BIOLOGIA PLANTARUM 48 (4): 597-600, 2004

BRIEF COMMUNICATION

Responses of *Camellia sinensis* to drought and rehydration

H. UPADHYAYA* and S. K. PANDA**,[1]

*Plant Biochemistry Laboratory, Department of Life Science, Assam (Central) University, Silchar-788011, India**
*Research Institute for Bioresources, Okayama University, Kurashiki-7100046, Japan***

Abstract

The effects of drought and rehydration on tea seedlings were significant. After five days of drought imposition the contents of chlorophylls, carotenoids, ascorbate and glutathione, and activities of guaiacol peroxidase and glutathione reductase decreased. Simultaneously, contents of proline, H_2O_2 and superoxide anion, lipid peroxidation and activities of catalase and superoxide dismutase increased. These parameters recovered to different degrees during subsequent rehydration.

Additional key words: ascorbate, carotenoid, catalase, chlorophyll, glutathione, guaiacol peroxidase, superoxide anion, superoxide dismutase.

Tea plant being perennial shrubs can grow under diverse climatic conditions and is always subjected to environmental stress. Plant may suffer either from excessive soil moisture or moisture deficit. Drought being an important limitation for plant impairs severely growth, crop yield and various morphological, anatomical, physiological and biochemical processes (Kefei *et al*. 1997, Egert and Tevini 2002). The drought resistance mechanisms can be categorised as *1*) drought avoidence, *2*) dehydration tolerance, and *3*) dehydration postponement (Kramer and Boyer 1995). Plant may perceive osmotic adjustment as a survival mechanism, which enable physiological activity to be maintained at a lower level throughout a period of water deficit (Turner 1997). Drought tolerance mechanisms have been compared in the clones of different plant species (*e.g*. in *Coffea canephora*; DaMatta *et. al.* 2003). The post drought recovery by the plant is also a subject of much concern.

Drought is known to cause oxidative damage in plants as a result of production of reactive oxygen species (ROS) like, superoxide radical, hydroxyl radical, hydroperoxide radical, alkoxyl radical, and hydrogen peroxide, which are inevitable products of natural redox reactions occurring in various cellular compartments (Zhang and Kirkham 1994, Alscher *et al.* 1997, Panda 2002). However, plants possess both enzymic [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) guiacol peroxidase (GPX), and glutathione reductase (GR)] and non enzymic (carotenoids, ascorbate, glutathione, α-tocopherol) antioxidants to overcome the toxic effect of ROS.

The level of osmolytes or osmoprotectants are increased in plant subjected to drought. Increase in total free amino acids and free proline were reported in wheat (Levitt 1980, Kathju *et al*. 1988) and in tea (Handique and Manivel 1990), respectively. The molecular mechanism of quenching of ROS by proline under stresses, which includes water stress is well reviewed by Matysik *et al.* (2002). Besides the contents and composition of osmolytes the antioxidant property also varies between the drought susceptible and drought resistant plants. The present experiment was undertaken to understand the drought imposed damage and its recovery in the developing clonal tea plant like other crop plant.

Healthy and uniform 1-year-old clonal seedlings of tea [*Camellia sinensis* (L.) O. Kuntze] were procured from Tocklai Tea Research station, Silchar, and grown under a natural light in a greenhouse. Drought was induced by

Received 19 September 2003, *accepted* 12 May 2004.
Abbreviations: APX - ascorbate peroxidase; CAT - catalase; GPX - guaiacol peroxidase; GR - glutathione reductase; PDR - post-drought rehydration; ROS - reactive oxygen species; RWC - relative water content; SOD - superoxide dismutase; TBA - thiobarbituric acid; TBARS - thiobarbituric acid reactive substance; TCA - trichloroacetic acid.
[1] Corresponding author, fax: (+81) 86 434 1210, e-mail: drskp@rib.okayama-u.ac.jp

withholding the watering for five days under controlled conditions. On the sixth day leaves from the control and drought imposed tea seedlings were sampled for various biochemical analysis and plants were rehydrated for another five days. On the sixth day of rehydration leaves were again sampled.

Leaves were extracted in cold 80 % acetone and chlorophylls and carotenoids were extracted and estimated by spectrophotometer type *106*, (*Systronics*, India) as per the methods of Arnon (1949). Extraction and estimation of H_2O_2 content was done according to Sagisaka (1976). Lipid peroxidation was measured as the amount of thiobarbituric acid reactive substance (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Glutathione was extracted and estimated according to Griffith (1980). For the extraction and estimation of ascorbate method of Oser (1979) was used. Proline content in leaves was determined following the method of Bates *et al.* (1973). Presence of superoxide anion ($O_2^{\bullet-}$) was determined as described by Elstner and Heupel (1976). Relative water content (RWC), defined as water content of tissue as a percentage of that in water saturated leaf tissue, was determined by the method of Weatherley (1950). Samples of fresh tissue were floated in distilled water at 25 ± 1 °C for 4 h.

The leaf tissues were homogenised with phosphate buffer pH 6.8 (0.1M) in prechilled mortar and pestle. The extract was centrifuged at 4 °C for 15 min at 17 000 *g* in a cooling centrifuge. The supernatant was used for the assay of CAT, GPX, SOD, and GR. Extractions and assay of CAT, GPX, GR, and SOD were done as per the methods described in Chance and Maehly (1955), Giannopolitis and Reis (1977) and Smith *et al.* (1988) respectively. Each experiment was repeated five times and data presented are means ± SE.

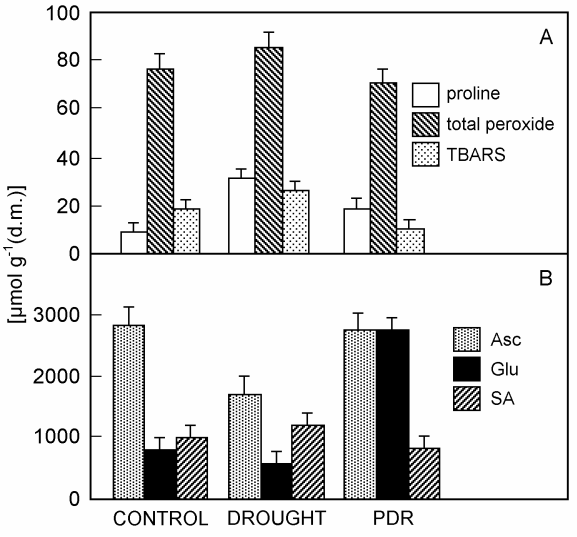

Fig. 1. Changes in contents of proline, H_2O_2, thiobarbituric acid reactive substances (TBARS) (*A*), ascorbate (Asc), glutathione (Glu) and superoxide anion (SA) (*B*) in *Camellia sinensis* leaves subjected to drought stress and rehydration (PDR). Means of 5 separate experiments ± SE.

RWC decreased with drought stress, but only a slight increase in RWC was observed on rehydration for 5 d. A decrease in chlorophyll (Chl) and carotenoid (Car) contents (42 and 51.96 % of that in control plants, respectively) was observed after 5 d of drought treatment. Such observation suggested a drought induced pigment degradations (Baisak *et. al*. 1994) and/or inhibition of their synthesis. Decrease in net photosynthetic rate by water stress in tea seedlings was observed by Sobrado (1996) and Yordanov *et al*. (2000). After rehydration, Chl and Car contents were 34.18 and 7.25 %, respectively, less than those in the control.

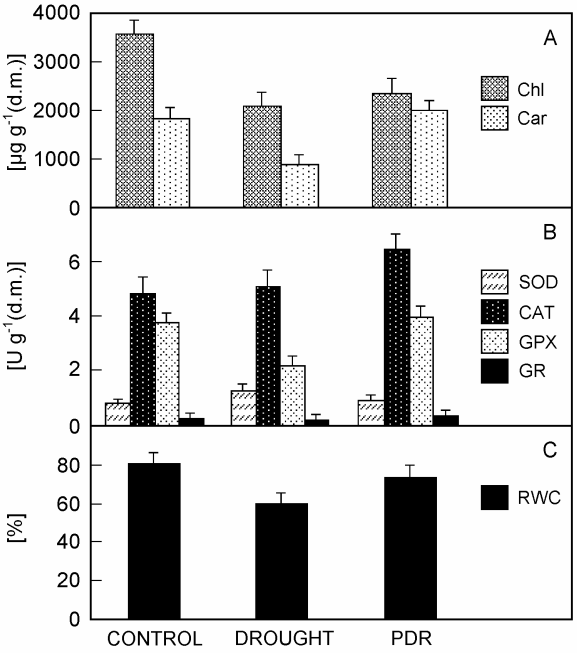

Fig. 2. Changes in chlorophyll (Chl) and carotenoid (Car) contents (*A*), and superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and glutahione reductase (GR) activities (*B*), and relative water content (RWC) (*C*) in *Camellia sinensis* leaves subjected to drought stress and rehydration (PDR). Means ± SE, *n* = 5.

An increase in proline content (251.64 %) was observed, whereas after rehydration it decreased to 108.07 % of that in control. Such proline accumulation in response to water deficit stress was reported in wheat (Kathju *et al.* 1988, Levitt 1980) and in tea (Handique and Manivel 1990). However, this accumulation may not be sufficient to reduce completely the damaging effect of dehydration on membrane disintegration or enzyme inactivation (Bohnert and Jenson 1996).

TBARS content is the measure of lipid peroxidation. It significantly increased in drought treated plants, but rehydration showed decrease of the same. An increase in

H_2O_2 content with simultaneous increase in lipid peroxidation in drought imposed tea plant suggested a loss of membrane function and induction of oxidative damage (Zhang and Kirkham 1994, Baisak *et al.* 1994, Sairam *et al.* 1997, Fu and Huang 2001, Egert and Tevini 2002). But post drought recovery analysis suggested that rehydration minimizes the negative effect of drought, as was evidenced by 44.57 % decreased lipid peroxidation and 7.8 % decrease in H_2O_2 content observed after rewatering the drought imposed plant.

Although an increase in SOD activity was visible with simultaneous increase in CAT activity, significant decrease in GPX and GR activities and a decrease in contents of non-enzymic antioxidants, ascorbate and glutathione suggested the incomplete ability of tea seedlings to overcome a drought induced oxidative stress. This is in agreement with results of Mukherjee and Choudhuri (1983), Jagtap and Bhargava (1995), Egert and Tevini (2002), and Fu and Huang (2001). The post drought recovery analysis suggested a marked increase in GPX, GR and CAT activities after rehydration of drought imposed tea seedlings with significant decrease in SOD activity. The increased amount of superoxide anion in drought imposed tea seedlings and its little recovery on subsequent rehydration also confirmed oxidative stress

In conclusion, imposition of drought caused serious damage in tea seedlings and only moderate recovery was noticed upon rehydration.

References

Alscher, R.G., Donahue, J.L., Cramer, C.L.: Reactive oxygen species and antioxidants: relationships in green cells. - Physiol. Plant. **100**: 224 -233, 1997.

Arnon, D.I.: Copper enzymes in isolated chloroplast polyphenoloxidase in *Beta vulgaris.* - Plant Physiol. **24**: 1-15, 1949.

Baisak, R., Rana, D., Archarya, P.B.S, Kar, M.: Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. - Plant Cell Physiol. **35**: 495-499, 1994.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-208, 1973.

Bohnert, H.J., Jensen, R.G.: Strategies for engineering water stress tolerance in plants. - TIBTECH **14**: 89-97, 1996.

Chance, B., Maehly, A.C.: Assay of catalase and peroxidase. - Methods Enzymol. **2**: 764-775, 1955.

DaMatta, F.M., Chaves, A.R.M., Pinheiro, A.H., Ducatti, C., Loureiro, E.M.: Drought tolerence of two field-grown clones of *Coffea canephora*. - Plant Sci. **164**: 111-117, 2003.

Egert, M., Tevini, M.: Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). - Environ. exp. Bot. **48**: 43-49, 2002.

Elstner, E.F., Heupel, A.: Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. - Anal. Biochem. **70**: 616-620, 1976.

Fu, J., Huang, B.: Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. - Environ. exp. Bot. **45**: 105-112, 2001.

Giannopolitis, C.N., Reis, S.K.: Superoxide dismutase. I. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Griffith, O.W.: Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. - Anal. Biochem. **106**: 207-211, 1980.

Handique, A.C., Manivel, L.: Selection criteria for drought tolerance in tea. - Assam Rev. Tea News **79**: 18-21, 1990.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1968.

Jagtap, V., Bharagava, S.: Variation in antioxidant metabolism of drought tolerant in and drought susceptible varieties of *Sorghum bicoler* (L) Moench. exposed to high light, low water and high temperature stress. - J. Plant Physiol **145**: 195-197, 1995.

Kathju, S., Vyas, S.P., Garg, B.K., Lahiri, A.N.: Fertility induced improvements in performance and metabolism of wheat under different intensities of water stress. - In: Proceedings of the International Congress of Plant Physiology. Pp. 854-858. New Delhi 1988.

Kefei, Z., Ma, Q., Zang, H..: Effect of water deficit on physiological activities of paddy rice and upland rice seedlings. - J. Shandong agr. Univ. **28**: 53-57, 1997.

Kramer, P.J., Boyer, J.S.: Water Relations of Plants and Soils. - Academic Press, San Diego 1995.

Levitt, J.: Responses of Plants to Environmental Stress. Volume 1. - Academic Press, London 1980.

Matysik, J., Ali Bhalu, B., Mohanty, P.: Molecular mechanism of quenching of reactive oxygen species by proline under water stress in plants. - Curr. Sci. **82**: 525-532, 2002.

Mukherjee, S.P., Choudhury, M.A.: Implication of water stress – induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. - Physiol. Plant. **58**: 166-171, 1983.

Oser, B.L.: Hawks Physiological Chemistry. - McGraw-Hill, New York 1979.

Panda, S.K.: The biology of oxidative stress in green cells. - In: Panda, S.K. (ed.): Advances in Stress Physiology of Plants. Pp. 1-13. Scientific Publisher, Jodhpur 2002.

Sagisaka, S.: The occurrence of peroxide in a perennial plant *Populus gelrica*. - Plant Physiol. **57**: 308-309, 1976.

Sairam, R.K., Deshmukh, P.S., Shukla, D.S.: Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. - J. Agron. Crop Sci. **178**: 171-171, 1997.

Smith, I.K., Vierheller, T.L. Thorne, C.A.: Assay of glutathione reductase in crude tissue homogenates using 5.5- dithiobis (2-nitrobenzoic acid). - Anal. Biochem. **175**: 408-413, 1988.

Sobrado, M.A.: Leaf photosynthesis and water loss as influenced by leaf age and seasonal drought in an evergreen tree. - Photosynthetica **32**: 563-568, 1996.

Turner, N.C.: Further progress in crop water relations. - Adv.

Agron. **58**: 293-338, 1997.
Weatherley, P.F.: Studies in the water relations of plant. I. The field measurement of water deficit in leaves. - New Phytol. **49**: 81-97, 1950.
Yordanov, I., Velikova, V., Tsonev, T.: Plant responses to drought, acclimation, and stress tolerance. - Photosynthetica **38**: 171-186, 2000.
Zhang, J., Kirkham, M.B.: Drought stress induced changes in activities of superoxide dismutase, catalase and peroxide in wheat species. - Plant Cell Physiol. **35**: 785-791, 1994.