ACTA BIOLOGICA CRACOVIENSIA Series Botanica 48/2: 89–96, 2006

DROUGHT STRESS TOLERANCE AND THE ANTIOXIDANT ENZYME SYSTEM IN *CTENANTHE SETOSA*

RABIYE TERZI* AND ASIM KADIOGLU

Department of Biology, Karadeniz Technical University, 61080 Trabzon, Turkey

Received June 7, 2006; revision accepted September 10, 2006

We studied the relationship of the antioxidant enzyme system to drought stress tolerance during leaf rolling in the leaf, petiole and root of *Ctenanthe setosa* (Rosc.) Eichler. Chlorophyll and carotenoid content and the chlorophyll stability index decreased in the early period of drought stress but increased in later periods, approaching the control level as leaf rolling increased. Relative water content decreased, while the root:shoot ratio increased during drought stress. Lipid peroxidation also increased and then declined in the same drought period, contrary to photosynthetic pigment content. Superoxide dismutase (SOD) activity did not significantly change in leaves. In the petiole and root, however, it decreased in the early drought period but increased later. Glutathione reductase (GR) activity did not significantly change in the leaf and petiole versus the control, but increased in root. Peroxidase (POD) activity increased in the leaf and petiole but decreased in the root. A peroxidase isoenzyme activity band present in the control leaves did not appear in leaves exposed to 32 days of drought, but in the later periods that activity increased. Tolerance of drought stress apparently is closely associated with the antioxidant enzyme system as well as leaf rolling in *C. setosa*.

Key words: *Ctenanthe setosa*, chlorophyll, lipid peroxidation, superoxide dismutase, glutathione reductase, peroxidase, isoenzyme.

INTRODUCTION

Plants respond and adapt to environmental abiotic stresses. Leaf rolling is one of the most familiar responses to drought stress (Begg, 1980); it increases resistance to stress in some plants (Townley-Smith et al., 1979). Drought is the most severe abiotic stress factor limiting plant growth and crop production. When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) are produced (Li and Staden, 1998). These ROSs may initiate destructive oxidative processes such as lipid peroxidation, chlorophyll bleaching, protein oxidation, and damage to nucleic acids (Scandalios, 1993). However, antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), catalase and peroxidase, and low-molecular antioxidants such as ascorbic acid, glutathione, α-tocopherol, flavonoids and carotenoids play a key role in scavenging those activated species (Sgherri et al., 2000). Modulation of the activity of these enzymes may be an important factor in the tolerance of various plants to environmental stress (Rensburg and Kruger, 1994). The relation between drought stress and enzymatic antioxidant systems has been studied in some plant species (Sairam et al., 2001). It has been reported that glutathione reductase helps in the resistance of plants to desiccation or drought (Gamble and Burke, 1984).

Researchers have also linked various physiological responses of plants to drought with their tolerance mechanisms, such as root:shoot dry weight ratio (Huang and Fry, 1998), pigment content and stability, and high relative water content (Clarke and McCaig, 1982). When water availability is limited, the root:shoot ratio increases because roots are less sensitive than shoots to growth inhibition by low water potentials (Wu and Cosgrove, 2000). Drought can also lead to pigment degradation (Hendry et al., 1987), thus causing irreversible water-deficit damage to the photosynthetic apparatus (Clarke et al., 1996). The chlorophyll stability index is an indicator of the stress tolerance capacity of plants (Koleyoreas, 1958).

*e-mail: rabiyeterzi@yahoo.com.tr

Abbreviations: CSI – Chlorophyll stability index; EDTA – ethylenediaminetetraacetic acid; GR – glutathione reductase; MDA – malondialdehyde; NBT – nitro blue tetrazolium; POD – peroxidase; PVPP – polyvinylpolypyrrolidone; ROS – reactive oxygen species; RWC – relative water content; SOD – superoxide dismutase.

PL ISSN 0001-5296

Ctenanthe setosa is one plant that shows a leaf rolling response to drought stress (Turgut and Kadioglu, 1998). Recent years have seen some studies on resistance to drought stress during leaf rolling in *C. setosa* (Kadioglu and Turgut, 1999; Ayaz et al., 2000; Kadioglu et al., 2002) This species has been taken as a model plant for examination of leaf rolling, one of the mechanisms of resistance to drought stress, but there is no available information about the antioxidant enzyme system during leaf rolling in this plant during extended drought.

This study investigates the relationship between tolerance to drought stress and the antioxidant system during leaf rolling in *Ctenanthe setosa*.

MATERIALS AND METHODS

Ctenanthe setosa (Rosc.) Eichler (Marantaceae) was vegetatively propagated and grown in plastic pots (16 cm high, 18 cm top and 12 cm bottom diameter) containing soil and sand (5:1) in a growth chamber under the following treatment conditions: 16 h day at 25°C, 8 h night at 21°C, relative humidity 70%, and photon flux density 400 μmol $s^{-1}m^{-2}$ at leaf surface. Some plants were watered well (control), and the other plants were subjected to drought stress by withholding water for 32, 40, 48, 56 and 64 days. Leaves, petioles and roots were sampled up to day 64 after initiation of drought, by which time the leaf lamina of the plant was extremely rolled, and all data were compared with those of the control plants, which never developed rolled leaves. The degree of leaf rolling was determined according to Premachandra et al. (1993). The width of the mid-portion of the leaves was measured and the degree of leaf rolling was calculated as the percentage reduction in leaf width by rolling.

CHLOROPHYLL AND CAROTENOID CONTENT, CHLOROPHYLL STABILITY INDEX

Leaf samples were selected randomly from the plants and homogenized in a mortar in acetone. The extract was centrifuged at 5000 g for 5 min. Absorbance of the supernatant was recorded at 663, 645 and 450 nm spectrophotometrically (Techcomp 8500 II, South Korea). Chlorophyll (Chl) content was determined following the method of Arnon (1949). The chlorophyll stability index (CSI) was determined according to Sairam et al. (1997) and calculated as follows:

$$CSI = (\text{Total Chl under stress}/\text{Total Chl under control}) \times 100$$

RELATIVE WATER CONTENT

Relative water content (RWC) was estimated according to the method of Castillo (1996) and calculated in the leaves for each drought period. Samples (0.5 g) were saturated in 100 ml distilled water for 24 h at 4°C in the dark and their turgid weights were recorded. Then they were oven-dried at 65°C for 48 h and their dry weights were recorded. RWC was calculated as follows:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100,$$

where FW, DW, and TW are fresh weight, dry weight and turgid weight, respectively.

ROOT:SHOOT RATIO

Shoots were weighed as soon as possible after their excision. Roots were removed carefully, washed with tap water and blotted with paper towels before weighing. Dry weights of roots and shoots were determined after drying in a forced draft oven at 70–80°C for 48 h.

LIPID PEROXIDATION

Lipid peroxidation was measured in terms of content of malondialdehyde (MDA, $\varepsilon = 155\ mmol^{-1}\ cm^{-1}$), a product of lipid peroxidation, following the method of Heath and Packer (1968). Leaf, petiole and root samples (0.5 g) were homogenized in 10 ml 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 g for 5 min. To 1 ml aliquot of supernatant, 4 ml 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. MDA content was expressed as nmol MDA per g fresh weight.

ANTIOXIDANT ENZYME ACTIVITY

The superoxide dismutase activity assay was based on the method of Beauchamp and Fridovich (1971) as modified by Dhindsa and Matowe (1981). Samples (0.5 g) were homogenized in 5 ml cold 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.05% (v/v) triton, 2% (w/v) polyvinylpolypyrrolidone (PVPP) and 1 mM ascorbic acid. In the spectrophotometric assay, 1 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionin, 75 μM NBT, 2 μM riboflavin and 50 μl plant extract. Riboflavin was added last and the reaction was initiated by placing the tubes under white fluorescent light. The reaction was terminated after 10 min by removal from the

TABLE 1. Changes in chlorophyll (Chl) and carotenoid content, chlorophyll stability index, relative water content and root/shoot ratio in *Ctenanthe setosa* in response to drought stress. Means ± SD of three replicates

Drought period (day)	Rolling degree (%)	Chl content (mg g^{-1} fresh weight)	Carotenoid content	Chl stability index (%)	Relative water content (%)		Root : Shoot ratio
					Leaf	Petiole	
0 (Control)	0	4.1 ± 0.3	1.5 ± 0.3	–	94 ± 2	94 ± 2	0.83 ± 0.03
32	19	3.8 ± 0.1	1.3 ± 0.05	84.72 ± 5.5	89 ± 0.5	92 ± 2	0.78 ± 0.02
40	36	3.2 ± 0.2	1.1 ± 0.1	71.36 ± 6.4	87 ± 1	84 ± 4	1.16 ± 0.25
48	55	3.5 ± 0.1	1.0 ± 0.2	77.27 ± 4.9	83 ± 2	72 ± 3	1.60 ± 0.04
56	72	3.5 ± 0.3	1.2 ± 0.1	78.49 ± 0.6	79 ± 1	69 ± 3	1.84 ± 0.11
64	79	4.1 ± 0.4	1.4 ± 0.1	91.24 ± 4.1	78 ± 2	67 ± 4.5	2.67 ± 0.19

light source. The reaction product was measured at 560 nm. The volume of supernatant corresponding to 50% inhibition of the reaction was assigned a value of 1 enzyme unit.

The glutathione reductase activity assay was based on the method of Foyer and Halliwell (1976). The extraction solution for GR was the same as that used for SOD. Activity was determined by monitoring the oxidation of NADPH at 340 nm in 1 ml of solution that contained 50 mM Tris-HCl, pH 7.8, 0.5 mM EDTA, 0.25 mM NADPH, 1 mM oxidized glutathione, and 50 µl of the enzyme extract.

For peroxidase activity, the leaf, petiole and roots were homogenized on ice in 10 ml cold sodium phosphate buffer (pH 7.0). Activity was determined spectrophotometrically according to Rodriguez and Sanchez (1982). POD activity was analyzed in 50 mM phosphate buffer (pH 6.5) containing 40 mM guaiacol and 26 mM H_2O_2. The increase of absorbance at 420 nm was recorded within 180 sec after adding 26 mM H_2O_2. Protein content was determined according to Bradford (1976). Bovine serum albumin was used as the standard.

ELECTROPHORESIS FOR PEROXIDASE ISOENZYMES

Polyacrylamide gel electrophoresis was conducted as described by Liu (1973). A separating gel of 8% acrylamide was used. Enzyme extract in 50% glycerol with 1% bromophenol blue was applied to the gel. Electrophoresis was performed in a cold room at 4°C using reservoir buffer (14.4 g glycine and 3 g Tris per liter, pH 8.3) at 10 mA. For analysis of peroxidase isoenzymes, the gel was incubated for 1 h at 35°C in solution containing 0.1 g benzidine/100 ml 0.2 M sodium acetate buffer (pH 5.0) and 2.5 ml 3% H_2O_2/100 ml benzidine solution.

STATISTICAL ANALYSIS

ANOVA of the means of three replicates was performed with the Duncan Multiple Comparison test, and significance was determined at $p < 0.05$.

RESULTS

CHLOROPHYLL AND CAROTENOID CONTENT, CHLOROPHYLL STABILITY INDEX, RELATIVE WATER CONTENT AND ROOT:SHOOT RATIO

Leaf rolling began on day 32 of the drought period, and the degree of leaf rolling gradually increased in *C. setosa* as the drought period increased. Leaf pigment content was also affected by drought stress. Chlorophyll and carotenoid content and the chlorophyll stability index decreased up to day 40 of drought and increased later, approximately reaching the control level on day 64 (Tab. 1). Relative water content decreased in the leaf and petiole during the drought period. On day 64, relative water content was 94% in the control leaves and 78% in leaves of the treated plants. RWC decreased more in the petiole than in the leaves. Unlike RWC, the root:shoot ratio increased during drought, reaching 0.83 in the control plants and 2.67 in treated plants by the end of the experiment (Tab. 1).

LIPID PEROXIDATION

As seen in Figure 1, lipid peroxidation, measured as MDA content, increased up to day 40 of drought in the leaf and petiole but decreased later. For example, MDA content was 30.8 nmol g f.w. in control leaves, 46.5 nmol g f.w. in 40-day-drought plants and 25.1 nmol g f.w. in 64-day-drought plants. In the root, however, lipid peroxidation increased up to day 48 of drought (Fig. 1).

ENZYME ACTIVITY

The activity of superoxide dismutase did not significantly change in drought-stressed leaves versus control leaves. SOD activity significantly decreased on day 32 of drought in the petiole and root but increased later, approximately reaching the control level. The increases started on day 56 in petiole and day 40 in root (Fig. 2).

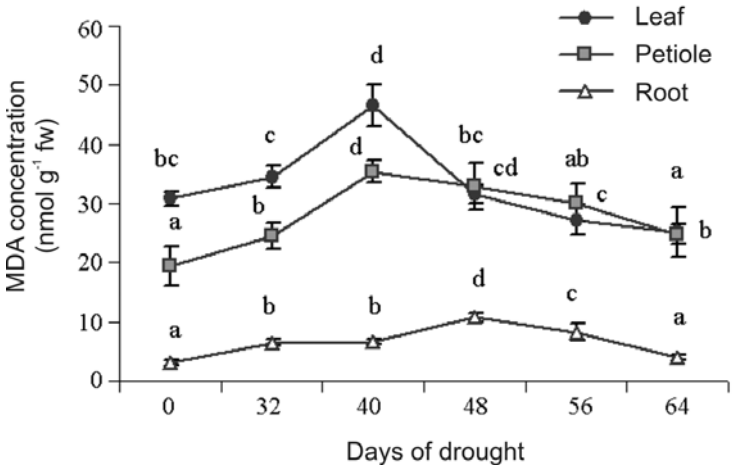

Fig. 1. Effects of drought stress on lipid peroxidation (malondialdehyde content) in *Ctenanthe setosa*. Different letters indicate significant differences between means of three replicates ($p < 0.05$).

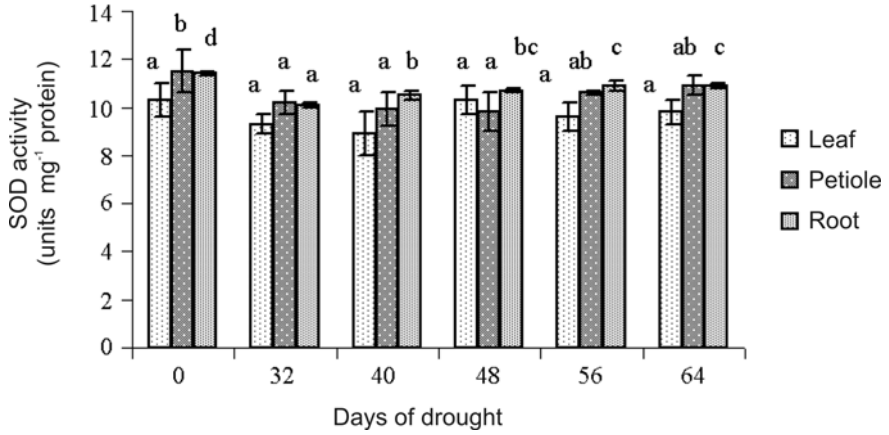

Fig. 2. Effects of drought stress on activity of superoxide dismutase in *Ctenanthe setosa*. Different letters indicate significant differences between means of three replicates ($p < 0.05$).

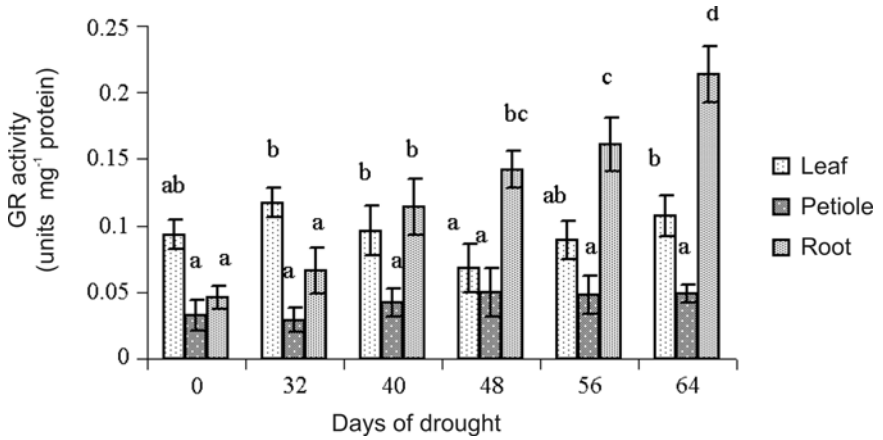

Fig. 3. Effects of drought stress on activity of glutathione reductase in *Ctenanthe setosa*. Different letters indicate significant differences between means of three replicates ($p < 0.05$).

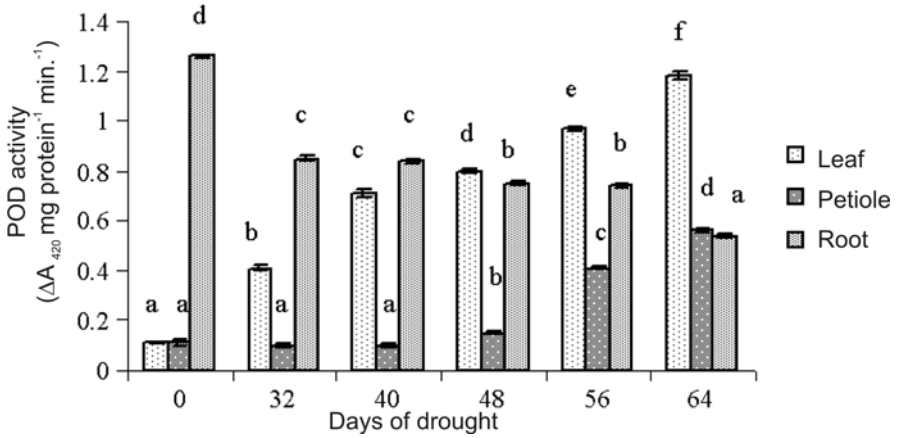

Fig. 4. Effects of drought stress on activity of peroxidase in *Ctenanthe setosa*. Different letters indicate significant differences between means of three replicates ($p < 0.05$).

Most changes in glutathione reductase activity in leaf and petiole versus the control were not statistically significant. It was 0.093 U per mg protein in control leaves and 0.117 U per mg protein in 32-day-drought leaves; GR activity decreased on day 48 of drought, but not significantly. In root, GR activity increased on day 32 of drought, again not significantly. Later increases of GR activity in root were statistically significant (Fig. 3).

Peroxidase activity changed significantly as drought progressed. POD activity strongly increased in leaf and petiole but decreased in root (Fig. 4).

PEROXIDASE ISOENZYMES

We determined the changes in the number and activity of peroxidase isoenzymes in leaves of *C. setosa* subjected to drought stress. No new isoenzyme band appeared for drought-stressed leaves. The activity of the peroxidase isoenzyme represented by the band at Rf 0.05 did not change over the drought period. There was no significant difference in the activity of the Rf 0.17 band isoenzyme between the control and 32-day-drought leaves, but later the activity of this isoenzyme increased under drought stress. Another isoenzyme band at Rf 0.20 was present for control leaves; it was not observed for 32-day-drought leaves, but appeared later (Fig. 5).

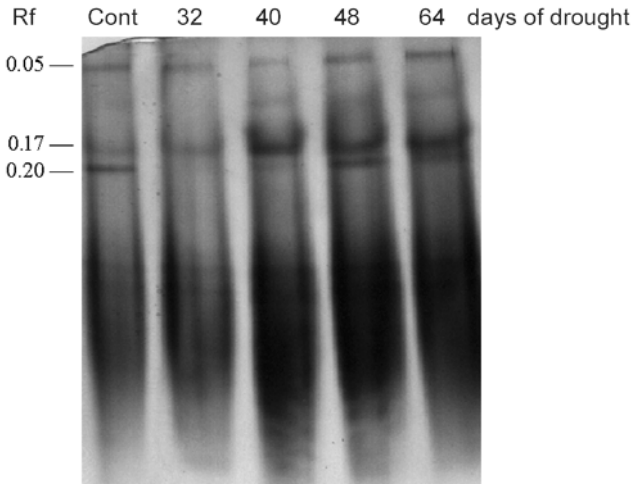

Fig. 5. Effect of drought stress on peroxidase isoenzyme pattern in leaves of *Ctenanthe setosa*. Each well was loaded with extract containing 20 μg protein.

DISCUSSION

Drought stress caused significant declines in chlorophyll and carotenoid content and in the chlorophyll stability index at early intervals of drought stress. Decreased or unchanged Chl level during drought stress has been reported in other species, depending on the duration and severity of drought (Kyparissis et al., 1995). Higher Chl and Car content have also been associated with the stress tolerance of plants (Pastori and Trippi, 1992; Sairam, 1994; Kraus et al., 1995). In the present study, the decrease of pigment content may have resulted from the decrease of RWC. The later increases may be attributable to higher moisture at the inner surface as a result of leaf rolling. The creation of a microclimate with higher humidity near leaf surfaces by leaf rolling may help protect scarce water resources, boosting resistance to stress (Matthews et al., 1990).

Most research has shown decreased RWC in response to drought stress (e.g., Fu and Huang, 2001; Shaw et al., 2002). In the present study, RWC declined as the drought period increased but the decrease of RWC was not higher despite the longer

drought period. Higher RWC has been reported to play a role in the stress tolerance of maize (Pastori and Trippi, 1992) and wheat genotypes (Kraus et al., 1995), and to be a good indicator of drought stress tolerance (Shaw et al., 2002). *C. setosa* decreases transpiration and water loss by leaf rolling, inhibiting the decrease in RWC.

On the other hand, the root:shoot ratio increased during drought stress in our experiments. It has been reported that the root:shoot dry matter ratio increases when drought stress develops (Huang and Fry, 1998; Wu and Cosgrove, 2000). Matthews et al. (1990) reported that resistant sorghum lines were able to maintain higher water status by increasing their root:shoot ratio as drought stress intensified.

MDA accumulation is often used as an indicator of lipid peroxidation (Smirnoff, 1995). In the present study, MDA content increased in the early period of drought but then decreased. Similarly, Sairam et al. (1997/1998) reported a general increase in lipid peroxidation and a decrease in the content of total chlorophyll and carotenoids. Increased MDA accumulation has been correlated with reduction of RWC and photosynthetic pigment content under prolonged drought (Jiang and Huang, 2001).

SOD, GR and POD play a protective role in scavenging ROS (Lin and Kao, 1998). In the present study, SOD activity did not significantly change in leaves. One mode of ROS formation is closure of stomata as a result of drought and a consequent decrease in CO_2 concentration in leaf mesophyll tissue, resulting in accumulation of NADPH; oxygen acts as an alternative acceptor of electrons, leading to the formation of superoxide radicals (Cadenas, 1989). Stomata were found to be open in rolled leaves of *C. setosa* (Turgut and Kadioglu, 1998). In the present study, then, one reason for the unchanged SOD activity may be that the stomata were open during drought stress, lowering the level of ROS formation. No significant changes in SOD activity were found in *Triticum aestivum* (Bartoli et al., 1999) and *Lotus corniculatus* leaves (Borsani et al., 2001) exposed to drought stress. SOD activity increased in *Hordeum*, *Armeria* and *Deschampsia* (Smirnoff, 1993), decreased in *Corchorus* (Chowdury and Choudhuri, 1985) and did not change in alfalfa (Irigoyen et al., 1992) and sunflower (Smirnoff, 1993) in response to water stress. Thus there is no single, clear finding in the literature. The level of response to drought depends on the species, the developmental and metabolic state of the plant, and the duration and intensity of the stress (Smirnoff, 1993; Castillo, 1996).

GR activity did not significantly change in drought-stressed leaf and petiole versus the control. Unlike SOD and GR activity, POD activity increased during drought stress in leaf and petiole. A peroxidase isoenzyme activity band at Rf 0.20 present for control leaves was not present for 32-day-drought leaves, but appeared later. The activity of an isoenzyme represented by the Rf 0.17 band increased versus the control during the drought period. Increasing POD activity may contribute to drought stress tolerance in this plant. Higher POD activity has also been correlated with the relative drought tolerance of crop plants (Gillham and Dodge, 1987). In root, however, POD activity diminished but GR activity increased. GR and POD are involved in scavenging the products of oxidative stress such as H_2O_2 and thus help in ameliorating the adverse effects of oxidative damage (Sairam et al., 1997/1998). Tanaka et al. (1990) reported results similar to ours: increased POD activity and little change in SOD activity in water-stressed spinach leaf.

We found *Ctenanthe setosa* to be tolerant to the administered drought stress, protecting itself from oxidative damage by increasing POD activity in leaves and GR activity in root.

ACKNOWLEDGMENTS

We thank the Research Fund of Karadeniz Technical University for its support (2001.111.004.5).

REFERENCES

Arnon DI. 1949. Copper enzymes in isolated chloroplasts, polyphenoloxidases in *Beta vulgaris*. *Plant Physiology* 24: 1–15.

Ayaz FA, Kadioglu A, and Turgut R. 2000. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rosc.) Eichler (Marantaceae). *Canadian Journal of Plant Science* 80: 373–378.

Bartoli CG, Simontacchi M, Tambussi E, and Beltrano J. 1999. Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. *Journal of Experimental Botany* 50: 375–383.

Beauchamp C, and Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44: 276–287.

Begg JE. 1980. Morphological adaptation of leaves to water stress. In: Turner NC, and Kramer PJ [eds.], *Adaptation of plants to water and high temperature stress*, 33–42. John Wiley & Sons, New York.

Borsani O, Diaz P, Agius MF, Valpuesta V, and Monza J. 2001. Water stress generates an oxidative stress through the induction of a specific Cu/Zn superoxide dismutase in *Lotus corniculatus* leaves. *Plant Science* 161: 757–763.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.

CADENAS E. 1989. Biochemistry of oxygen toxicity. *Annual Review of Biochemistry* 58: 79–110.

CASTILLO FJ. 1996. Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. *Oecologia* 107: 469–477.

CHOWDURY SR, and CHOUDHURI MA. 1985. Hydrogen peroxide metabolism as an index of water stress tolerance in jute. *Physiologia Plantarum* 65: 503–507

CLARKE JM, and MCCAIG TN. 1982. Evaluation of techniques for screening for drought resistance in wheat. *Crop Science* 22: 503–506.

CLARKE NA, HETSCHKUN HM, and THOMAS TH. 1996. Stress mechanisms in sugar beet. In: Fenwick GR, Hedley C, Richards RL, and Khokhar S [eds.], *Agric food quality, an interdisciplinary approach*, 75–78. The Royal Society of Chemistry, Cambridge.

DHINDSA RS, and MATOWE W. 1981. Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. *Journal of Experimental Botany* 32: 79–91.

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

FU J, and HUANG B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany* 45: 105–114.

GAMBLE PE, and BURKE JJ. 1984. Effect of water stress on the chloroplast antioxidant system I. Alterations in glutathione reductase activity. *Plant Physiology* 76: 615–621.

GILLHAM DJ, and DODGE AD. 1987. Chloroplast superoxide and hydrogen peroxide scavenging systems from pea leaves: Seasonal variations. *Plant Science* 50: 105–109.

HEATH RL, and PACKER L. 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125: 189–198.

HENDRY G, HOUGHTON JD, and BROWN SB. 1987. The degradation of chlorophyll a biological enigma. *New Phytologist* 107: 255–302.

HUANG B, and FRY JD. 1998. Root anatomical, physiological, and morphological responses to drought stress for tall fescue cultivars. *Crop Science* 38: 1017–1022.

IRIGOYEN JJ, EMERICH DW, and SANCHEZ-DIAZ M. 1992. Alfalfa leaf senescence induced by drought stress: photosynthesis, hydrogen peroxide metabolism, lipid peroxidation and ethylene evolution. *Physiologia Plantarum* 84: 67–72.

JIANG Y, and HUANG B. 2001. Drought and heat injury to two cool-season turf grasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Science* 41: 436–442.

KADIOGLU A, and TURGUT R. 1999. Some biochemical changes during leaf rolling in *Ctenanthe setosa* (*Marantaceae*). *Acta Physiologiae Plantarum* 21: 209–214.

KADIOGLU A, TURGUT R, PALAVAN-UNSAL N, and SARUHAN N. 2002. Effect of polyamines on leaf rolling in *Ctenanthe setosa*. *Israel Journal of Plant Science* 50: 19–23.

KOLEYOREAS SA. 1958. A new method for determining drought resistance. *Plant Physiology* 33: 22.

KRAUS TE, MCKERSIE BD, and FLETCHER RA. 1995. Paclobutrazole induced tolerance of wheat leaves to paraquat may involve antioxidant enzyme activity. *Journal of Plant Physiology* 145: 570–576.

PYOARISSIS A, PETROPOULOU Y and MANETAS Y. 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. *Journal of Experimental Botany* 46: 1825–1831.

LI L, and STADEN JV. 1998. Effects of plant growth regulators on the antioxidant system in callus of two maize cultivars subjected to water stress. *Plant Growth Regulation* 24: 55–66.

LIN JN, and KAO CH. 1998. Effect of oxidative stress caused by hydrogen peroxide on senescence of rice leaves. *Botanical Bulletin of Academia Sinica* 39: 161–165.

LIU EH. 1973. A simple method for determining the relative activities of individual peroxidase isoenzymes in a tissue extract. *Analytical Biochemistry* 56: 149–154.

MATTHEWS RB, AZAM-ALI SN, and PEACOCK JM. 1990. Response of four sorghum lines to mid-season drought: II. leaf characteristics. *Field Crops Research* 25: 297–308.

PASTORI GM, and TRIPPI VS. 1992. Oxidative stress induces high rate of glutathione reductase synthesis in a drought-resistant maize strain. *Plant Cell Physiology* 33: 957–961.

PREMACHANDRA GS, SANEOKA H, FUJITA K, and OGATA S. 1993. Water stress and potassium fertilization in field grown maize (*Zea mays* L.): effects of leaf water relations and leaf rolling. *Journal of Agronomy and Crop Science* 170: 195–201.

RENSBURG LV, and KRUGER GHJ. 1994 Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of *Nicotiana tabacum* L. *Journal of Plant Physiology* 143: 730–737.

RODRIGUEZ R, and SANCHEZ TR. 1982. Peroxidase and IAA oxidase in germinating seeds of *Cicer arientium* L. *Revista Espanola de Fisiologia* 38: 183–188.

SAIRAM RK. 1994. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian Journal of Experimental Biology* 32: 594–597.

SAIRAM RK, DESHMUKH PS, and SHUKLA DS. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *Journal of Agronomy and Crop Science* 178: 171–178.

SAIRAM RK, SHUKLA DS, and SAXENA DC. 1997/98. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. *Biologia Plantarum* 40: 357–364.

SAIRAM RK, CHANDRASEKHAR V, and SRIVASTAVA GC. 2001. Comparison of hexaploid and tetraploid wheat cultivars in their responses to water stress. *Biologia Plantarum* 44: 89–94.

SCANDALIOS JG. 1993. Oxygen stress and superoxide dismutase. *Plant Physiology* 101: 7–12.

SGHERRI CLM, MAFFEI M, and NAVARI-IZZO F. 2000. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. *Journal of Plant Physiology* 157: 273–279.

SHAW B, THOMAS TH, and COOKE DT. 2002. Responses of sugar beet (*Beta vulgaris* L.) to drought and nutrient deficiency stress. *Plant Growth Regulation* 37: 77–83.

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

Smirnoff N. 1995. Antioxidant systems and plant response to the environment. In: Smirnoff N. [ed.], *Environment and plant metabolism: Flexibility and acclimation*, 217–243. Bios Scientific Publishers, Oxford.

Tanaka K, Masuda R, Sugimoto T, Omasa K, and Sakaki Z. 1990. Water deficiency-induced changes in the contents of defensive substances against active oxygen in spinach leaves. *Agricultural and Biological Chemistry* 54: 2629–2634.

Townley-Smith TF, and Hurd EA. 1979. Testing and selecting for drought resistance in wheat. In: Mussell H, and Staples RC [eds.], *Stress physiology in crop plants*, 447–464. John Wiley & Sons, New York.

Turgut R, and Kadioglu A. 1998. The effect of drought, temperature and irradiation on leaf rolling in *Ctenanthe setosa*. *Biologia Plantarum* 41: 629–663.

Wu Y, and Cosgrove DJ. 2000. Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. *Journal of Experimental Botany* 51: 1543–1553.