BIOLOGIA PLANTARUM 45 (4): 557-562, 2002

Response of tea plants to water stress

U. CHAKRABORTY*, S. DUTTA* and B.N. CHAKRABORTY**

Plant Biochemistry Laboratory and Immuno-Phytopathology Laboratory**, Department of Botany, University of North Bengal, Siliguri-734 430, India*

Abstract

Two-year-old potted plants of six *Camellia sinensis* cultivars (TV-18, TV-26, UPASI-3, UPASI-26, T-78 and HV-39) were subjected to water stress for 4, 8 and 12 d. Relative water content (RWC) of leaves of all cultivars declined with water stress, but in the two drought tolerant cultivars (UPASI-3 and UPASI-26), higher RWC were maintained in comparison to the others. Phenol content and activities of phenylalanineammonialyase, polyphenoloxidase and peroxidase initially increased, but decreased during extended drought. Chlorophyll contents decreased, whereas proline contents increased during water stress. SDS-PAGE analysis of proteins revealed increased accumulation of proteins of intermediate molecular masses (42 - 44 kDa) and low molecular masses (14 - 26 kDa). After 12 d of water stress, most of these proteins disappeared in T-78 and HV-39, but in the other cultivars they were still detectable.

Additional key words: *Camellia sinensis*, chlorophyll, peroxidase isozymes, phenols, phenylalanineammonialyase, polyphenoloxidase, proline, protein, relative water content.

Introduction

Water deficit affects many physiological and biochemical processes. The effect of water stress varies with the plant species, degree and duration of water stress and growth stage of the plant (*e.g.* Kramer and Boyer 1995). The tea plant is a perennial, and as such, encounters a large number of environmental stresses throughout its life span. The main climatic variables influencing the growth and yield of tea are temperature, vapour pressure deficits of the air, and soil water deficit (Ng'etich 1997).

Many higher plants, accumulate proline as a consequence of water stress and some studies have shown that drought tolerant cultivars accumulate more proline (*e.g.* Carceller *et al.* 1999, Nanjo *et al.* 1999). The induction of drought stress responsive proteins during water stress has also been worked out in several cases (*e.g.* Pareek *et al.* 1999, Sinha *et al.* 1999). Chlorophyll content controls the photosynthetic activity and contributes to the blackness of tea (Liyanage and Penyasiri 1993). The oxidation of tea polyphenols, the key step in tea processing, is dependent on polyphenoloxidase (PPO), phenylalanineammonialyase (PAL), *etc.*

Considering all the above, the present investigation was undertaken to determine the effect of water stress on major biochemical components and metabolism of different cultivars of tea.

Materials and methods

Plants: Six tea [*Camellia sinensis* (L.) O. Kuntze] cultivars, two from Assam (TV-18 and TV-26), Darjeeling (T-78 and HV-39) and UPASI (UP-3 and UP- 26) were selected for the present work. Plants grown in pots in a glasshouse at a temperature range of 30 - 34 °C, RH 65 - 70 %, 16-h photoperiod, and irradiance of 400 μmol $m^{-2}s^{-1}$ were taken for experimental purposes. Plants were watered daily with tap water and once a week with Hoagland and Knop nutrient solution.

Received 3 May 2001, *accepted* 29 November 2001.
Acknowledgement: Financial assistance received from the Council of Scientific & Industrial Research, New Delhi, India, is gratefully acknowledged.
Fax: (+91) 353 581546; e-mail: chakrabortyvsha@hotmail.com

Two-year old seedlings were subjected to progressive water stress by withholding water supply for 4, 8 and 12 d. Control plants were watered daily. After each period of water stress treatments, samplings were done, morphological changes noted, and relative water content (RWC) of leaves was determined as described by Farooqui *et al.* (2000). Soil moisture content was determined by noting the difference in fresh and dry mass of soil, expressed as percentage (Gardner 1965). Leaf samples were then used for following biochemical analyses.

Extraction and estimation of phenols: Total and *o*-dihydroxyphenols were extracted from the tea leaves according to Harborne (1973) and estimated as described by Mahadevan and Sridhar (1982). Both total and *o*-dihydroxyphenols were extracted from fresh tea leaves in boiling absolute alcohol followed by re-extraction in 80 % alcohol. Phenolic extract was mixed with equal volume of 1 M Folin Ciocalteau and double volume of 20 % Na_2CO_3. Absorbance (A) of the blue colored solution was measured at 650 nm in a UV-VIS spectrophotometer (*Digispec-200 GL*, *SICO*, India). For *o*-dihydroxyphenol, phenolic extract was mixed with 1 M NaOH, 0.5 M HCl and Arnow's reagent and A_{560} was noted. Both phenol quantities were estimated by comparison with the standard curve of caffeic acid.

Extraction and estimation of enzyme activities: Phenylalanine ammonia lyase (EC 4.3.1.5.) was extracted from tea leaves in 0.1 M sodium borate buffer (pH 8.8) containing 2 mM β-mercaptoethanol and assayed as described by Chakraborty *et al.* (1993). Assay mixture consisted of 0.5 cm^3 of enzyme extract, 0.1 cm^3 of 30 μM L-phenylalanine, 0.1 cm^3 of 300 μM sodium borate buffer (pH 6.8), which was incubated at 40 °C for 1 h and A_{290} was read.

Polyphenol oxidase (EC 1.10.3.2.) was extracted from tea leaves and estimated as described by Mahadevan and Sridhar (1982). Extraction was done in 0.2 M sodium phosphate buffer (pH 6.6). For assay, 1 cm^3 of enzyme extract was mixed with 2 cm^3 of 0.2 M sodium phosphate buffer of pH 6 containing 0.01 M pyrogallol. PPO activity was assayed as ΔA_{495}.

Peroxidase (EC 1.11.1.7) was extracted in 0.1 M sodium borate buffer following the method of Chakraborty *et al.* (1993) with modification. Assay buffer consisted of 0.2 M sodium phosphate buffer (pH 5.4), 4 mM H_2O_2, *o*-dianisidine [5 mg cm^{-3}(methanol)] andenzyme extract. Peroxidase activity was assayed spectrophotometrically at A_{460} by monitoring the oxidation of *o*-dianisidine in presence of H_2O_2. Specific activity was expressed as ΔA_{460} mg^{-1}(protein) min^{-1}.

Peroxidase isozyme analysis by PAGE: Peroxidase enzyme extract was prepared in 0.1 M sodium phosphate buffer (pH 7). Polyacrylamide gel electrophoresis was performed according to the method of Davis (1967). Native PAGE system consisted of 7.5 % resolving gel. Enzyme extract (0.03 cm^3) were loaded on the gel and separated for 3 h using 75 V and 15 mA current at 4 °C. For staining, the gel was incubated in a solution of 1 % benzidine, 2 % acetic acid, and 3 % H_2O_2 for 5 min. The reaction was stopped after the appearance of clear blue colored bands by 7 % acetic acid. Analysis of isozyme bands was done immediately by determing the R_m values.

Extraction and estimation of free proline: Proline was extracted from the leaves as described by Bates *et al.* (1973) in 3 % sulphosalicylic acid. For estimation the extract was treated with ninhydrin and incubated over a water bath for 20 min. The ninhydrin derivative was extracted with toluene and analysed spectrophotometrically at A_{520} and quantified using standard curve of proline.

Extraction and estimation of protein: Soluble proteins were extracted from tea leaves as described by Chakraborty *et al.* (1995). Leaf tissues were ground in 0.05 M phosphate buffer (pH 7.2), containing 10 mM $Na_2S_2O_5$, 0.5 mM $MgCl_2$, 2 mM soluble polyvinyl pyrrolidone phosphate (PVPP) and 2 mM polymethyl-sulphonylfluoride (PMSF). The extracts were centrifuged at 12 000 *g* at 4 °C for 10 min and the supernatants were used as crude protein extract. Protein content was estimated following Bradford's (1976) method using bovine serum albumin as standard.

Analysis of crude protein extract was carried out on 10 % SDS-PAGE gels following Laemmli's (1970) method. Proteins (50 μg) as well as standard markers were loaded on the gel and separated for 5 h at 200 V and 30 mA. Following electrophoresis the gel was fixed, stained in a Coomassie Brilliant Blue (R250, *Sigma*) staining solution and finally destained in a solution of methanol, acetic acid and water (4.5:4.5:1).

Results

Different tea cultivars responded to the periods of water stress differently. In the Tocklai (TV-18 and TV-26) and Darjeeling (T-78 and HV-39) cultivars, prolonged water stress led to a wilting of leaves, whereas, the UPASI cultivars did not exhibit morphological wilting even after 12 d of stress, where the soil moisture content decreased from 98 to 38 %. The relative water contents of the control leaves ranged from 75 - 85 %. Following water

stress the RWC declined to 42, 50, and 58 % in the Darjeeling, Tocklai and UPASI cultivars.

The content of both total and *o*-dihydroxyphenols increased after 4- and 8-d water stress but declined when the stress period was prolonged upto 12 d (Fig. 1*A*,*B*). The response was more or less similar in all cultivars, though the inherent phenol content varied among them. However, after 12-d water stess, in the UPASI cultivars, the phenol contents were much higher than in either Tocklai or Darjeeling cultivars.

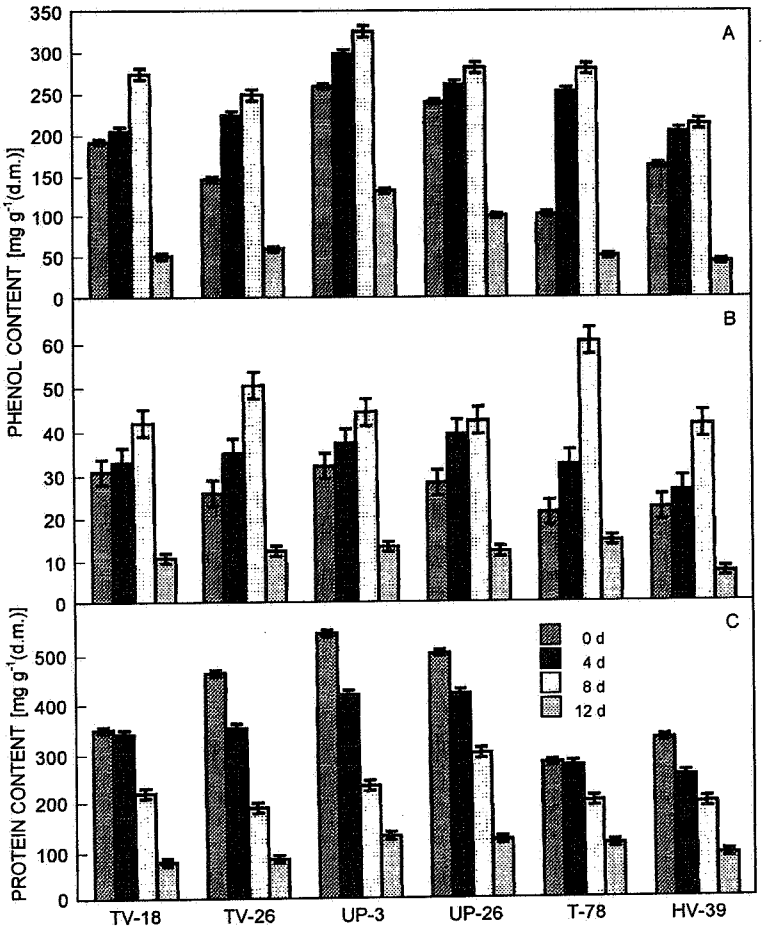

Fig. 1. Contents of total phenol (*A*), *o*-dihydroxyphenol (*B*), and proteins (*C*) in leaves of tea cultivars subjected to water stress for 4, 8 and 12 d. Means ± SE, $n = 5$.

Table 1. Effect of water stress (4, 8 and 12 d) on phenylalanine-ammonialyase activity [μg(cinnamic acid) mg^{-1}(protein) min^{-1}] in leaves of tea cultivars. Means ± SE, $n = 5$.

	control	4 d	8 d	12 d
TV-18	8.3 ± 0.9	10.5 ± 1.1	8.1 ± 0.9	5.9 ± 0.5
TV-26	5.4 ± 0.5	9.1 ± 1.0	7.7 ± 1.1	5.4 ± 0.3
UP-3	4.6 ± 0.7	6.3 ± 0.7	7.6 ± 0.6	3.5 ± 0.1
UP-26	3.5 ± 0.3	6.0 ± 0.4	3.9 ± 0.1	3.9 ± 0.1
T-78	7.2 ± 0.8	10.4 ± 1.0	7.9 ± 0.6	7.2 ± 0.4
HV-39	5.7 ± 0.2	7.5 ± 0.9	9.2 ± 0.9	5.5 ± 0.7

Table 2. Effect of water stress (4, 8 and 12 d) on polyphenol oxidase activity [$\times 10^{-2}$ ΔA_{495} mg^{-1}(protein) min^{-1}] in leaves of tea cultivars. Means ± SE, $n = 5$.

	control	4 d	8 d	12 d
TV-18	5.6 ±0.1	16.3 ± 0.7	5.6 ± 0.1	3.4 ± 0.1
TV-26	1.4 ± 0.8	6.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.4
UP-3	1.6 ± 0.9	3.3 ± 0.2	1.8 ± 0.1	0.7 ± 0.4
UP-26	2.1 ± 0.1	4.9 ± 0.3	1.5 ± 0.2	0.2 ± 0.1
T-78	4.1 ± 0.3	5.6 ± 0.1	2.5 ± 0.1	0.2 ± 0.1
HV-39	4.2 ± 0.1	4.5 ± 0.1	2.0 ± 0.2	0.6 ± 0.1

PAL and PPO activities initially increased (4 d) after which the activity declined steadily (Tables 1, 2). Peroxidase activity also increased in all cultivars following 4-d water stress. In the Tocklai and Darjeeling cultivars, activity declined after 8 and 12 d, but in the UPASI cultivars there was an increase in activity even after prolonged drought (Table 3). Analysis of the isozymes of peroxidase by PAGE revealed the presence

of three isozymes with relative mobility (R_m) of 0.375, 0.500 and 0.604 in both control and stressed plants. Following 12-d drought, only two isozymes were discernible (0.375 and 0.604).

Table 3. Effect of water stress (4, 8 and 12 d) on peroxidase activity [ΔA_{460} mg^{-1}(protein) min^{-1}] in leaves of different tea cultivars. Means ± SE, $n = 5$.

	control	4 d	8 d	12 d
TV-18	3.9 ± 0.5	5.6 ± 0.5	5.3 ± 0.5	3.1 ± 0.1
TV-26	2.1 ± 0.3	2.8 ± 0.5	3.3 ± 0.1	1.8 ± 0.4
UP-3	2.7 ± 0.4	3.2 ± 0.4	5.2 ± 0.4	6.2 ± 0.3
UP-26	2.7 ± 0.8	3.4 ± 0.2	5.4 ± 0.3	6.6 ± 0.5
T-78	3.6 ± 0.1	5.6 ± 0.5	3.1 ± 0.5	1.0 ± 0.1
HV-39	3.8 ± 0.1	4.7 ± 0.6	5.1 ± 0.4	2.1 ± 0.1

Table 4. Chlorophyll (Chl) contents [mg g^{-1}(d.m.)] of leaves of tea cultivars subjected to water stress (4, 8 and 12 d). Means ± SE, $n = 5$.

		control	4 d	8 d	12 d
TV-18	Chl *a*	9.0±0.10	6.3±0.27	2.1±0.21	0.4±0.04
	Chl *b*	4.0±0.70	4.5±0.45	0.7±0.07	0.4±0.04
TV-26	Chl *a*	7.0±0.80	5.4±0.36	1.4±0.07	0.4±0.04
	Chl *b*	4.0±0.30	3.6±0.27	1.4±0.14	0.4±0.16
UP-3	Chl *a*	9.0±0.50	9.0±0.36	6.3±0.21	2.4±0.12
	Chl *b*	6.0±0.30	4.5±0.45	2.8±0.35	0.8±0.20
UP-26	Chl *a*	9.0±0.50	9.0±0.36	6.3±0.42	2.4±0.24
	Chl *b*	6.0±0.40	5.4±0.45	2.1±0.35	1.2±0.32
T-78	Chl *a*	7.0±0.50	5.4±0.27	4.9±0.21	0.4±0.08
	Chl *b*	4.0±0.40	3.6±0.18	2.8±0.28	0.4±0.08
HV-39	Chl *a*	4.0±0.30	0.4±0.05	0.2±0.01	0.1±0.01
	Chl *b*	0.3±0.20	0.3±0.07	0.1±0.01	0.1±0.01

Chlorophyll content decreased after 8- and 12-d stress in all the cultivars (Table 4). Water stress induced accumulation of proline. After 4 d the increase in proline content was not very significant but after 12 d the increase was approximately 6 - 7 fold (Table 5).

Prolonged period of drought resulted in a decrease in protein content in all the tested cultivars and the decrease was more significant in Tocklai and Darjeeling cultivars. In general, the UPASI cultivars had higher inherent protein content in comparison to the Darjeeling or Tocklai cultivars (Fig. 1*C*). SDS-PAGE analysis of soluble proteins from water stressed and control plants revealed that increased accumulation of proteins of molecular mass of about 43 kDa could be observed after 4- and 8-d water stress. In Tocklai and UPASI cultivars, after 12 d of water stress, most of the proteins were still detectable, whereas in the Darjeeling cultivars, the proteins were almost undetectable after 12 d of drought. Some of the low molecular mass proteins bands ranging from 14 - 26 kDa were also more prominent following water stress (Fig. 2).

Table 5. Effect of water stress (4, 8 and 12 d) on proline content [mg g^{-1}(d.m.)] in leaves of different tea cultivars. Means ± SE, $n = 5$.

	control	4 d	8 d	12 d
TV-18	43.0 ± 2.4	54.9 ± 2.7	88.2 ± 2.2	96.4 ± 2.4
TV-26	41.0 ± 5.8	38.7 ± 2.5	113.4 ± 2.9	222.4 ± 2.9
UP-3	14.0 ± 3.4	22.5 ± 0.7	156.1 ± 4.8	195.2 ± 3.8
UP-26	21.0 ± 3.2	21.6 ± 2.4	92.4 ± 3.6	192.8 ± 1.9
T-78	65.0 ± 2.1	67.5 ± 3.4	84.7 ± 2.8	337.6 ± 2.6
HV-39	71.0 ± 1.2	81.9 ± 4.2	99.8 ± 3.6	584.2 ± 3.2

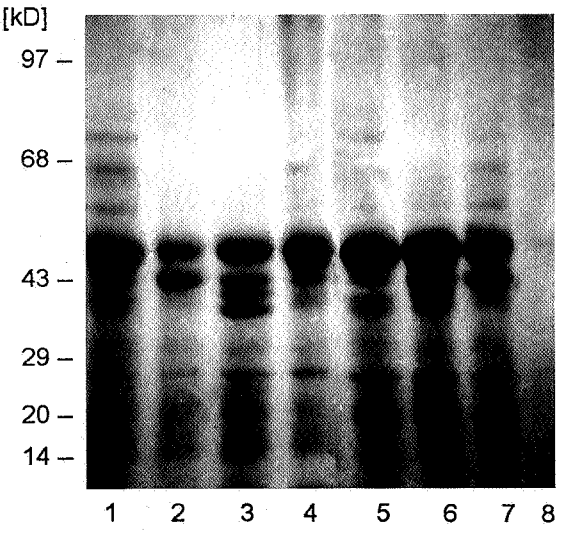

Fig. 2. SDS-PAGE analysis of leaf proteins from control and water-stressed tea plants. *Lanes 1 - 4*: UP-26; *5 - 8*: T-78. *Lanes 1, 5* - control; *2, 6* - 4 d; *3, 7* - 8 d, and *4, 8* - 12 d of water stress.

Discussion

In the present study, severe wilting symptoms were exhibited by Tocklai and Darjeeling cultivars, whereas, comparatively, the UPASI cultivars were more drought resistant. RWC of leaves decreased in all cultivars due to water stress but the decrease was least in the UPASI cultivars. Maintenance of high RWC in drought resistant cultivars has also been reported to be an adaptation to water stress in several crop species (*e.g.* Schonfield *et al.* 1988, Farooqui *et al.* 2000).

Water stress resulted in the accumulation of total and

o-dihydroxyphenols after 4 and 8 d followed by a decline under prolonged water stress in all tested cultivars. Polyphenols are considered to be involved in the plant defense to various stresses (Leinhos and Bergmann 1995). In the UPASI cultivars, which were most tolerant to drought, relatively high phenol content was observed even after 12 d of water stress. In all the six cultivars PAL and PPO activity initially increased (at 4 d) and then the activity declined steadily to a lower values at 12-d drought. Among the three enzymes studied maximum increase was observed in peroxidase activity after drought. In the Darjeeling and Tocklai cultivars after the initial increase at 4 d of water stress there was a decline in activity, whereas in the two UPASI cultivars the activity was higher even after 12 d of water stress. However, increased activity could not be correlated to induction of isozymes, since, after 12 d of drought, there was the disappearance of one of the isozymes. Ability to withstand prolonged water stress could in part be due to the induction of high PO activity. Increased activity of peroxidase was also reported following chemical sprays and infection (Chen *et al.* 2000, Curtis *et al.* 1997).

Severe decrease of chlorophyll content was noticed after 8-d stress onwards. According to Kaur and Deshmukh (1980) the reduction of chlorophyll contents may be due to stimulation of chlorophyllase which degrades chlorophyll (Janave 1997).

Water stress induced increased accumulation of proline. The increase was maximum in case of Darjeeling cultivars though all the cultivars showed a significant increase. Several authors have obtained increased accumulation of proline in water stressed plants (*e.g.* Andrade *et al.* 1995, Girousse *et al.* 1996, Sanchez *et al.* 1998, Barathi *et al.* 2001). A decrease in protein content in all the tested cultivars after prolonged drought was observed which was more significant in Tocklai and Darjeeling cultivars. However, appearance of drought induced proteins along with the constitutive ones were visualized on SDS-PAGE gels, specially after 4 and 8 d of drought, while after 12 d most of the constitutive proteins were undetectable. Induction of drought induced proteins has also been reported by previously. Sinha *et al.* (1999) reported the accumulation of polypeptides of 46, 40, 35 and 28 kDa in *Lathyrus* seedlings subjected to water stress. Barathi *et al.* (2001) also reported the accumulation of additional proteins (78 and 92 kDa) in mulberry leaves subjected to water stress.

Results obtained here point to the fact that among the three groups, UPASI cultivars are more drought tolerant, which could be correlated to maintenance of high RWC in leaves, higher amounts of phenolic compounds and higher peroxidase activity.

References

Andrade, J.L., Larque-Saavedra, A., Truju, C.L.: Proline accumulation in four cultivars of *Phaseolus vulgaris* L. with different drought resistance. - Phyton **57**: 149-157, 1995.

Barathi, P., Sundar, D., Reddy, R.A.: Changes in mulberry leaf metabolism in response to water stress. - Biol. Plant. **44**: 83-87, 2001.

Bates, H.S., Waldren, R.P., Treare, I.D.: Rapid estimation of free proline for water stress determination. - Plant Soil **39**: 205-207, 1973.

Bradford, K.: A rapid and sensitive method for the quantification of microgramme quantities of protein utilising the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Carceller, M., Prystupa, P., Lemcoff, J.H.: Remobilization of proline and other nitrogen compounds from senescing leaves of maize under water stress. - J. Agron. Crop Sci. **183**: 61-66, 1999.

Chakraborty, B.N., Basu, P., Das, R., Saha, A., Chakraborty, U.: Detection of cross reactive antigens between *Pestalotiopsis theae* and tea leaves and their cellular location. - Ann. appl. Biol. **127**: 11-21, 1995.

Chakraborty, U., Chakraborty, B.N., Kapoor, M.: Changes in the levels of peroxidase and phenyl alanine ammonia lyase in *Brassica napus* cultivars showing variable resistance to *Leptosphaeria maculans*. - Folia microbiol. **38**: 491-496, 1993.

Chen, C., Belanger, R.R., Benhamou, M., Paulitz, T.: Defense enzyme induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. - Physiol. mol. Plant Pathol. **56**: 13-23, 2000.

Curtis, M.D., Rac, A.L., Rasu, A.G., Harrison, H.J., Manners, J.M.: A peroxidase gene promoter induced by phytopathogens and methyl jasmonate in transgenic plants. - Mol. Plant Microbiol. Interact. **10**: 326-338, 1997.

Davis, B.J.: Disc electrophoresis methods and application to human serum proteins. - Ann. nat. Acad. Sci. **121**: 404, 1967.

Farooqui, A.H.A., Kumar, R., Fatima, S., Sharma, S.: Response of different genotype of lemon grass (*Cymbopogon flexuosus* and *C. pendulus*) to water stress. - J. Plant Biol. **27**: 277-282, 2000.

Gardner, W.H.: Water contents. - In: Blank, C.A. (ed.): Methods of Soil Analysis. Part I. Pp. 84. American Society for Agronomy, Madison 1965.

Girousse, C., Bournoville, R., Bonnenain, N.: Water deficit induced changes in concentrations in proline and some other amino acids in the phloem sap of alfalfa. - Plant Physiol. **111**: 109-113, 1996.

Harborne, J.B.: Phytochemical Methods. - Chapman and Hall, London 1973.

Janave, M.T.: Enzymatic degradation of chlorophyll in cavendish bananas - *in vitro* evidence for two independent degradative pathways. - Plant Physiol. Biochem. **35**: 837-846, 1997.

Kaur, M., Deshmukh, K.B.: Photosynthetic activities of cowpea plants infected with *Erysiphe polygoni*. - Indian Phytopathol. **33**: 344-345, 1980.

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

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the bacteriophage T_4. - Nature **227**: 680-685, 1970.

Leinhos, V., Bergmann, H.: Changes in the yield, lignin content and protein patterns of barley (*Hordeum vulgare* cv. Alexis) induced by drought stress. - Angew. Bot. **69**: 206-210, 1995.

Liyanage, A.C., Penyasiri, P.A.N.: High performance liquid chromatography (HPLC) of chlorophylls in tea (*Camellia sinensis*). - Sri Lanka J. Tea Sci. **62**: 32-37, 1993.

Mahadevan, A., Sridhar, R.: Methods in Physiological Plant Pathology. 2nd Edition. - Sivakami Publications, Madras, 1982.

Nanjo, T., Kobayashi, M., Yoshiba,Y., Sanada, Y., Wada, K., Tsukaya, H., Kakubari, Y., Yamaguchi-Shinozaki, K., Shinozaki, K.: Biological functions of proline in morphogenesis and osmotolerance revealed in antisense *Arabidopsis thaliana*. - Plant J. **18**: 185-193, 1999.

Ng'etich, W.K.: Responses of tea to environment. - Tea **18**: 149-155, 1997.

Pareek, A., Singla, S.L., Grover,A. Analysis of stress proteins at four different developmental stages in field-grown rice, *Oryza sativa* L. (cv. Pusa 169) plants. - Curr. Sci. **76**: 81-86, 1999.

Sanchez, F.J., Manzanares, M., Deandres, E.F., Tenorio, J.L., Ayerbe, L.: Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. - Field Crops Res. **59**: 225-235, 1998.

Schonfield, M.A., Johnson, R.C., Carver, B.F., Mornhinweg, W.: Water relations in winter wheat as drought resistance indicators. - Crop Sci. **28**: 526-531, 1988.

Sinha, K.M., Sachdev, A., Johri, R.P., Mehta, S.L. Stress induced polypeptides in *Lathyrus sativus*. - J. Plant Biochem. Biotechnol. **8**: 47-51, 1999.