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EFFECT OF WATER AND UV-B STRESSES ON ANTIOXIDANT SYSTEMS IN TOBACCO GENOTYPES

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The role of antioxidants in protecting the tobacco cultivars having different response to water stress from deleterious effect of oxidative stress induced by water and UV-B stress was investigated. The cultivars of tobacco to two cultivated species were able to adopt themselves to water and UV-B stresses by increasing the activities of antioxidant enzymes. The differences in the peroxidase (POX) activity among the tobacco cultivars were significant under both stresses except in the variety Pyruvithanam which was susceptible to water stress. The cultivars Kanchan, Banket A1 and Ratna (*N. tabacum*) showed maximum increase in POX activity in response to both stresses. Polyphenol oxidase activity increased significantly in all the cultivars in water stress whereas it increased non-significantly under UV-B stress. Thiobarbituric acid reactive substances (TBARS) content varied considerably under stress condition. A coordinated response involving the high activity of SOD, CAT, POX and ascorbic acid is triggered in the varieties Kanchan, Banket A1 and Ratna which are having water stress tolerance to withstand the oxidative stress.

INTRODUCTION

Plants are exposed to both abiotic and biotic stresses throughout their life span. In addition to growth, various metabolic processes are affected at the advent of stress and the magnitude of effects varies (Levitt, 1972). Any stress is known to induce production of reactive oxygen species (ROS) that causes damage to the cell and/or signal the start of biochemical responses (Scandaleos, 1993). Many plant species are able to acclimatize to changes in ultraviolet-B radiation (UV-B) (290-320 nm) exposure. Due to the wide range of targets of UV-B, plants have evolved diverse repair and protection mechanisms (Arora *et al.*, 2002). These include increased biosynthesis of UV-B screening compounds (POX, TBAS, CAT, Ascorbate) and elevated antioxidant activity and increased rates of DNA repair (Rao *et al.*, 1996).

Abiotic stress particularly water deficit stress associated with high light intensity generates toxic radicals which react with biomolecules like proteins and DNA and cause denature. The water stress and UV-B exposure induce the production of reactive oxygen species such as super oxide radicals (O^{-}_{2}), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). The plants intern enhance the activities of enzymatic and non enzymatic antioxidants viz. superoxide dismutase, ascorbate peroxidase, catalase and metabolites like ascorbate, glutathione, á-tocopherol and carotnoids *etc* to combat the oxidative stress injury under the stress conditions (Scandaleos, 1993).

The objective of the present study was to investigate the role of antioxidant enzymes and metabolites under water and UV-B stresses in the tobacco cultivars of two *Nicotiana* species (*Nicotiana tabacum* and *Nicotiana rustica*) in order to evaluate the importance of these oxidative systems in controlling the levels of ROS species generated during the above stress.

MATERIALS AND METHODS

Four cultivars belonging to *N. tabacum* species *viz.*, Kanchan, Ratna, Banket A1 (water stress tolerant) and Pyruvithanam (water stress susceptible) and two cultivars of *N. rustica* species (GC-1 and HD 60-45) differing in their ability to withstand drought under field condition were subjected to short term water stress and UV-B stress. Seeds of the above varieties were surface sterilized with 0.1% mercuric chloride solution for 3 minutes and then thoroughly washed with double distilled water. The seeds were placed in Petri dish lined with double layer of Whatman No. 1 filter paper. Seeds were germinated at 28±2°C under laboratory conditions. Water stress was induced at 10 days old seedlings by giving polyethylene

glycol solution (-9 bars) for two days before the analysis. For UV-B treatment, 10 days old seedlings were exposed to UV-B light for one hour before analysis.

Enzyme extract for superoxide dismutase (SOD) was prepared by grinding 0.25 g seedlings with 5 ml of chilled phosphate buffer (0.1M, pH 7.8) while for catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO), the extract was prepared by grinding 0.25 g seedlings with 5 ml of chilled phosphate buffer (0.1 M, pH 6.8). The homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant served as an enzyme source. The SOD activity determined according to the method of Beauchamp and Fridovich (1971). Catalase, peroxidase and polyphenol oxidase activities were assayed by the method of Chance and Maehly (1955).

For the determination of ascorbate (Mukherjee and Choudhuri, 1983) the seedlings (0.5 g) were homogenized in cold mortar with 10 ml of 6% trichloroacetic acid and centrifuged at 2000 rpm for 10 min. To 4.0 ml of the supernatant, 2.0 ml of 2% dinitrophenylhydrazine (in acidic medium) and 1 drop of 10% thiourea (in 70% ethanol) were added. The mixture was kept in boiling water bath for 15 min and after cooling to room temperature, 5.0 ml of 80% (v/v) H_2SO_4 was added to it at 0°C and the absorbance recorded at 530 nm. The concentration of ascorbate was calculated from a standard curve plotted with known concentration of ascorbic acid.

To measure the content of Thiobarbituric acid reactive substances (TBARS), the seedlings (0.5 g) were homogenized in 10 ml of 0.1% trichloroacetic acid and the homogenate was centrifuged at 10,000 rpm for 20 min. To 1 ml of the supernatant, 4.0 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added. The mixture was heated at 95°C for 30 min and quickly cooled in ice bath. The contents were centrifuged at 10,000 rpm for 10 min and the absorbance of the supernatant was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of TBARS was calculated using TBRAS extinction coefficient of 155 $mmol^{-1}cm^{-1}$ and expressed as nmol g^{-1} fresh weight following the method of Heath and Packer (1968). The proline content was estimated by the method of Bates *et a*l. (1973).

RESULTS AND DISCUSSION

The role of antioxidants in protecting the tobacco cultivars having different response to water stress from deleterious effect of oxidative stress induced by water and UV-B stress was investigated. The effects of reactive oxygen species (ROS) on membrane and cellular damages are likely to differ depending on the stress imposed. Interaction of antioxidant enzymes SOD, CAT, POX and PPO and their expression and involvement in scavenging ROS are very complex.

Differences in the POX activity among the tobacco cultivars were significant under both stresses compared to control except in the variety Pyruvithanam which was susceptible to water stress (Table 1). The cultivars Kanchan, Banket A1, and Ratna (*N. tabacum*) showed maximum increase in POX activity in response to both stresses. Variety GC-1 showed significant increase in POX activity under both stresses compared to the variety HD 60-45. Peroxidase decomposes H_2O_2 by oxidation of phenolic compounds. Increased activity of POX in response to water stress was reported in maize (Zhang *et al.*, 1995), *Cassia* (Sheela and Panday, 2003), *Cucumis sativus* (Tekchandani and Guru Prasad, 1988), decreased in sunflower and sorghum seedlings (Zhang and Kirkhan, 1996) and no change in wheat (Fagmeir *et al.*, 1994) while, Norieki and Mika (2000) have reported an increase in POX activity in cucumber seedlings under UV-B stress.

Catalase (CAT) activity increased non significantly in water and UV-B stress in all the cultivars except variety HD60-45 where it decreased (Table 1). Catalase scavenges H_2O_2 by breaking it directly to form water and oxygen and an increase in its activity is related with increase in stress tolerance (Kraus *et al.*, 1995). Previous studies have shown that response of CAT activity to water stress may be varied. CAT activity was not affected by mild drought in sorghum (Zahng and Kirkham, 1996), decreased in pea (Moran *et al.*, 1994) and increased in wheat genotype C306 (Sairam and Srivastava, 2001). Dawar *et al.* (1998)

have reported increased activities of CAT and SOD during first hour of UV-B exposure in wheat. The increased levels of H_2O_2 presumably stimulate CAT activity.

Polyphenol oxidase activity increased significantly in all the cultivars under water stress whereas it was increased non-significantly under UV-B stress (Table 1). Polyphenol oxidase and peroxidase are the two major enzymes responsible for oxidation of phenolic compounds (Balakrishna *et al.*, 2008). Namiki (1990) observed that plyphenols from dry bean may act as anti-oxidants to inhibit the formation of damaging free radicals. Increase in the activities of these enzymes under water stress and UV-B stress could be indicative of an increased production of ROS and a build up of a protective mechanism to reduce oxidative damage triggered by stress in plants (Balakrishna *et al.*, 2008).

The activity of superoxide dismutase (SOD) varied with the stress imposed on the seedlings of two species (Table 1). Under water stress, the activity was significantly increased in all the cultivars except variety Pyruvithanam. Under UV-B stress, SOD activity was significantly increased in the cultivars Kanchan and Banket A1 whereas in other cultivars there was non significant increase in the activity. Increase the activity of SOD was more in the water stress tolerant cultivars compared to other cultivars. The increased activity of SOD might protect the plants from oxidative injury would not favor accumulation of (O^-_2). SOD catalyses the dismutation of O^-_2 to H_2O_2 and plays a key role in quenching the active oxygen. A varied response of SOD activity to water stress has been reported. SOD activity was not influenced by water stress in sorghum (Zhang and Kirkham, 1996) and wheat (Sairam *et al.*, 1998; Sairam and Srivatsava, 2001). Activity of SOD increased in cucumber seedlings exposed to 6 h of UV-B radiation (Noriaki and Mika, 2000) whereas in *Cassia* and wheat seedlings it increased within 1 h (Sheela and Varsha, 2003; Dawar *et al.*, 1998).

Proline content increased under water stress in all the cultivars whereas it significantly increased in the variety Ratna under UV-B stress, the proline content increased significantly in Ratna cultivar (Table 2). However, the extent of increase in proline content over initial value was significantly higher in the water stress tolerant cultivars *i.e.* Kanchan, Banket A1 and Ratna than the Pyruvittanam, GC-1 and HD 65-40 (susceptible). Increased proline content was reported in the water stressed *Calamagrotis arundinaceae* plants (Kramarov *et al.*, 1999). Proline accumulation confers adaptive mechanism to water stress and helps in stress tolerance either by rehydration of protoplasm or by providing energy for recovery of plants (Kramarov *et al.*, 1999).

Ascorbic acid is often considered as stress antioxidant since it is formed in large quantities under the influence of abiotic stresses such as moisture stress, heat or salinity stress. In the present study, ascorbic acid content was estimated in six genotypes of tobacco both under control and stress situation. Ascorbic acid content significantly increased in the water stress tolerant varieties (Kanchan, Ratna and Banket A1) whereas, it non-significantly increased in other cultivars indicating that tolerant types are better equipped to synthesize and accumulate higher quantities of ascorbic acid than the susceptible types (Table 2). Under UV-B stress, ascorbate content increased significantly in the cultivars Kanchan, Ratna and decreased in the variety Banket A1. Ascorbic acid participates in the removal of H_2O_2 as a substrate of ascorbic peroxidase directly and reduces superoxide, quenching singlet oxygen and generate reduced α –tocopherol. Reduction in ascorbic acid in response to drought was reported in *Vigna catjang* (Mukherjee and Choudhari, 1983), sorghum (Zhang and Kirkham, 1996) and wheat (Bartoli *et al.*, 1999). The increased content of ascorbate in varieties Kanchan and Ratna could be postulated as a key factor to control the oxidation at membrane level, limiting the increase in lipid radical content.

The amount of lipid peroxidation has also long been considered as one of the factors which indicate the severity of stress experienced by the plant (Dhindsa *et al.*, 1982). Lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS) in six genotypes of tobacco under control and stress situations. The TBARS content was almost same in normal condition in all genotypes of tobacco but varied considerably

Table 1: Effect of water stress and UV-B stress on enzyme activities

Variety	Peroxidase			Polyphenol oxidase			Catalase			Superoxide dismutase			Acid phosphotase		
	Control	WS	UV-B	Control	WS	UV-B	Control	WS	UV-B	Control	WS	UV-B	Control	WS	UV-B
Kanchan*	0.205	0.297	0.285	0.098	0.128	0.03	0.134	0.172	0.135	0.600	0.946	0.880	17.71	18.90	17.40
BanketA1*	0.216	0.296	0.256	0.154	0.170	0.164	0.146	0.183	0.161	0.670	1.060	1.020	19.20	19.80	18.06
Ratna*	0.186	0.252	0.221	0.134	0.174	0.150	0.080	0.133	0.117	0.520	0.960	0.560	17.33	17.40	18.26
Pyruvthanam	0.147	0.158	0.160	0.124	0.137	0.136	0.090	0.111	0.108	0.446	0.546	0.506	15.06	15.57	15.44
GC-1	0.189	0.290	0.278	0.187	0.255	0.203	0.172	0.189	0.186	0.613	0.826	0.720	19.27	22.34	19.48
HD60-45	0.199	0.230	0.228	0.180	0.201	0.200	0.168	0.178	0.164	0.513	0.800	0.606	21.53	21.26	21.41
SEm±	**0.092**			**0.074**			**0.006**			**0.040**			**0.384**		
CD (P=0.05)	**0.025**			**0.008**			**NS**			**0.110**			**1.065**		

*Water stress tolerant genotypes

Table 2: Effect of water and UV-B stresses on biochemical constituents

Variety	Proline			Ascorbic acid			TBARS		
	Control	Water	UV-B	Control	Water	UV-B	Control	Water	UV-B
Kanchan*	20.05	34.48	23.48	57.84	74.17	72.38	50.29	50.80	65.34
BanketA1*	18.48	29.84	19.91	55.14	63.83	52.14	59.70	60.65	61.02
Ratna*	21.74	35.57	24.87	56.57	71.34	63.64	56.36	54.18	59.68
Pyruvithanam	18.07	24.71	20.92	55.56	59.83	57.17	60.85	71.72	68.36
GC-1	21.35	29.59	22.82	59.84	60.66	63.90	59.90	75.74	68.29
HD 60-45	20.97	29.51	21.09	63.08	65.17	62.44	55.12	70.11	65.08
SEm±	**0.90**			**2.31**			**3.72**		
CD (P=0.05)	**3.28**			**6.54**			**NS**		

*Water stress tolerant genotypes
TBARS: Thiobarbituric acid reactive substances

under stress condition. The susceptible varieties Pyruvithanam, GC-1 and HD 60-45 had more TBARS compared to the tolerant types Kanchan, Ratna and Banket A1. This showed that tolerant types have lower levels of lipid peroxidation. Similar genetic differences for lipid peroxidation were also reported in rice (Reddy *et al.*, 1980) and in tobacco (Nataraju *et al.*, 2001).

In conclusion, the cultivars of tobacco belonging to two cultivated species were able to adopt themselves to water and UV-B stresses by increasing the activities of antioxidant enzymes. A coordinated response involving the higher activity of SOD, CAT, POX and ASA was triggered in the varieties Kanchan, Banket A1 and Ratna which are having water stress tolerance to prevent the oxidative stress. It is apparent that not only superoxide radical scavenging enzyme SOD is important, but also the H_2O_2 scavenging enzymes CAT and POX are also equally important in imparting tolerance against drought and UV-B stress induced oxidative stress. The higher activity of PPO in water stress might also participate in enhancing tolerance to oxidative stress.

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