tubes lacking the plant extract. Catalase (CAT) activity was assayed using the method of Mishra *et al*. (1993). The reaction mixture of 1 cm^3 contained 0.5 cm^3 of 100 mM potassium phosphate buffer (pH 7.0), 0.1 cm^3 of 110 mM H_2O_2 and enzyme extract. The decrease in absorbance at 240 nm was recorded as a result of H_2O_2 degradation (coefficient of absorbance, ε = 36 mM^{-1} cm^{-1}). POD activities were measured using different substrates: ascorbate and guaiacol. POD activity with ascorbate as hydrogen donor (ascorbate-peroxidase; APOD) was determined by measuring the decrease in absorbance at 290 nm (ε = 2.8 mM^{-1} cm^{-1}) according to Chen and Asada (1989). One unit of enzyme activity was defined as the amount of enzyme that causes the formation of 1 μM ascorbate oxidized per min. For guaiacol-peroxidase (GPOD) activity, the oxidation of guaiacol was estimated by measuring the increase in absorbance at 470 nm for 1 min and the activity was calculated using an absorption for tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}) (Lee and Lin 1995). One unit of enzyme activity was defined as the amount of enzyme that causes the formation of 1 μM tetraguaiacol per min.

Detection of antioxidant isoenzymes by PAGE: To determine changes in the SOD isozymes, the gel after native polyacrylamide gel electrophoresis (PAGE) (Beauchamp and Fridovich 1971) was soaked in 50 mM potassium phosphate (pH 7.8) containing 2.5 mM NBT

for 25 min at room temperature. Then the gel was rinsed in distilled water and then incubated in 50 mM potassium phosphate buffer (pH 7.8) containing 28 mM N,N,N',N'-tetramethylethylenediamine (TEMED) and 28 μM riboflavin for 30 min. The gel was laid under irradiance of 70 μmol m^{-2} s^{-1} for 50 min. The CAT isoenzymes were followed by gel incubation with 0.003 % H_2O_2 for 25 min, and a treatment with a solution containing 1 % $FeCl_3$ and 1 % $K_3Fe(CN)_6$ for 10 min, as described by Woodbury *et al*. (1971). For APOD isoenzymes, the gels were immersed in 50 mM potassium phosphate buffer (pH 7.0) containing 4 mM ascorbate and 2 mM H_2O_2 for 20 min. The gel was rinsed in distilled water and then incubated in 50 mM KPO_4 buffer (pH 7.8) containing 28 mM TEMED and 2.5 mM NBT for 15 min. Staining reaction was stopped with 7.5 % glacial acetic acid. The gels were then stored at 4 °C in 7.5 % acetic solution until photographic recording (Rao *et al*. 1996). For GPOD isoenzymes, the gels were soaked for 10 min in 50 mM Tris buffer (pH 7.4) then incubated with 0.46 % (v/v) guaiacol, and 13 mM H_2O_2 in the same buffer at room temperature until red bands appeared and subsequently fixed in water/methanol/acetic acid (6.5:2.5:1, v/v/v) (Caruso *et al.* 1999).

Statistical analysis: A completely randomized design was used with three replicates for two water levels and five sampling dates. Duncan's multiple range test or Student's *t*-test was used to compare the means of separate replicates. Regression analysis was used to determine the significance of relationships among the measured variables. Unless otherwise stated, conclusions are based on differences between means significant at $P \leq 0.05$.

Results

Leaf water status, dry mass, $O_2^{\bullet-}$, H_2O_2 and MDA content: The leaf water potential (Ψ_w) reached minimum value of -2.33 MPa after 28 d of water-deficit treatment, corresponding to a 30 % decrease in relative water content (RWC). No significant changes occurred in the control (Table 1).

Table 1. Changes in leaf water potential (Ψ_w) and relative water content (RWC) of white clover plants well-watered (C) or water-stressed (WS) for 28 d. Values are means of three replicates with three plants each. Values in a row followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

Parameters	Treatment	0 d	7 d	14 d	21 d	28 d
Ψ_w	C	0.43^a	0.49^a	0.51^a	0.46^a	0.50^a
[-MPa]	WS	0.46^a	1.44^b	1.67^c	2.27^d	2.33^d
RWC	C	91.50^a	90.72^a	90.33^a	91.11^a	90.17^a
[%]	WS	91.11^a	76.84^b	73.43^c	64.62^d	63.79^d

Leaf DM was not significantly affected by water deficit during the first 7 d, but decreased to 72.1 % of control at day 28 (Fig. 1*A*). Water deficit gradually increased $O_2^{\bullet-}$ content during the first 14 d and subsequently maintained this concentration until day 28 (Fig. 1*C*). A significant increase (+22 %) in H_2O_2 concentration occurred at day 14 (Fig. 1*B*). The MDA content was not significantly affected over the first 7 d but then continuously increased until day 28 (+44.6 %) (Fig. 1*D*).

Activities of antioxidant enzymes and their isoenzymes: Drought treatment significantly increased SOD activity throughout the experimental period. During the early 14 d, SOD activity in leaves increased to about 2-fold higher activity as compared to the control. This activity was then maintained until day 28 (Fig. 2*A*).

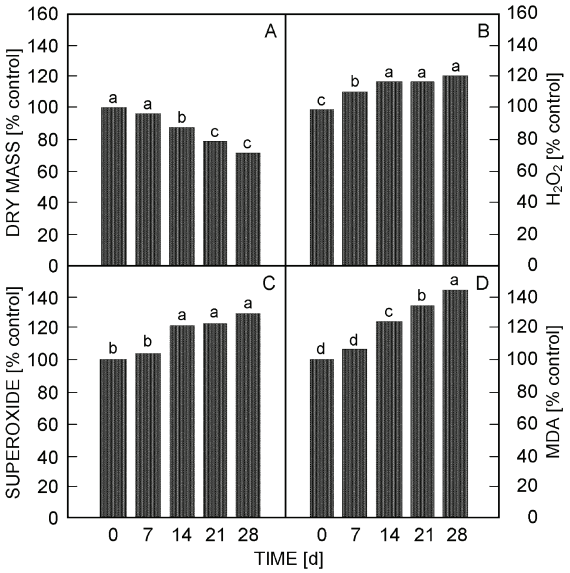

Fig. 1. Water-deficit effects on dry mass (*A*), H_2O_2 (*B*), $O_2^{\bullet-}$ (*C*) and MDA content (*D*) in white clover leaves during the 28 d experimental period. The experimental data obtained in water-stressed leaves were expressed as a percentage of those found in the well-watered (control) leaves. *Bars* labeled with different letters are significantly different ($P < 0.05$) according to Student's *t*-test.

Under water stress CAT activity also increased linearly during the early 14 d (Fig. 2*B*). The increase (+73 %) in APOD activity induced by water stress was much more rapid (occurring within the first 7 d) as compared to those of other enzymes but then the activity gradually decreased until day 28 d (Fig. 2*C*). GPOD activity was not significantly different between treat-ments for the first 7 d. However, subsequently GPOD activity in drought-stressed leaves increased resulting in 1.9-fold higher level compared to the control by day 28 (Fig. 2*D*).

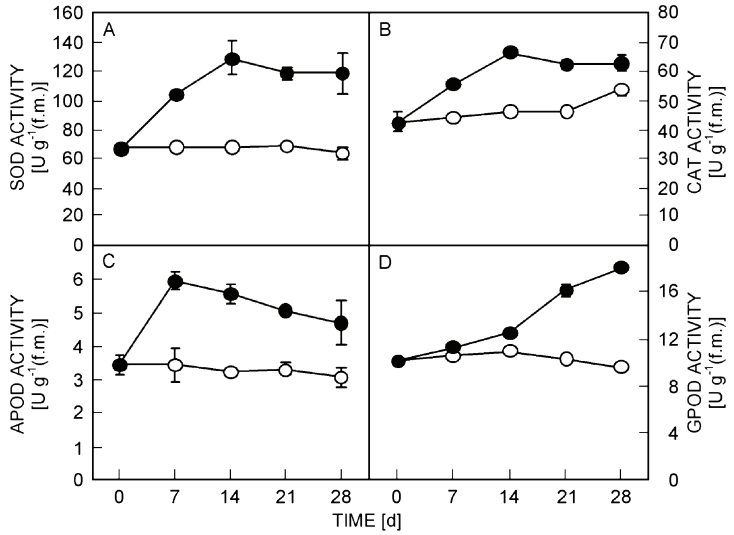

Fig. 2. Changes in the activities of SOD (*A*), CAT (*B*), APOD (*C*) and GPOD (*D*) in water-stressed (*closed circles*) and well-watered (*open circles*) leaves of white clover. *Vertical bars* indicate SE for $n = 3$.

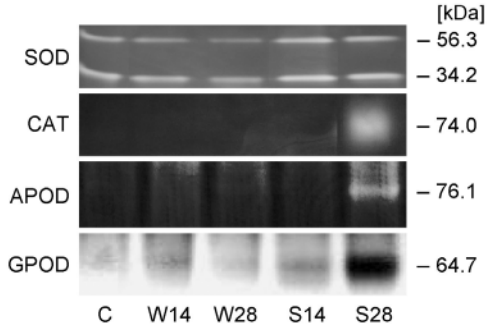

Fig. 3. Activity staining of SOD, CAT, APOD and GPOD in white clover leaves. Samples were taken at the day of treatment (control, d 0), 14 and 28 d after well-watered (W) or water-deficit (S) treatment.

Two SOD isozymes of molecular mass 56.3 and 34.2 kDa were commonly detected in both drought-stressed and control leaves. However, expression of these two isozymes was much stronger in drought-stressed as compared to control leaves (Fig. 3). The CAT isozyme of 74.0 kDa was only present in drought-stressed leaves after 28 d of treatment (Fig. 3). The APOD isozyme of 76.1 kDa was active in drought-stressed leaves after 28 d but not in control leaves (Fig. 3). The GPOD isozyme of 64.7 kDa was also active only in drought-stressed leaves (Fig. 3).

Table 2. Coefficient of linear correlations (r) between the activity of antioxidant enzymes and $O_2^{\bullet-}$, H_2O_2, MDA, or DM in two distinct phases of water stress development. Values are normalized to the difference (Δ) between the values measured at the water-stressed leaves and those of the control (n.s - not significant, * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$).

Period		Δ $O_2^{\bullet-}$	Δ H_2O_2	Δ MDA	Δ DM
0 - 14 d	Δ SOD	0.936^{***}	0.858^{**}	0.830^{**}	-0.818^{**}
	Δ CAT	0.912^{***}	0.882^{**}	0.899^{***}	$-0.642^{n.s}$
	Δ APOX	0.798^{**}	$0.554^{n.s}$	$0.500^{n.s}$	$-0.483^{n.s}$
	Δ GPOX	0.751^{*}	0.877^{**}	0.867^{**}	$-0.611^{n.s}$
14 - 28 d	Δ SOD	$0.230^{n.s}$	$0.228^{n.s}$	$0.421^{n.s}$	$-0.270^{n.s}$
	Δ CAT	0.702^{*}	0.784^{*}	0.861^{**}	-0.951^{***}
	Δ APOX	$0.331^{n.s}$	$0.400^{n.s}$	$0.444^{n.s}$	$-0.365^{n.s}$
	Δ GPOX	$0.591^{n.s}$	0.740^{*}	0.868^{**}	-0.922^{***}

Discussion

Drought-stress was induced in white clover leaves during this experiment, as Ψ_w fell to a minimum value of -2.33 MPa and RWC decreased to 70 % at day 28 (Table 1). Significant decreases in DM occurred following 14 d of drought treatment ($\Psi_w \leq$ -1.67 MPa) which were consistent with inhibition of growth (Foyer *et al*. 1998, Costa França *et al*. 2000). The significant decline of Ψ_w (Table 1) may also result in stomatal closure, causing a reduction in CO_2/O_2 ratio in leaves and CO_2 fixation. This, in turn, leads to NADPH accumulation and subsequent leakage of electrons leading to enhance generation of ROS (Foyer and Noctor 2003). Indeed, significant ($P \leq 0.05$) increases in $O_2^{\bullet-}$ and H_2O_2 caused by water deficit were apparent (Fig. 1). However, $O_2^{\bullet-}$ responded much more rapidly than did H_2O_2. This result suggests that $O_2^{\bullet-}$ could be a more sensitive indicator of ROS accumulation caused by water stress, because $O_2^{\bullet-}$ is a precursor of the formation of other ROS and it readily responds to stress factors (Selote *et al*. 2004).

In this study, the quadratic relationships between Ψ_w and the activity of antioxidative enzymes examined (SOD, CAT, APOD and GPOD) were highly significant. The activities of SOD, CAT and APOD increased to their maximum during the first 14 d when the Ψ_w was above -1.67 MPa. In particular, the increase in SOD activity, which catalyses the dismutation of superoxide radicals to H_2O_2 and O_2 (Foyer and Noctor 2000), was high during this period. A high activation of CAT, also apparent during the first 14 d following water deficit treatment, suggests that the H_2O_2 produced by SOD, would in turn be scavenged mostly by CAT. In this study, the activation of APOD was highest during the first 7 d but the absolute level of increase was lower than that of the other enzymes examined. These results are consistent with those of Foyer and Noctor (2003) who reported that CAT removed the bulk of H_2O_2, whereas ascorbate peroxidase (APOD) could scavenge H_2O_2 that was inaccessible for CAT because APOD has a higher affinity for H_2O_2. The activation of GPOD was relatively low until day 14. Thus GPOD contrasts with APOD in that APOD was active throughout the stress period in an antioxidant role by reducing H_2O_2 to water using ascorbate as reducing agent in the ascorbate-glutathione cycle (Asada 1992, Foyer *et al*. 1997). Thus the difference in activation of APOD and GPOD, following water-deficit treatment, might indicate a physiologically different response as oxidative stress develops. In addition, for the early period of treatment (day 0 - 14), the activity of antioxidant enzymes was significantly and positively correlated with $O_2^{\bullet-}$, H_2O_2, or MDA content (except for ΔAPOD activity with ΔH_2O_2 or ΔMDA) (Table 2). The data from the first 14 d suggest that a rise of SOD, CAT and APOD activity is a stress induced ROS scavenging mechanism preserving membrane integrity and leaf growth.

From day 14, the increased contents of $O_2^{\bullet-}$ (+17 %) and H_2O_2 (+22 %) induced by water deficit were maintained until day 28. The increase in ROS content was accompanied with a significant decrease in DM and a gradual increase in MDA content during this period. Thus the antioxidant system in white clover leaves was not sufficiently effective in scavenging the $O_2^{\bullet-}$ and H_2O_2. This was reflected in the weaker correlations between Δ activity of enzymes and ΔROS content for the later sampling period (not significant in most cases) than those for the earlier 14 d period (Table 2). A strong activation, after 14 d of water-deficit treatment, was apparent only for GPOD activity. The increased GPOD activity for this period was closely associated with a decrease in DM, as evidenced by a negative correlation (r = -0.922, $P \leq 0.001$; Table 2). This increased GPOD activity might be involved in the cell wall lignification, rather than in protection of plant tissues against the oxidative damage. Silva *et al*. (2004) reported that the high POD activities in pathogen-infected tomato plants were usually found at a later stage of infection and was linked to lignification. A similar role for POD has been proposed in rice seedling (Lin and Kao 2002) and cowpea leaves (Cavalcanti *et al*. 2004) subjected to salt and osmotic stress.

Taken together, the data obtained from this study indicate that the responses of some physiological parameters symptomatic for oxidative stress and the related enzymes strongly depend on the severity of water-stress. In addition, kinetic analyses performed distinguish two distinct phases of water stress development: *1*) day 0 - 14 ($\Psi_w \geq$ - 1.65 MPa) is characterized by high activations of SOD, CAT and APOD; *2*) day 14 - 28 ($\Psi_w \leq$ - 1.65 MPa), characterized by gradual increase in lipid peroxidation and growth restriction. Further work is required to elucidate the roles of SOD, CAT, and POD during recovery from water stress.

References

Asada, K.: Ascorbate peroxidase - a hydrogen peroxide scavenging enzyme in plant. - Physiol. Plant. **85**: 235-241, 1992.

Beauchamp, C., Fridovich, I.: Superoxide dismutase: improved assays and assay applicable to acrylamide gels. - Anal. Biochem. **44**: 276-287, 1971.

Bradford, M.M.: A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of proteins-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Cakmak, I., Horst, J.H.: Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). - Physiol. Plant. **83**: 463-468, 1991.

Caruso, C., Chilosi, G., Caporale, C., Leonardi, L., Bertini, L., Magro, P., Buonocore, V.: Induction of pathogenesis-related proteins in germinating wheat seeds infected with *Fusarium culmorum*. - Plant. Sci. **140**:107-120, 1999.

Cavalcanti, F.R., Oliveira, J.T.A., Martins-Miranda, A.S., Viégas, R.A., Silvira, J.A.G.: Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. - New Phytol. **163**: 563-571, 2004.

Chazen, O, Neumann, P.M.: Hydraulic signals from roots and rapid cell-wall hardening in growing maize (*Zea mays* L.) leaves are primary responses to polyethylene glycol-induced water deficits. - Plant Physiol. **104**: 1385-1392, 1994.

Chen, G., Asada, K.: Ascorbate peroxidase in tea leaves: Occurrence of two isozymes and the differences in their enzymatic and molecular properties. - Plant Cell Physiol. **30**: 987-998, 1989.

Chen, Y.A., Shin, J.W., Liu, Z.H.: Effect of light on peroxidase and lignin synthesis in mungbean hypocotyls. - Plant Physiol. Biochem. **40**: 33-39, 2002.

Costa França, M.G., Thi, A.T.P., Pimntel, C., Pereyra Rossiello, R.O., Zuily-Fodil, Y., Laffray, D.: Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. - Environ. exp. Bot. **43**: 227-237, 2000.

Foyer, C.H., Lopez-Delgado, H., Dat, J.F., Scott, I.M.: Hydrogen peroxide- and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. - Physiol. Plant. **100**: 241-254, 1997.

Foyer, C.H., Noctor, G.: Oxygen processing in photosynthesis: regulation and signaling. - New Phytol. **146**: 359-388, 2000.

Foyer, C.H., Noctor, G.: Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. - Physiol. Plant. **119**: 355-364, 2003.

Foyer, C.H., Valadier, M.H., Andre, M., Becker, T.W.: Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. - Plant Physiol. **117**: 283-292, 1998.

Giannopolities, C.H., Ries, S.K.: Superoxide dismutase. I. Occurrence in higher plant. - Plant Physiol. **59**: 309-314, 1977.

Jiang, Y., Huang, B.: Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. - Crop Sci. **41**: 436-442, 2001.

Jung, S.: Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. - Plant Sci. **166**: 459-466, 2004.

Kim, T.H., Lee, B.R., Jung, W.J., Kim, K.Y., Avice, J.C., Ourry, A.: *De novo* protein synthesis in relation to ammonia and proline accumulation in water stressed white clover. - Funct. Plant Biol. **31**: 847-855, 2004.

Lee, T.M., Lin, Y.H.: Changes in soluble and cell wall-bound peroxidase activities with growth in anoxia-treated rice (*Oryza sativa* L.) coleoptiles and roots. - Plant Sci. **106**: 1-7, 1995.

Lin, C.C., Kao, C.H.: Cell wall peroxidase activity, hydrogen peroxidase level and NaCl-inhibited root growth of rice seedling. - Plant Soil **230**: 135-143, 2001.

Lin, C.L., Kao, C.H.: Osmotic stress-induced changes in cell wall peroxidase activity and hydrogen peroxide level in roots of rice seedlings. - Plant Growth Regul, **37**: 177-184, 2002.

Mishra, N.P., Mishra, R.K., Singhal, G.S.: Changes in the activities of antioxidant enzymes during exposure of intact wheat leaves of strong visible light at different temperature in the presence of protein synthesis inhibitors. - Plant Physiol. **102**: 903-910, 1993.

Peltzer, D., Dreyer, E., Polle, A.: Differential temperature dependencies of antioxidative enzymes in two contrasting species: *Fagus sylvatica* and *Coleus blumei*. - Plant Physiol. Biochem. **40**: 141-150, 2002.

Rao, M.V., Paliyath, G., Ormrod, D.P.: Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. - Plant Physiol. **110**: 125-136, 1996.

Selote, D.S., Bharti, S., Khanna-Chopra, R.: Drought acclimation reduces $O_2^{\bullet -}$ accumulation and lipid peroxidation in wheat seedlings. - Biochem. biophys. Res. Commun. **314**: 724-729, 2004.

Silva, H.A.S., Romeiro, R.S., Macagnan, D., Halfeld-Vieira, B.A., Pereira, M.C.B., Mounteer, A.: Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection a decrease in enzyme activities. - Biol. Control **29**: 288-295, 2004.

Sivritepe, N., Erturk, U., Yerlikaya, C., Turkan, I., Bor, M., Ozdemir, F.: Response of cherry rootstock to water stress induced *in vitro*. - Biol. Plant. **52**: 573-576, 2008.

Smirnoff, N.: The role of active oxygen in the response of plants to water deficit and desiccation. - New Phytol. **125**: 27-58, 1993.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R., Thomas, G.: Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) - differential response in salt-tolerant and sensitive varieties. - Plant Sci. **165**: 1411-1418, 2003.

Wang, A.G., Luo, G.H.: Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. - Plant Physiol. Commun. **6**: 55-57, 1990.

Woodbury, W., Spencer, A.K., Stahmann, M.A.: Improved procedure using ferricyanide for detecting catalase isozymes. - Anal. Biochem. **44**: 301-305, 1971.