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Alterations In Enzyme Activities Due To Drought In Pot-grown Tea *(Camellia spp.)* Cultivars of Southern India

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ABSTRACT

The impact of different levels of water stress on various enzyme activities was studied in selected drought tolerant (DT) and drought susceptible (DS) pot-grown tea (*Camellia* spp.) cultivars. Activities of peroxidase (POD), acid phosphatase (APH), L-aspartate: 2 oxoglutarate aminotransferase (AOAT) and superoxide dismutase (SOD) were found to increase with a decrease in soil moisture in all the cultivars investigated. Although there was an initial increase in the activity of polyphenol oxidase (PPO) during the onset of drought stress, but it declined as the stress extended. Activity of nitrate reductase (NRA) was found to decrease with an increase in soil moisture deficit. DT cultivars recorded higher POD, AOAT, SOD and NRA activities while DS cultivars showed higher PPO and APH activities, under non-stress and stressed conditions. Hence, POD, AOAT, SOD and NRA could be used as markers for drought tolerance in tea plant breeding programs.

Key words: Peroxidase (POD); Polyphenol oxidase (PPO); Acid phosphatase (APH); L-aspartate : 2 oxoglutarate aminotransferase (AOAT); Superoxide dismutase (SOD); Nitrate reductase (NRA); drought tolerant (DT); drought susceptible (DS):

INTRODUCTION

Water stress usually reduces crop growth and productivity. Although several important physiological processes can be dramatically altered by water stress, it is uncertain which processes are important with respect to tolerance or resistance to drought. It seems probable that there is no single unique characteristic that will unequivocally convey drought tolerance or resistance in plants. Consequently, the development of

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more drought tolerant crops through plant breeding has been difficult. Nonetheless, genotypic differences in response to water stress with respect to stomatal activity in coconut palm (Shivashankar et al., 1991), osmotic adjustment in mung bean (Itoh et al., 1987), abscisic acid and proline levels in rice (Chou et al., 1991; Kim and Yuk, 1992) have been identified and could be used as selection criteria. Although adaptive roles for stress-induced physiological changes have been inferred from many crops, before a metabolic trait can be recommended for use in plant breeding work of a particular crop, its adaptive worth must be established beyond reasonable doubt (Hanson and Hitz, 1982). One approach to establish the adaptive worth of a metabolic response to stress is by comparing different cultivars of known drought tolerance/susceptibility.

All South Indian tea growing areas experience drought stress from mid-December to mid-March. In certain years, early cessation of northeast monsoon or delay in the receipt of summer showers stretches drought from end November to end-April/early May. Since tea plants are perennial, they have to withstand drought stress year after year. Hence, the development of drought tolerant (DT) tea cultivars is the only option to overcome the effect of drought, which requires characterization of the existing tea varieties. Once the characterization is over, the specific traits of DT plants can be used as markers for drought tolerance in plant improvement programs.

The present investigation deals with activities of certain important enzymes that are assumed important to drought tolerance, during different levels of water stress. The enzymes studied include the scavengers of toxic intermediates (POD; SOD), the key enzyme for black tea quality (PPO), the important enzyme for Pi nutrition (APH), the vital enzyme in the assimilation of C and N compounds (AOAT) and the fundamental enzyme for nitrate reduction (NRA). As the varying environmental parameters in any given day may alter enzyme activities adversely, this study on enzyme alterations was carried out using pot-grown tea cultivars under controlled environment, inside a green house.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse maintained at the Experimental Farm of UPASI Tea Research Foundation, located in Anamallais, Coimbatore District, India, at an elevation of 1050 m amsl. Pot-grown tea plants were used in this study of which some were DT (UPASI-1, UPASI-2 and UPASI-9) and others were drought susceptible (DS) (UPASI-3, UPASI-8 and UPASI-17) based on the assessment of their survival and field performance during soil moisture stress periods over several years (Satyanarayana *et al.*, 1992) and based on morphological and physiological factors (Rajasekar *et al.*, 1988).

Aperiodic shoots from each clone were used to raise plants in polythene bags during January 2002. After 12 months growth in the nursery, twelve plants from each cultivar was transferred to pots during January 2003 and acclimatized under greenhouse conditions. An acidic (pH: 4.8) sandy loam soil was used for filling the pots, which had an electrical conductivity of 0.03 dS m^{-1}. The water holding capacity of the soil was 55 per cent. Plants were maintained in the greenhouse throughout the experimental period.

Day temperatures ranged from 24 to 29 °C and night temperatures from 12 to 17 °C. The relative humidity was between 50 and 80%. Although irradiation varied, the photosynthetically active radiation (PAR) was generally $\geq 1400 \mu Em^{-2} sec^{-1}$. In order to induce more laterals, these plants were centered (removal of apical bud) at 15 to 20 cm height. Immediately after induction of adequate branches/crop shoots, drought stress was induced at different levels by withholding watering. Control plants were watered (150 ml) once in two days. At the end of each treatment, soil moisture was determined gravimetrically and the shoots (three leaves and a bud) were excised (between 08:00 Hrs to 10:00 Hrs), weighed, and immersed immediately in ice-cold acetone. The tissue was ground with a pestle in a pre-chilled mortar. The slurries were suspended in 180 ml ice-cold acetone and filtered through Whatman No.1 filter paper. The residues were washed once with ice-cold ethyl ether. Dried, pigment-free acetone powders were stored in aluminium foils at –20 °C and used for the extraction of all the enzymes except NRA.

Statistical design

Effect of water stress was studied in six clones (12 plants/clone). There were four treatments viz., drought stress induced for 5 days, for 8 days and for 10 days, besides watered control. There were three samplings from three different plants to study the effect of each treatment in each cultivar. All assays were repeated thrice from each sampling and data were statistically analysed to calculate the critical difference between the tea cultivars and moisture regimes at 0.05 level.

Following assays were conducted:

1. *Peroxidase* (EC. 1.11.1.7) and *Polyphenol oxidase* (EC. 1.10.3.1)
   POD and PPO in the acetone powder was assayed by the method of Gregory and Bendall (1973), using a sensitive Clark's type oxygen electrode (Model 97-08-99, Orion Inc., USA) fitted with a voltmeter. POD and PPO activities were expressed as μmole O_2 released and consumed, respectively min^{-1} g^{-1} fresh wt.

2. *Acid phosphatase* (EC. 3.1.3.2)
   APH activity was assayed at 410 nm using the method of Baker and Takeo (1974). One unit of APH activity was defined as 1μ mole of PNP produced h^{-1} g^{-1} fresh wt and expressed as units mg^{-1} protein.

3. *L-aspartate: 2 oxoglutarate aminotransferase* (EC. 2.6.1.1)
   AOAT was assayed according to Sadasivam and Manickam (1996), by measuring the oxaloacetic acid at 510 nm. The enzyme activity was expressed as μg oxaloacetic acid formed h^{-1} mg^{-1} protein.

4. *Superoxide dismutase* (EC. 1.15.1.1)
   The method of SOD assay, described by Lee and Bennett (1982), was based on SOD inhibition of superoxide-mediated ferri-cytochrome *c* reduction. The rate of reaction was read (at 550 nm) at 15 s intervals for 1 to 2 min. One unit of SOD activity was defined as that which inhibited 50% of the reaction rate and expressed as units mg^{-1} protein.

5. *Nitrate reductase* (EC. 1.6.6.1)

NRA was assayed in the crop shoots using the method of Jaworski (1971) at 540 nm. The activity of the NRA was expressed as µmoles nitrite produced g^{-1} fresh wt h^{-1}.

RESULTS

Changes in POD and PPO due to water stress

POD activity increased with a decrease in soil moisture in the ranges studied (19.7 to 3.2 % soil moisture), in all the cultivars investigated. Under non-stress and stressed conditions, DT cultivars showed higher POD activities. The mean values of the two categories of cultivars indicate that the proportionate increase was much pronounced in tolerant cultivars than susceptible ones (Table 1). PPO activity was found to be higher in DS cultivars than tolerant ones during stress and non-stress conditions. Although there was an initial increase in the activity when the soil moisture decreased to 15.3%, and thereafter all the cultivars exhibited a sudden drop in the enzyme activity (Table 2).

Table 1: Changes in peroxidase (µmole O_2 released min^{-1} g^{-1} fresh wt) activity due to drought stress

Clones	Soil moisture (%)			
	19.7 (Control)	15.3 (5 days)	6.1 (8 days)	3.2 (10 days)
Drought tolerant				
UPASI-1	256 ± 5.51	263 ± 4.0	267 ± 4.0	274 ± 4.16
UPASI-2	255 ± 8.19	260 ± 7.8	265 ± 2.9	270 ± 3.79
UPASI-9	243 ± 4.51	243 ± 7.0	249 ± 3.2	255 ± 2.65
Mean	251	255	260	266
Drought susceptible				
UPASI-3	219 ± 5.00	227 ± 4.0	232 ± 5.0	235 ± 3.61
UPASI-8	189 ± 7.00	191 ± 4.6	193 ± 3.1	195 ± 1.53
UPASI-17	188 ± 7.81	192 ± 4.0	195 ± 1.5	197 ± 3.51
Mean	199	203	207	209
Statistical significance		SE	CD (5%)	CV
between clones		2.06	4.03	2.2
between regimes		1.68	3.29	
Regime x clone		4.11	8.06	
Mean ± SD				

Table 2: Changes in polyphenol oxidase (μmole O_2 consumed min^{-1} g^{-1} fresh wt) activity due to drought stress

Clones	Soil moisture (%)			
	19.7 (Control)	15.3 (5 days)	6.1 (8 days)	3.2 (10 days)
Drought tolerant				
UPASI-1	15.3 ± 3.21	18.3 ± 4.2	13.0 ± 1.0	11.0 ± 2.65
UPASI-2	25.0 ± 3.00	25.3 ± 2.1	22.0 ± 3.6	19.0 ± 2.65
UPASI-9	15.7 ± 2.52	17.3 ± 3.1	14.0 ± 2.0	10.3 ± 2.52
Mean	18.7	20.3	16.3	13.4
Drought susceptible				
UPASI-3	30.3 ± 2.08	31.3 ± 3.1	28.0 ± 3.0	21.7 ± 3.51
UPASI-8	36.0 ± 2.65	36.3 ± 1.5	32.0 ± 1.0	28.7 ± 3.06
UPASI-17	33.7 ± 2.52	35.0 ± 2.0	31.0 ± 3.0	28.3 ± 3.22
Mean	33.3	34.2	30.3	26.2
Statistical significance		SE	CD (5%)	CV
between clones		1.23	2.40	12.45
between regimes		1.00	1.96	
Regime x clone		2.45	4.80	
Mean ± SD				

Changes in APH due to water stress

In the case of APH, although the trend with respect to moisture stress was similar to that of POD, susceptible clones exhibited higher activity compared to tolerant cultivars (Table 3) under stress and non-stress conditions. UPASI-8 and UPASI-17 possessed more APH activity at reduced soil moisture levels.

Table 3: Changes in acid phosphatase (units mg^{-1} protein) activity due to drought stress

Clones	Soil moisture (%)			
	19.7 (Control)	15.3 (5 days)	6.1 (8 days)	3.2 (10 days)
Drought tolerant				
UPASI-1	83.0 ± 1.36	84.3 ± 1.3	88.4 ± 1.5	93.9 ± 1.22
UPASI-2	89.6 ± 1.41	91.9 ± 1.3	95.9 ± 1.3	98.4 ± 1.28
UPASI-9	83.3 ± 1.41	84.7 ± 1.5	88.6 ± 1.5	92.5 ± 1.36
Mean	85.3	87.0	91.0	95.0
Drought susceptible				
UPASI-3	90.3 ± 1.40	92.9 ± 1.7	96.9 ± 1.4	99.4 ± 1.19
UPASI-8	92.6 ± 1.35	94.7 ± 1.3	98.3 ± 1.2	104.3 ± 1.20
UPASI-17	92.3 ± 1.36	95.4 ± 1.3	97.0 ± 1.3	105.6 ± 1.35
Mean	91.7	94.3	97.4	103.1
Statistical significance		SE	CD (5%)	CV
between clones		0.22	0.43	0.6
between regimes		0.18	0.35	
Regime x clone		0.43	0.85	
Mean ± SD				

Changes in AOAT due to water stress

The activity of AOAT enzyme followed the same trend as in POD with respect to moisture stress i.e., an increase in AOAT activity with an increase in water deficit. Under all soil moisture regimes, the DT bushes possessed higher AOAT activity (mean 1.22 to 1.61 µg oxaloacetic acid formed h^{-1} mg^{-1} protein) than susceptible ones (mean 0.89 to 1.25 µg oxaloacetic acid formed h^{-1} mg^{-1} protein) (Table 4).

Table 4: Changes in AOAT (μ g oxaloacetic acid formed h^{-1} mg^{-1} protein) activity due to drought stress

Clones	Soil moisture (%)			
	19.7 (Control)	15.3 (5 days)	6.1 (8 days)	3.2 (10 days)
Drought tolerant				
UPASI-1	1.21 ± 0.27	1.42 ± 0.08	1.61 ± 0.20	1.83 ± 0.24
UPASI-2	1.33 ± 0.32	1.51 ± 0.27	1.41 ± 0.22	1.51 ± 0.25
UPASI-9	1.11 ± 0.28	1.30 ± 0.24	1.53 ± 0.34	1.51 ± 0.45
Mean	1.22	1.41	1.52	1.61
Drought susceptible				
UPASI-3	0.82 ± 0.29	0.91 ± 0.27	1.04 ± 0.23	1.12 ± 0.24
UPASI-8	0.82 ± 0.32	0.91 ± 0.07	1.12 ± 0.22	1.32 ± 0.25
UPASI-17	1.04 ± 0.33	1.04 ± 0.28	1.23 ± 0.22	1.31 ± 0.38
Mean	0.89	0.95	1.13	1.25

Statistical significance	SE	CD (5%)	CV
between clones	0.05	0.10	10.1
between regimes	0.04	0.08	
Regime x clone	0.10	0.20	

Mean ± SD

Changes in SOD due to water stress

With the increase in moisture stress, SOD activity increased in tolerant and susceptible cultivars. Here also, the rate of activity was higher in tolerant cultivars (Table 5) under stress and non-stress conditions. Among the DT cultivars, UPASI-9 showed the highest activity in all the stress regimes studied followed by UPASI-1 and UPASI-2. Likewise among the susceptible cultivars, UPASI-17 excelled with an activity of 326 units/mg protein in the least moisture condition while UPASI-3 stood last with 285 units/ mg protein in the above stress level.

Table 5: Changes in superoxide dismutase (units mg^{-1} protein) activity due to drought stress

Clones	Soil moisture (%)			
	19.7 (Control)	15.3 (5 days)	6.1 (8 days)	3.2 (10 days)
Drought tolerant				
UPASI-1	320 ± 4.34	329 ± 4.04	333 ± 4.73	344 ± 5.13
UPASI-2	307 ± 9.07	318 ± 3.00	325 ± 3.21	333 ± 4.51
UPASI-9	326 ± 9.28	332 ± 3.06	338 ± 2.65	346 ± 3.21
Mean	318	326	332	341
Drought susceptible				
UPASI-3	263 ± 4.33	273 ± 3.61	278 ± 6.51	285 ± 8.50
UPASI-8	278 ± 4.92	285 ± 9.00	293 ± 3.21	308 ± 3.00
UPASI-17	293 ± 7.46	312 ± 3.46	322 ± 4.04	326 ± 4.73
Mean	278	290	298	306
Statistical significance		SE	CD (5%)	CV
between clones		2.34	4.60	1.8
between regimes		1.91	3.75	
Regime x clone		4.69	9.19	
Mean ± SD				

Changes in NRA due to water stress

Unlike other enzymes discussed so far, NRA activity was found to decrease with the increase in moisture stress (Table 6). Although the proportionate decrease was higher in DT cultivars, they were able to maintain higher activities of NRA than susceptible ones at all soil moisture regimes.

Table 6: Changes in nitrate reductase (μmoles nitrite produced g^{-1} fresh wt h^{-1}) activity due to drought stress

Clones	Soil moisture (%)			
	19.7 (Control)	15.3 (5 days)	6.1 (8 days)	3.2 (10 days)
Drought tolerant				
UPASI-1	0.84 ± 0.06	0.76 ± 0.02	0.65 ± 0.04	0.59 ± 0.02
UPASI-2	0.87 ± 0.06	0.75 ± 0.04	0.64 ± 0.04	0.57 ± 0.05
UPASI-9	0.82 ± 0.02	0.72 ± 0.04	0.65 ± 0.04	0.53 ± 0.04
Mean	0.84	0.74	0.65	0.56
Drought susceptible				
UPASI-3	0.73 ± 0.05	0.66 ± 0.02	0.57 ± 0.02	0.46 ± 0.02
UPASI-8	0.70 ± 0.05	0.64 ± 0.03	0.54 ± 0.03	0.48 ± 0.03
UPASI-17	0.66 ± 0.03	0.66 ± 0.01	0.56 ± 0.03	0.49 ± 0.08
Mean	0.70	0.65	0.55	0.48

Statistical significance	SE	CD (5%)	CV
between clones	0.02	0.03	6.6
between regimes	0.01	0.03	
Regime x clone	0.03	0.07	

Mean ± SD

DISCUSSION

Drought stress led to increased POD activity irrespective of DT nature of tea cultivars. Jong (1973) reported that POD activities are frequently malleable with environmental factors. An initial increase in PPO activity was observed during the onset of drought stress in different tea cultivars; thereafter it started to decline in its activity. PPO is a plastid enzyme which is in inactive form and gets activated only when released into the cytoplasm following cell injury (Vaughn *et al.*, 1988). Chakraborty *et al.* (2000) observed an increase in PPO activity in tea leaves up to 40 or 45 °C, after which there was a decline in the activity. PPO and POD are inter-related enzymes such that the increased levels of hydroperoxides associated with drought susceptibility can be attributed to the higher activity of PPO and lower activity of POD (Kasturibai *et al.*, 1996).

An increase in the activity of APH was observed in both categories of tea cultivars during drought stress. Water deficit may cause increased APH activities in leaves of

higher plants, e.g. cowpeas (Takaoki, 1968) and cotton (Vieira-da-Silva, 1969). It is not known whether this is due to the effect of water deficit on P nutrition. Allowing tomato plants to drought by making the soil dry to 50 % of the field capacity over 6-8 days caused a 15-25% reduction in the concentration of phosphate in the leaves (Gates, 1957). Furthermore, exposure of tomato roots to a solute potential of -540 kPa for 1 h caused a 70 % reduction in P transport into the shoot (Greenway *et al.*, 1969).

Disturbances in Pi-nutrition appear to be partially responsible for the reduced growth of plants undergoing moderate water deficit (Greenway *et al.*, 1969). Not surprisingly, drought-stressed plants therefore appear to induce enzymes, such as APH, that are characteristic of the Pi starvation response (Duff *et al.*, 1994). APH, which is a hydrolytic enzyme, usually gets released from the chloroplast into cytoplasm due to the drought induced membrane damage (Vieira da Silva, 1976).

The transaminases play an adaptive role under environmental stress conditions. Further, this forms an important link between carbohydrate and protein metabolism under stressful conditions. Ketoacids are important intermediary metabolites and provide carbon skeletons for the synthesis of amino acids and thus help in detoxifying the ammonia that accumulated; however, this is largely decided by the transamination reactions (Knox and Gregnard, 1965). AOAT mediated the utilization of excessive ammonia by converting ketoacids into amino acids under stress conditions (Sheoran *et al.*, 1981). The elevated activities of AOAT noticed in the present study may further explain the accumulation of amino acids during drought as observed by earlier workers (Dubey, 1994; Thakur and Thakur, 1987). AOAT plays an important role in the synthesis of secondary metabolites such as proline (Fruton and Simmonds, 1965). Enhanced activity of APH and AOAT during induced stress as well as with the development of moisture stress under field condition in the tolerant than the susceptible types has been reported in coconut earlier (Shivashankar *et al.*, 1991).

SOD and POD play important roles in the scavenging of toxic intermediates of the incomplete oxidation of tissues (Dhindsa *et al.*, 1982), thus maintaining cell membrane integrity (Elstner *et al.*, 1988). The lower activity of these enzymes can result in the formation of superoxide radical $(O^2)^+$ and H_2O_2. The result of the present investigation was in accordance with the findings of Chempakam *et al.* (1993) who reported increased activity of SOD in DT than the DS coconut types with respect to lower lipid peroxidation levels.

NRA is known to be modulated rapidly in response to change in environmental conditions (Kaiser and Huber, 1994). Impaired nitrate reduction as a result of decline in NRA activity during water deficit (Kenis *et al.*, 1994) with a concurrent nitrate accumulation in the tissue (Kaiser and Forster, 1989) has been reported. It has been shown that NRA activity was relatively less inhibited in the DT wheat cultivar (Sairam, 1994). It can be deduced that the tolerant genotypes may exhibit relatively less inhibition in the activities of NRA during periods of drought stress, which may be due to the genotype's ability to deal with stress more effectively.

Activities of POD, AOAT, SOD and NRA were higher in the DT cultivars while activities of PPO and APH were higher in DS cultivars, under non-stress and stressed conditions. Higher activities of POD, AOAT, SOD and NRA may allow greater drought tolerance and hence, could be used as selection criteria. Transgenic plants over-expressing these traits will be promising in the future under conditions of recurring drought. This paper has provided evidence that genetic differences do exist in tea cultivars with respect to response of several bio-chemical pathways to water stress.

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