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Influence of drought stress on cellular ultrastructure and antioxidant system in tea cultivars with different drought sensitivities

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Abstract

Drought is the major yield-limiting abiotic factor of tea cultivation. In the present study, influence of drought stress on cellular ultrastructure and antioxidants was studied drought-tolerant (TV-23) and -sensitive (S.3/A3) tea cultivars by imposing drought stress for 21 days. Drought stress led to considerable structural alterations in mitochondria, chloroplast and vacuole. Lesser membrane integrity and higher structural damage was observed in S.3/A3. Chlorophyll a, chl-b and carotenoids content in leaves decreased in each cultivar; however, the decrement was more brisk in S.3/A3. Proline, total soluble sugar, ascorbic acid and abscisic acid were elevated in TV-23 whereas hydrogen peroxide, superoxide anion, lipid peroxidation and electrolyte leakage increased rapidly in S.3/A3. Starch content decreased both in leaves and roots of each cultivar and was more pronounced in roots of TV-23. Under drought, enhanced activities of ascorbate peroxidase, catalase, peroxidase and superoxide dismutase were recorded in both roots and leaves of each cultivar, but the rate of enhancement was more in TV-23. This indicated that tolerant cultivar exhibited higher antioxidant capacity and a stronger protective mechanism such that their ultrastructural integrity was better maintained during exposure to drought stress.

Key words

Antioxidant, *Camellia sinensis*, Drought, Ultrastructure

Introduction

Drought stress is a major yield-limiting factor of crop plants. The exposure of plants to drought and other environmental stresses can result in oxidative damage due to the enhanced production of reactive oxygen species (ROS). The damages occur due to peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition and finally leading to death of cells (Sharma *et al.,* 2012). ROS include free radicals such as superoxide anion (O_2^-), hydroxyl radical (OH), as well as non-radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and so on. ROS are formed by inevitable leakage of electrons onto O_2 from the electron transport activities of cellular organelles and plasma membranes or as a byproduct of various metabolic pathways localized in different cellular compartments (Sharma *et al.* 2012). Structural studies have shown that drought stress may provoke substantial modifications in the ultrastructural organisation (*Stoyanova et al., 2002*). To protect their cells from oxidative damage, plants possess an antioxidant mechanism comprising enzymatic and non-enzymatic defence.

Enzymatic antioxidants include superoxide dismutase, ascorabate peroxidase, catalase, peroxidase etc. and non-enzymatic antioxidants include ascorbate, glutathione, tocopherol, proline, and so forth (Hong-bo *et al.,* 2008; Upadhyaya *et al.,* 2008; Mukhopadyay *et al.,* 2012). Phytohormone abscisic acid (ABA) is an endogenous signal that ensures development and plays a crucial role during

environmental stress. ABA signalling comprises of various cellular events including pressure potential regulation and differential biochemical synthesis in response to stress (Zhu *et al.,* 2002).

Tea is an economically important plantation crop, which is under large-scale cultivation in several countries including India. Drought is the most important recurrent limiting factor of tea cultivation which incurred around 40% crop loss (Jain, 1999; Das *et al.,* 2012). Wijeratne *et al.* (1998) found that drought stress reduced relative water content and water potential and increased diffusive resistance in tea leaves. In their study, it was shown that the clones having efficient stomatal control for reducing water loss and osmotic adjustment for absorbing water from drier soils can withstand drought. Moreover, a significant (P<0.001) correlation of total polyphenol content with shoot growth and water stress of tea, and a linear relationship between soil water content and both water stress index and shoot polyphenol content was reported by Cheruiyot *et al.* (2007). However, these studies were made mostly on leaves of tolerant cultivar with various degrees of drought tolerance. In the present study, influence of drought stress on cellular ultrastructure and antioxidant profiles in tea cultivars were compared with contrasting level of drought tolerance in both roots and leaves. There are lack of studies on structural and antioxidant responses of tea plants considering both drought-tolerant and -sensitive cultivars. The present study would help to broaden up the scope to comprehend the biochemical profiles of drought stress responses and enhance our understanding in our on-going research on functional genomics of drought stress tolerance in tea plants (Das *et al.,* 2012; Das and Mondal, 2010). The results would also help the scientific community to study abiotic stress responses in other woody crop plants.

Materials and Methods

Plant growth, drought stress induction and sampling : Two-yr-old, vegetatively propagated well-rooted, tea [*Camellia sinensis*(L.) O. Kuntze] seedlings (~36-inch height) of S.3/A3 (drought-sensitive) and TV-23 (drought-tolerant) cultivars (Das *et al.* 2012) were planted in earthen pots (12-inch diameter) and maintained under controlled greenhouse conditions at a light intensity of 300 µmol m^{-2} s^{-1} and 25 ± 2ºC with relative humidity of 65%-70%. Initially, all the plants (16 of each cultivar) were watered consistently for two months following the commencement of new growth. Based on the previous findings (Das *et al.* 2012), roots and leaves were collected on 21st day of stress induction at 7% soil moisture -1.2 Mpa ± 0.20), 8.73 µmol m^{-2} s^{-1} photosynthesis rate (*Pn*) and 0.42 mmol m^{-2} s^{-1} (*gs*) stomatal conductance. Unless mentioned, tissues (100 mg each) from 3rdphysiologically mature leaves from top of the shoot and young white fibrous roots of control and drought stressed plants were sampled, and subsequently various assays were performed with four replicates in each treatment.

TEM analysis : For ultra-structure study, 1-2 mm sections of fresh leaves and roots were prepared after washing with 50 mM potassium phosphate buffer (pH 7). Samples were fixed with 2.5% glutaraldehyde solution prepared in 50 mM potassium phosphate buffer (pH 6.9) overnight followed by washing three times for 15 min each with 100 mM sodium cacodylate buffer (pH 6.9) (Sandalio *et al.,* (2001). Ultrathin sections were collected onto grids and dried for overnight. Grids were stained with uranyl acetate for 15 min and lead citrate for 5 min and observed under TEM (JEM 100C x II, Jeol) at an accelerating voltage of 80 kV.

Biochemical estimations and enzyme assays : In order to analyze the biochemical changes under drought stress, pigments such as Chl-a, Chl-b and carotenoids were assayed following the protocol of Lichtenthaler (1987) in acetone extract and absorbance was read at 470, 663, and 645 nm. Total soluble sugar (TSS) was estimated using anthrone reagent (Yem and Willis, 1954). Starch was measured as liberated glucose using anthrone reagent following the hydrolysis of extracted powder with perchloric acid (MacRae *et al.,* 1974). Proline and ascorbic acid were estimated following the methods of Bates *et al.* (1973) and Oser (1973), respectively. For measuring enzyme activities, leaves and roots' samples were ground under liquid nitrogen and later resuspended in 1.0 ml buffer solution containing 50 mMTris-HCl (pH 7.8) fortified with 1% polyvinylpyrrolidone (PVP). Homogenates were centrifuged at 10500x g for 20 min at 4ºC. The supernatant was collected to measure the activities of SOD, POX and CAT. However, for APX, the extraction buffer contained 50 mM phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM AA and 1% PVP. SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium at 560 nm (Vyas *et al.,* 2007). CAT and APX activities were determined using H_2O_2 as a substrate and reduction of H_2O_2 was monitored at 240 nm and 290 nm, respectively (Chance and Maehly, 1959). POX activity was determined using pyrogallol as substrate and purpurogallin formed was read at 430 nm (Mukhopadhyay *et al*. 2013a).

Abscisic acid (ABA) extraction and quantification : For ABA determination, leaves and roots' samples were homogenized separately in 2 ml chilled 80% methanol, containing butylated hydroxy toluene (BHT) (100 mgl^{-1}(Cabot *et al.,* 1986). Finally, 20 µl filtered sample was injected into a RP 18 (250 x 4.6 mm ID) column protected by a guard column in a HPLC system (Waters). Elution was carried out with methanol/water (v/v) with 1% acetic acid at a flow rate of 1 ml min^{-1}. Solvents were filtered through 0.45 µm filter (Millipore) and column was monitored by an UV detector at 254 nm ABA was measured by referring to a pure (±) standard ABA (Sigma). Authentic ABA was run for four times. The retention time of ABA was found to be 3.18 min.

Determination of ROS, lipid peroxidation and membrane stability levels : Estimation of O^{2-} was done by monitoring nitrate

formation from hydroxylamine (Jordan and Devay, 1990). Lipid peroxidation was determined by measuring malonaldehyde content, following the method of Ohkawa *et al.* (1979). H_2O_2 content and electrolyte leakage were assessed according to modified protocol of Bernt (1974). For estimating H_2O_2 content, samples were homogenized in 2 ml of 5% cold trichloroacetic acid followed by centrifugation at 13500x g and 40°C for 10 min. In 1.6 ml of the supernatant, 0.4 ml of 50% TCA, followed by 0.4 ml of 10 mM ferrous ammonium sulphate was added. Colour was developed by adding 0.2 ml of 2.5 M potassium thiocyanate, and subsequently reading was taken at 436 nm. Electrolyte leakage was determined by immersing one leaf in 30 ml distilled water and incubated at 25°C for 2 hr. The suspension medium was measured for initial electric conductivity (EC1). Samples were then autoclaved for 10 min to release all the electrolytes, cooled and the final electric conductivity (EC2) was measured. The percent leakage of electrolytes was calculated by the formula (EC1/EC2) ×100% (Bernt 1974).

Statistical analysis : All the quantitative data were subjected to one-way analysis of variance (ANOVA) for each parameter. The mean differences were evaluated for Least Significance Differences (<P 0.05) using Indostat statistical package (Hyderabad, India). Data were expressed as mean±SE error for four independent experiments.

Results and Discussion

In the present study, pigments such as Chl-a, Chl-b and carotenoid decreased in both the cultivars but more rapidly in S.3/A3 as compared to their respective control plants after 21 days of drought treatment (Table 1). Reduction of Chl-a, Chl-b and carotenoid was recorded as 22%, 3% and 14% in TV-23 and 25%, 8% and 16% in S.3/A3 with respect to control plants under drought stress. The Chl/Car ratio was enhanced to 113% in TV-23 and 110% in S.3/A3 in comparison to their respective control plants (Table 1). Drought stress not only cause substantial damage to photosynthetic pigments, but also leads to deterioration of thylakoid membranes due to accumulation of ROS (Ashraf and Harris, 2013).Thus, decrease in Chl content under drought stress is quite an obvious phenomenon which has been reported in a number of crop plants (Din *et al.,* 2011, Ashraf and Harris 2013). Carotenoids play an important role in protecting the photosynthetic apparatus against photo-inhibitory damage by singlet oxygen species, which are produced by the excited triplet state of chlorophyll (Loggini *et al.,* 1999). It is well known that carotenoids can directly deactivate $^1O_2$ and can also quench the excited triplet state of chlorophyll, thus indirectly reducing the formation of $^1O_2$ species (Loggini *et al.,* 1999). Hence, plants with higher capacity to maintain pigment degradation can tolerate drought stress.

Alteration in vacuolar structure was observed in both leaves and roots of TV-23 and S.3/A3 under drought stress. In leaves, though vacuolar structure gets deformed, membrane integrity was observed in TV-23 (Fig. 1B); however, pore formation or disruption in vacuolar membrane was observed in S.3/A3 (Fig. 1C). In roots, vacuole was shrunken and deformed in both the cultivar; however, there was membrane integrity in TV-23 (Fig. 1-D) on opposite to dissolved membrane in S.3/A3 (Fig. 1E). Due to high amount of compatible solutes in vacuole such as sugar and other derivatives for osmotic adjustment, it got swelled. On increasing stress level, the vacuolar membrane faces higher pressure and gradually, gets damaged (Blokhina *et al.,* 2003).

In the present study, disorientation of thylakoids and lamellar system was observed in both the cultivar under drought stress; however distortion was severe in S.3/A3 (Fig. 1H) in comparison to TV-23 (Fig. 1G).Shrinking of cells due to less water supply leads to loss of turgor, osmotic stress and change of membrane potentials in photosynthetic machinery (Mahajan and Tuteja, 2006).The results of the present study are in support with the previous findings in bean, maize and cotton where change in water supply seems to affect the structural characteristics of chloroplasts differentially in cultivars with different drought sensitivities (Stoyanova and Yordanov 1999). The ultrastructural changes reported in the above mentioned species include excessive swelling, distortion of thylakoids and appearance of lipid droplets due to accumulation of compatible solutes, antioxidants and detoxification of ROS (Da silva and Arrabaca, 2004)

The mitochondrial cristae were observed deformed in both roots and leaves of TV-23 (Fig. 1 J, L) and S.3/A3 (Fig. 1 K, M), however comparatively it was less in roots in between the tissues and in TV-23 in comparison to S.3/A3. Mitochondria are the site of ROS production through its electron transport chain (Chen *et al.,* 2005). Under stress condition, high production of ROS and their accumulation causes destruction of its own inner membranous structures. In susceptible cultivar, the mitochondrial structure was found almost destroyed due to comparatively higher production and accumulation of ROS and lour capability of detoxification.

In leaves, proline, TSS and ABA content increased in each cultivar after 21 days of drought stress (Table 1) but, the increment was more, such as, proline (28%), TSS (6%) and ABA (5%) in TV-23 in comparison to S.3/A3 (Table 1). However, starch (5%) and AA (10%) content decreased in TV-23 in comparison to S.3/A3 (Table 1). In roots, enhancement of proline, starch, TSS, AA and ABA content was registered in each cultivar (Table 1). However, enhancement of proline (68%), TSS (19%) and ABA (7%) was sharper in TV-23 in comparison to S.3/A3 (Table 1). In between the tissues, enhancement of proline was more in leaves i.e., 4% in TV-23 and 46% in S.3/A3;starch and AA were also enhanced more in leaves i.e., 9% and 61% in TV-23 as well as 17% and 74.6% in S.3/A3 respectively. In contrast, enhancement of TSS and ABA was more in roots, i.e., 17% and 6% in TV-23 as well as 4% and 4% in S.3/A3.

Table 1 : Effect of drought stress on pigments and other biochemical in tolerant (TV-23) and susceptible (S.3/A3) tea plantlets

Cultivars / Treatment	TV-23				S.3/A3			
	Control		Drought-stressed		Control		Drought-stressed	
	L	R	L	R	L	R	L	R
Chl a [µg g^{-1} f.wt.]	2.38±0.08^{a} (100)	--	1.85±0.05^{b} (78)	--	2.24±0.02^{a} (100)	--	1.67±0.04^{b} (75)	--
Chl b [µg g^{-1} f.wt.]	399 ±0.0^{ab} (100)	--	389±0.01^{ab} (97)	--	330 ±0.02^{a} (100)	--	303 ±0.01^{c} (92)	--
Car [µg g^{-1} f.wt.]	630 ±0.02^{a} (100)	--	540 ±0.01^{b} (86)	--	639 ±0.00^{a} (100)	--	534 ±0.01^{b} (84)	--
Chl / Car	0.64 ± 0.04 (100)	--	0.72 ± 0.06 (113)	--	0.52 ± 0.04 (100)	--	0.57 ± 0.05 (110)	--
Proline [µmol g^{-1} f.wt.]	2.38±0.1^{b} (100)	2.1±0.04^{c} (100)	4.68±0.4^{c} (194)	3.96±0.1^{a} (188)	5.61±0.3^{b} (100)	2.39±0.1^{c} (100)	9.34±0.3^{a} (166)	2.88±0.1^{b} (120)
Starch [mg g^{-1} f.wt.]	27.3±0.08^{a} (100)	29.13±0.7^{a} (100)	11.6±0.24^{c} (42)	9.54±1.41^{c} (33)	20.49±0.5^{b} (100)	25.5±0.82^{b} (100)	9.67±0.2^{d} (47)	7.56±0.5^{c} (30)
TSS [mg g^{-1} f.wt.]	31.8±1.02^{ab} (100)	34.5±0.2^{c} (100)	40.6±3.63^{a} (127)	49.7±1.04^{a} (144)	31.8±1.44^{ab} (100)	34.3±0.37^{c} (100)	38.8±2.54^{a} (121)	42.9±0.16^{b} (125)
AA [µmol g^{-1} f.wt.]	16.3±0.61^{bc} (100)	3.15±0.01^{c} (100)	28.8±1.22^{a} (176)	3.64±0.04^{a} (115)	10.5±1.12^{bc} (100)	2.97±0.02^{d} (100)	19.5±2.57^{b} (186)	3.31±0.0^{b} (111.4)
ABA [mg g^{-1} f.wt.]	90.5±0.26^{b} (100)	79.2±0.65^{b} (100)	96.3±0.53^{a} (106)	89.4±0.85^{a} (112)	79.4±0.85^{c} (100)	75.2±1.20^{c} (100)	80.04±0.5^{c} (101)	79.1±0.89^{b} (105)
MDA [nmol g^{-1} f.wt.]	29.4±0.05^{b} (100)	23.8±0.05^{ab} (100)	36.2±0.19^{a} (123)	27.9±0.03^{ab} (117)	23.2±0.0^{c} (100)	17.6±0.07^{c} (100)	31.5±0.05^{ab} (136)	27.8±0.07^{a} (158)
Electrolyte leakage [%]	8.6±0.35^{c} (100)	18.3±0.2^{b} (100)	15.4±0.7^{b} (180)	18.8±0.15^{b} (102)	8.95±0.3^{c} (100)	18.2±0.7^{b} (100)	32.7±0.0^{a} (365)	31.6±2.1^{a} (174)
Superoxide anion [A580]	0.8±0.03^{c} (100)	0.69±0.5^{b} (100)	1.53±0.1^{b} (188)	1.02±0.7^{a} (148)	0.89±0.02^{c} (100)	0.72±0.5^{b} (100)	1.82±0.05^{a} (204)	1.16±0.8^{a} (161)
H_2O_2 [nmolg^{-1} f.wt.]	6.8±0.95^{c} (100)	42.4±0.59^{c} (100)	12.8±0.91^{b} (188)	78.4±0.2^{b} (185)	6.50±1.79^{c} (100)	42.4±0.06^{c} (100)	26.12±2.0^{a} (401)	85.3±0.87^{a} (201)

(AA – ascorbic acid, ABA – abscisic acid, chl – chlorophyll, car – carotenoids, H_2O_2 – hydrogen peroxide, L – leaf, MDA – malondialdehyde, R – root, TSS – total soluble sugar ; Letters indicate Least Significant Differences by one-way analysis of variance). The figure in parenthesis indicates change in value as compared to their control treatment/tissue to the respective treatments and expresses in percentages.

Accumulation of soluble sugars and depletion of starch content in both leaves as well as roots of each cultivar in the present study finds support from the previous findings (Upadhyaya *et al.,* 2008, Hong-Bo *et al.,* 2008). A complex but essential role of soluble sugars in plant metabolism is well-known as products of hydrolytic processes, substrates in biosynthetic processes, energy production as well as in signalling systems (Hong-Bo *et al.,* 2008). Soluble sugars may also function as typical osmoprotectants and may stabilize cellular membranes and maintain turgor. Previous studies, with a variety of plants, demonstrate drought-induced conversion of hexoses and other carbohydrates such as sucrose and starch into sugar-alcohols (polyols) and proline (Upadhyaya *et al.,* 2008; Mohammadkhani and Heidari, 2008). In the present study, higher proline content in TV-23 confirmed that this cultivar was better protected under drought as it helped in maintaining osmotic stress; preventing membrane distortion and also scavenge hydroxyl radical. High proline accumulation in response to drought stress was also reported in several crop plants, including tea (Upadhyaya *et al.,* 2008). Ascorbate is a major primary antioxidant compound synthesized on the inner membrane of mitochodria which reacts chemically with reactive oxygen species and thiol radical and acts as a natural substrate of many plant peroxidases (Blokhina *et al.,* 2003). Drought stress damaged the membrane system of mitochondria along with other ultra-structures which may be a reason of finding the decreased level of ascorbate in the present study. Phytohormone ABA plays a key role in plant adaptation under adverse environmental conditions, including drought (de Carvalho, 2008). In the present study, the elevated ABA level in TV-23 may have a significant role in drought tolerance.

Activities of different antioxidative enzymes such as APX, SOD, POX and CAT are depicted in Fig. 2. There was an overall increase in APX activity which was increased 237.02% in leaves of S.3/A3, whereas it was 221.74% in TV-23. Although, APX activity was also showed a similar trend in roots, the enhancement was comparatively more in leaves. Similarly, SOD activity was found more in leaves of S.3/A3 (214.21 %) than TV-23

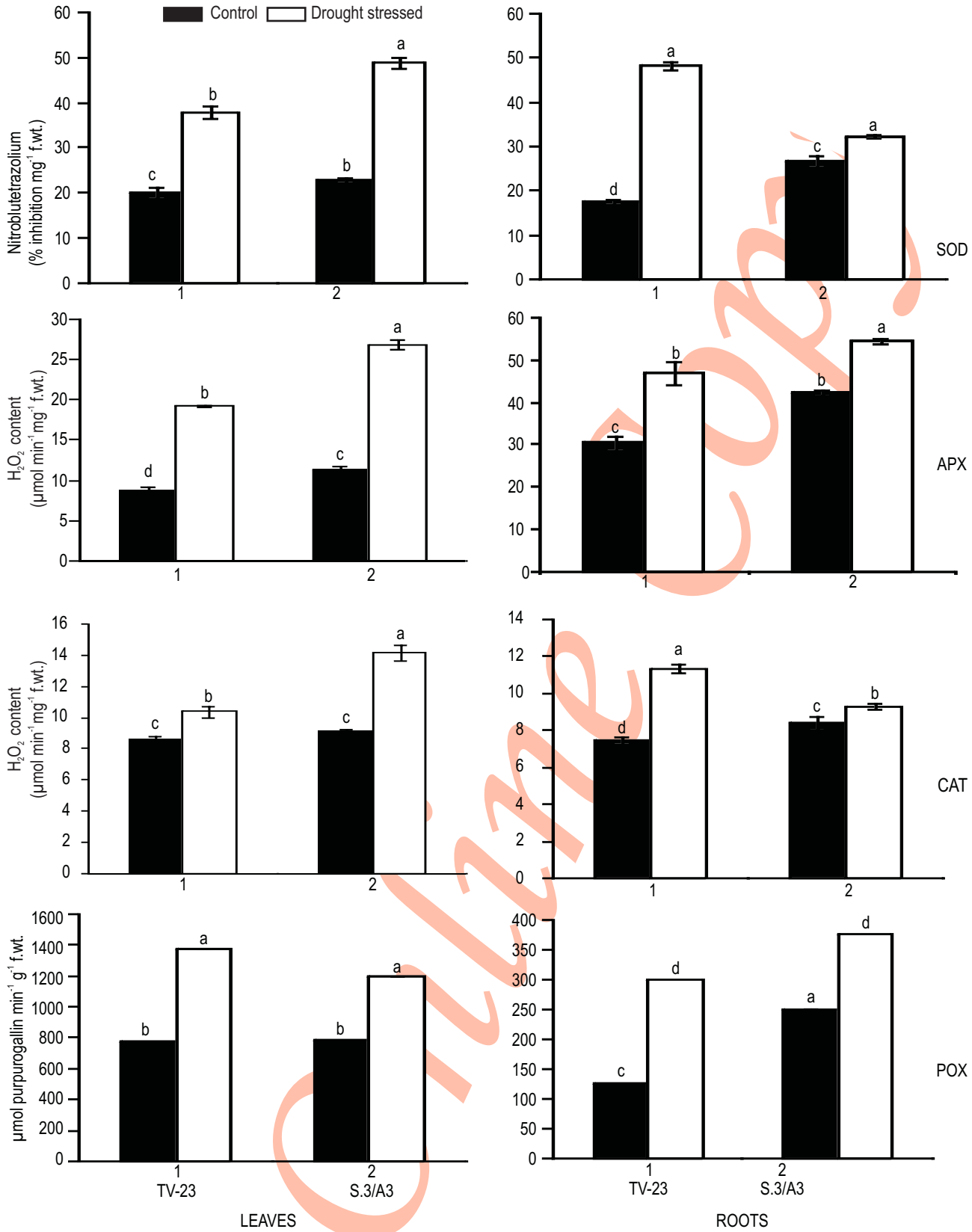

Fig. 2 : Antioxidative enzymes activitiy of tea roots and leaves under control and drought-stressed conditions (SOD=Superoxide dismutase, APX=Ascorbate peroxidase, CAT=Catalase, POX=Peroxidase; Letters indicate least significant differences by one-way analysis of variance)

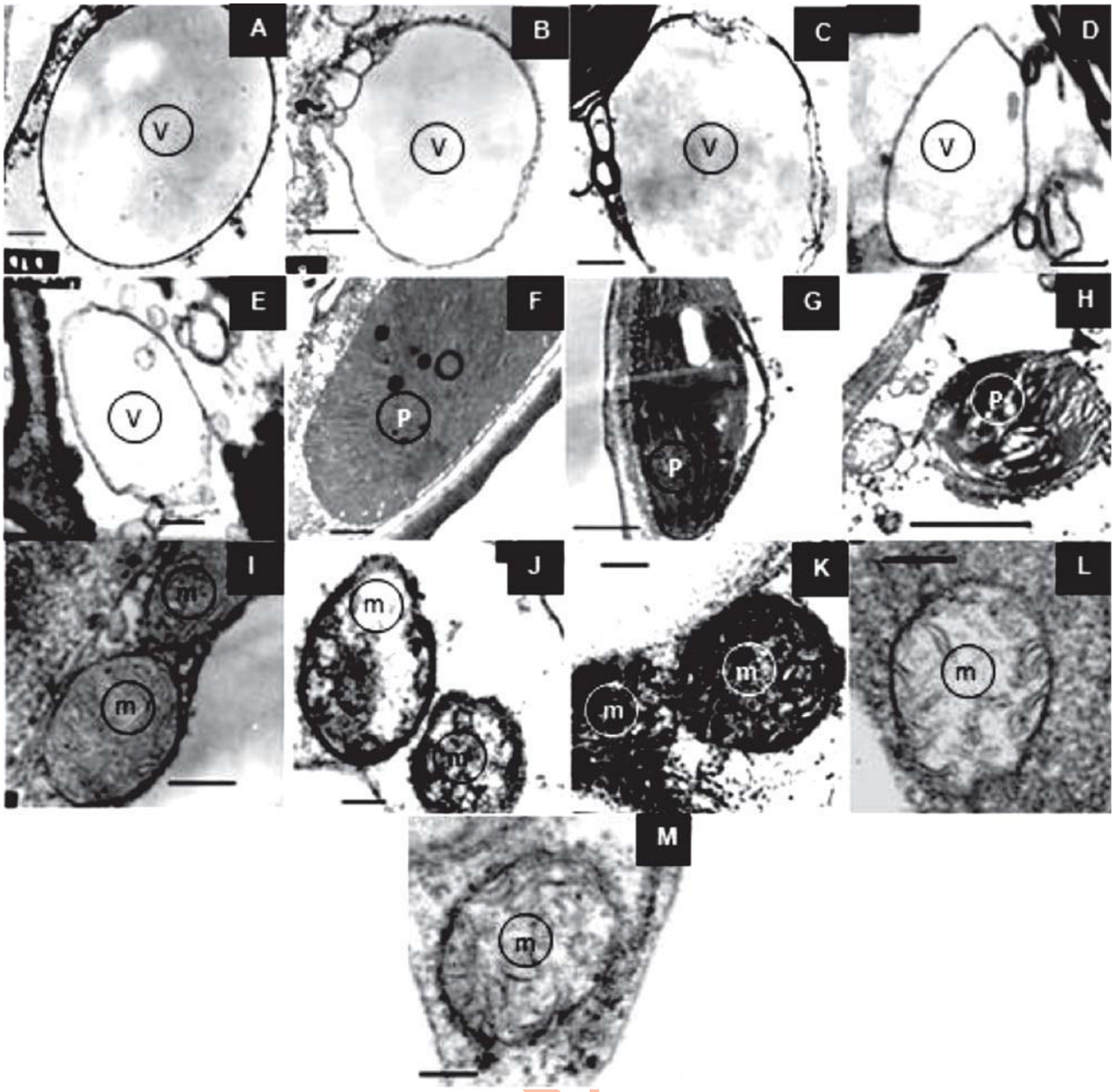

Fig. 1 : Ultrastructural changes in roots and leaves of tolerant and susceptible tea plantlets under drought stress (v=vacuoles, p=chloroplast, m=mitochondria, [A] normal vacuoles (1.25 cm = 0.37 μm). [B] vacuoles of drought stressed leaf of TV-23 with deformed structure (1.25 cm = 1 μm), [C] disruption of vacuolar wall of drought stressed leaf of S. 3/A3 (1.25 cm = 0.37 μm), [D] deformed structure of vacuoles of root fo TV-23 (1.25 cm = 0.37 μm), [E] dissolved vacuolar wall of root of S3/A3 (1.25 cm = 1μm), [F] normal chloroplast (1 cm = 0.71 mm), [G] chloropast with deformed grana of TV-23 (1.25 cm = 1μm), [H] chloroplast with distorted grana of S3/A3 (1.25 cm = 2.5 μm) [I] normal mitochondria (1.25 cm = 0.25 μm) [J] deformed sturcture of mitochondria of leaf of TV-23 (1 cm = 0.2 μm), [K] distorted structure of mitochondria of leaf of S.3/A3 (1 cm = 0.2 μm) [L] deformed structure of mitochondria of root of TV-23 root (1 cm = 0.25 μm) [M] deformed structure of mitochondria of root of S.A/A3 root (1 cm = 0.2 μm)

(188.81%), however, a reverse trend was registered in roots where it was 275.84% in TV-23 and 120.49% in S.3/A3. CAT activity was also enhanced in each cultivar in both types of tissues, but the increment was higher in TV-23. It increased by 177% and 153% in leaves as well as by 240% and 150% in roots of TV-23 and S.3/A3 respectively. Overall, CAT activity was found very high in leaves than in roots. Activity of POX registered a different trend. It was more in leaves of S.3/A3 (158%) in comparison to TV-23 (121%), however, in roots it was higher in TV-23 (151%) than in S.3/A3 (111%).

Antioxidant enzymes play an important role in defense

system of plants against oxidative stress. Balance between ROS production and activities of antioxidative enzymes determines whether oxidative signalling and/or damage will occur. The potential of scavenging ROS and reducing their damaging effects may correlate with drought tolerance of plants. In the present investigation, activity of POX enhanced with drought which has carrier been reported in various plant species including tea (Chakraborty *et al.,* 2002) suggesting that POX plays an important protective role against drought stress. Superoxide dismutase is an essential component of plant antioxidation system as it dismutates superoxide radicals to H_2O_2 and O_2 in cytosol, mitochondria and chloroplast (Hong-Bo *et al.* 2008). SOD activity was also found up-regulated by drought stress in a number of plant species including tea (Chakraborty *et al.,* 2002; Upadhyaya *et al.,* 2008). Similarly, CAT is also an important enzyme whose activity increased under drought stress. Enhancement of CAT activity with increasing stress indicated its role in front-line defense. Tea being a C_3 plant, higher CAT activity could scavenge H_2O_2 formed in the photo-respiratory pathway and thereby, reduce photorespiration rates (Upadhyaya *et al.* 2008). Increased level of APX activities in response to drought is well-established in literature (Srivalli *et al.,* 2003).

The oxidative biochemical markers such as MDA, O^{2-}, H_2O_2 content and percent electrolyte leakage increased under drought treatment in leaves of both TV-23 and S.3/A3 cultivars (Table 1). However, in between the cultivar, increment of percent electrolyte leakage (185%), O^{2-} (16%)and H_2O_2(213%) were more in S.3/A3 and statistically significant. Similarly, in roots, enhancement of MDA, O^{2-}, H_2O_2 content and percent electrolyte leakage were found in both the cultivars but the enhancement was more pronounced in S.3/A3 (Table 1).Enhancement was recorded as MDA (41%), O^{2-}(20%), H_2O_2(16%) in S.3/A3 in comparision to TV-23. In between tissues, enhancement of electrolyte leakage (78%) in TV-23 and (191%) in S.3/A3; O^{2-} (40%) in TV-23 and (43%) in S.3/A3 as well as H_2O_2(3%) in TV-23 and (200%) in S.3/A3 was recorded in leaves under drought stress. However, MDA content was enhanced by 6% in leaves of TV-23, whereas it was decreased by 22% in the leaves of S.3/A3 cultivar under drought stress.

Lipid peroxidation is often used as an indicator of increased oxidative damage. Low level of lipid peroxidation, H_2O_2 and O^{2-} content in leaves and roots of TV-23 further suggested that its drought-tolerance trail was due to better protection from cellular damages under stress. This result is in accordance with the previous findings of Upadhyaya *et al.* (2008) where tolerant-cultivar of tea showed lower membrane damage under drought stress. It was found that level of both MDA content and electrolyte leakage increased significantly in the leaves under drought stress. Higher level of lipid peroxidation found in the present study was probably due to the harmful effects of excess H_2O_2 or its ROS derivatives in cellular compartments. Excessive level of ROS may have resulted in damage to organelles, including photosynthetic apparatus ultimately leading to severe cellular damage and chlorosis of leaves (Mukhopadhyay *et al.,* 2013b).

The observation made in the present study suggested that both tolerant (TV-23) and susceptible (S.3/A3) cultivars responded to drought stress by adopting various biochemical changes, however, tolerant cultivar (TV-23) was more equipped in withstanding the stress. It was evident from the structural study of chloroplast, mitochondria and vacuoles, where less membrane integrity and higher structural damage were observed in the susceptible cultivar.

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