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Effect of Fluoride on Catalase, Guiacol Peroxidase and Ascorbate Oxidase Activities in Two Verities of Mulberry Leaves (*Morus alba* L.)

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Abstact: A regulated balance between oxygen radical production and destruction is required of metabolic efficiency and function is to be maintained either in normal or toxic conditions. A continually high anti oxidant capacities under toxicity conditions can present the damage correlated with resistance to that of particular toxicity. Hence the mechanisms that reduce oxidative stress are expected to play an important role in imparting toxicity tolerance in plant. A significant alleviation in activities of peroxidase, catalase and ascorbic acid oxidase was recorded on both cultivars on exposure to fluoride. Further the degree of increase was found to be dependent on severity and duration of exposure. Further degree of elevation in enzyme activity was relatively high in fluoride tolerant cultivar M_5 when compared to sensitive cultivar V_1. Mulberry cultivar M_5 on the whole, exhibited more fluoride toxic tolerance than mulberry cultivar V_1.

Key words: Senescence % Fluoride % Anti Oxidative Enzymes

INTRODUCTION

A variety of environmental stress like extremes of temperature drought, soil salinity, heavy metals, fluorine compounds are known to cause oxidative damage to plants either directly or indirectly by triggering an increased level of production of reactive oxygen species [1]. These reactive oxygen species include super oxide radical (Co_2G^1), hydroxyl radical (OHG^1) and hydrogen peroxide (H_2O_2) and products during membrane linked electron transport activities as well as by a number of metabolic pathways and in turn cause damage to the biomolecules such as membrane lipids proteins lipids chloroplast pigments, enzymes, nucleic acids [2]. Plants have antioxident defence mechanism comprising of enzymes catalase, peroxidases superoxide dismutase and the non enzymatic constituents "- tocopherol, ascorbate and reduced glutathione which remove or neutralize and scavenge the reactive oxygen species [3]. The enzymes of ascorbate – glutathione reductase also play a significant role in scavenging H_2O_2 mainly in chloroplasts and in maintaining the redox status of the cell [4]. The catalases are enzymes which cause the evolution of oxygen on attacking the substrate hydrogen peroxide generation during the photo-respiration and $-Oxidation of fatty acids [5]. Peroxidases are containing proteins that utilize H_2O_2 in the oxidation of various organic and inorganic substrates [6]. Peroxidases utilizing guajacol as electron donor in vitro are guiacol peroxidases and participate in developmental processes, lignifications ethylene biosynthesis, defense, wound healing etc. [7]. The other group of peroxidases scavenges H_2O_2 in cell and utilize glutathione, Cyt c, pyridine nucleotide and ascorbate as electron donors *in vitro* [7]. Guiacol peroxidases are glycoproteins, located in cytosol, vacuole, cell wall and in extra cellular space, while the other group is non glycosylated and localized in chloroplasts and cytosol [7]. The reactive species of oxygen have the potential to cause considerable damage to chloroplasts, other membrane systems and cellular macromolecules. Hydroxyl radicals, in particular, are so reactive; they can attack and damage almost every molecule of living cells [8]. The prolonged presence of these active oxygen species is not compatible with cell function or structure. The main cellular components susceptible to damage by free radicals of oxygen are lipids (Peroxidation of unsaturated fatty acids in membranes), proteins (denaturation of enzymes), carbohydrates (cleavage of polysaccharides) and nucleic acids (scission of DNA strands i.e., mutation) [9]. As fluoride is one of the toxic substance present in

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both aquatic as well as terrestrial environments and mulberry crop serves as chief feed for silkworms the present study was undertaken to determine the fluoride induced possible alteration in the oxidative damage to mulberry and likely alterations in behavior of the enzymes of antioxidant defense system in mulberry leaves.

MATERIALS AND METHODS

The study begins with by procuring the mulberry varieties V_1 and M_5 from the R S R S (Regional Sericulture Research Station), Anantapur, India. The experiment was conducted in leaf of 45 days old. They detached from the plants and washed in deionized water and were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds, then washed with distilled water.

The leaf bits were placed in Petri dishes of 20 cm diameter, containing distilled water or fluoride solutions. 4 leaf bits were in kept each Petri dish. Fluoride is available in many salts forms like, sodium fluoride, calcium fluoride, aluminum fluoride etc. Sodium fluoride with molecular formula weight 41.988, is used in the investigation to study the effects of fluoride on mulberry leaf. Different concentrations i.e. 100ppm, 200ppm and 300ppm solutions of the above salts were prepared in distilled water and distilled water was alone served as control. Three Petri dishes were placed for each concentration of fluoride. Petri dishes were kept under a light intensity of approximately 150 wmG^2 and temperature of 27° ± 3°C the solutions were replaced every day with fresh once. The samples, were estimated after, for physiological and biochemical studies, 48, 96, 144 and 192 hrs of incubation.

Catalase activity was assayed and estimated as per the method of Barber [10]. Guaiacol peroxidase (EC 1.11.1.7) was assayed according to Cakmak, I. and Horst, W.J. [11]. Ascorbic acid and 1mM PMSF as described by Moran *et al.* [12].

RESULTS AND DISCUSSION

From the data presented in the Table 1 and Figure 1 it is seen that the Catalase activities decreased in fluoride exposed mulberry leaves, relative to controls the Catalase activity was significantly decreased at all concentrations in both cultivars. However, the decrease in Catalase was greater in magnitude in V_1 than in M_5 at all the concentrations of fluoride on all days of exposure. From the data presented in the Table 2 and Figure 2 it is seen that relative to controls peroxidases activity increased in the leaves of fluoride exposed mulberry leaves. Peroxidases activity increased significantly in both V_1 and M_5. Further guaiacol peroxidases activities increased significantly in both cultivars at concentrations and at all exposure days. However, the percent increase in the activity was more in the cultivar V_1 than in M_5.

Table 1: Catalase (units gmG^1 fresh wt hG^1) in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2nd, 4th, 6th and 8th days

	V_1				M_5			
Day	Control	100ppm	200ppm	300ppm	Control	100ppm	200ppm	300ppm
2	1.352^a	1.323^b (-2.114)	1.276^c (-5.621)	1.213^d (-10.281)	1.213^a	1.193^b (-1.648)	1.127^c (-7.089)	1.016^d (-16.240)
4	1.512^a	1.174^b (-22.354)	1.018^c (-32.671)	0.965^d (-36.177)	1.521^a	0.974^b (-35.963)	0.912^c (-40.039)	0.889^d (-41.551)
6	1.610^a	0.823^b (-48.88)	0.772^c (-52.049)	0.665^d (-58.695)	1.691^a	0.795^b (-52.986)	0.753^c (-51.470)	0.701^d (-58.545)
8	1.819^a	0.788^b (-56.679)	0.717^c (-60.582)	0.623^d (-65.674)	1.736^a	0.677^b (-61.002)	$^{0.642c}$ (-63.018)	0.623^d (-63.594)

*Each value is a mean of eight estimations

** Percent decrease / increase over control is given in parenthesis.

*** Means within a row followed by the same letter are not significantly different (P>0.05) from each other according to Duncan's multiple range tests.

Table 2: Guiacol Peroxidase (units gmG^1 fresh wt hG^1) in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2nd, 4th, 6th and 8th days

	V_1				M_5			
Day	Control	100ppm	200ppm	300ppm	Control	100ppm	200ppm	300ppm
2	1.459^a	1.665^b (+14.11)	1.731^c (+18.642)	2.138^d (+46.538)	1.710^a	2.466^b (+44.210)	2.638^c (+54.269)	3.483^d (+103.684)
4	1.531^a	1.748^b (+14.173)	1.881^c (+22.860)	2.410^d (+57.413)	1.984^a	3.619^b (+82.409)	3.830^c (+93.044)	4.853^d (+144.606)
6	1.643^a	1.853^b (+12.781)	2.206^c (+34.266)	3.357^d (+104.32)	2.251^a	4.060^b (+80.364)	4.718^c (+109.595)	5.717^d (+153.976)
8	1.717^a	2.156^b (+23.411)	2.256^c (+29.135)	3.209^d (+83.686)	2.317^a	5.675^b (+144.928)	5.193^c (+124.126)	6.265^d (+170.392)

* Each value is a mean of eight estimations

** Percent decrease / increase over control is given in parenthesis.

*** Means within a row followed by the same letter are not significantly different (P>0.05) from each other according to Duncan's multiple range tests.

Table 3: Ascorbic acid oxidase (units gmG[1] fresh wt hG[1]) in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2nd, 4th, 6th and 8th days

	V_1				M_5			
Day	Control	100ppm	200ppm	300ppm	Control	100ppm	200ppm	300ppm
2	22.176[a]	27.276[b] (+229.97)	29.523[c] (+33.130)	32.630[d] (+47.1489.571)	27.533[a]	30.513[b] (+10.823)	49.130[c] (+78.440)	60.383[d](+119.22)
4	22.470[a]	28.553[b] (+27.071)	29.453[c] (+131.076)	36.303[d] (+161.562)	28.323[a]	44.260[b] (+56.268)	50.556[c] (+78.498)	71.296[d](+151.724)
6	22.606[a]	30.656[b](+22.800)	35.176[c] (+55.604)	40.683[d] (+79.965)	28.573[a]	49.426[b] (+72.981)	59.220[c] (+107.258)	79.490[d](+178.199)
8	22.800[a]	32.673[b] (+43.302)	39.420[c] (+72.894)	41.576[d] (+82.350)	39.203[a]	60.473[b] (+54.256)	70.330[c] (+79.399)	85.656[d](+118.493)

* Each value is a mean of eight estimations

** Percent decrease / increase over control is given in parenthesis.

*** Means within a row followed by the same letter are not significantly different (P>0.05) from each other according to Duncan's multiple range tests.

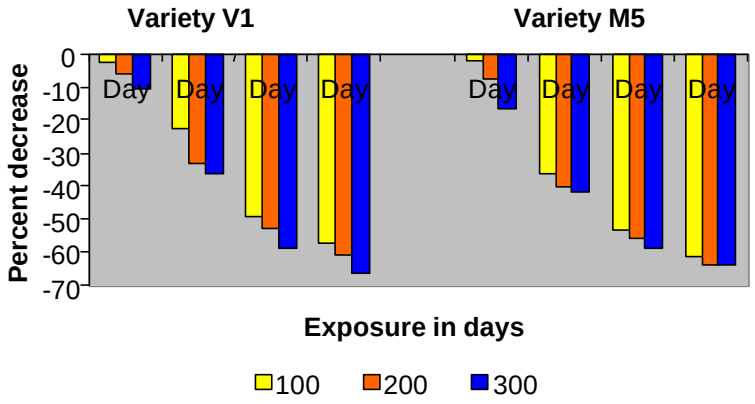

Fig. 1: Percent decrease over control in the Catalase in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2, 4, 6 and 8 days

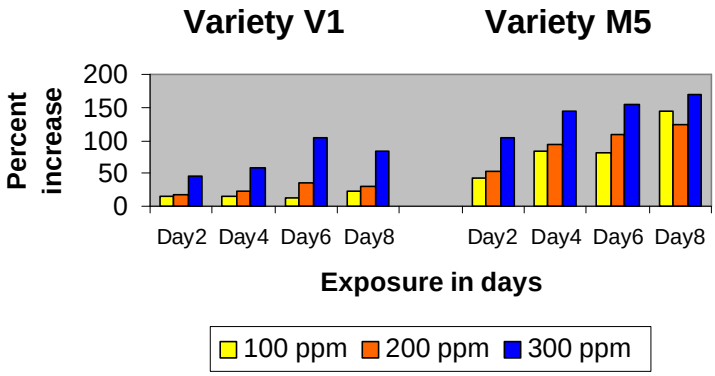

Fig. 2: Percent increase over control in the Guaiacol Peroxidase in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2, 4, 6 and 8 days

Ascorbate oxidase activity in the leaves was presented in the Table 3 and Figure 3, the Ascorbate oxidase activities levels significantly increased at all the concentrations of fluoride exposure in both V_1 and M_5. The ascorbate oxidase activities were significantly increased by all days of exposure. Further more, the degree of increase was dependent on concentration of exposure period. The shifts in ascorbate peroxidase were greater in M_5 than in V_1 cultivars.

Enzymic antioxidative defense system includes catalase guiacol peroxides and ascorbate oxidase. With increasing levels of fluoride treatment a concomitant decline in catalase activity was absorbed in leaves of mulberry at all exposure periods. Compared to M_5 mulberry variety, a greater inhibition in catalase activity was observed in V_1 cultivars. Our results showed a decrease in the level of catalase activity indicating that fluoride inhibits catalase activity. Our results are in

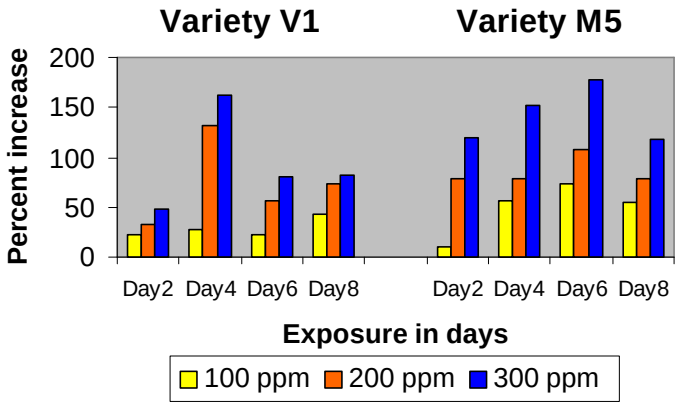

Fig. 3: Percent increase over control of Ascorbic acid oxidase in the leaves of mulberry verities on exposure to different concentrations of fluoride at 2, 4, 6 and 8 days

conformity with the observations of many other investigators who have also found that fluoride inhibited of catalase activity [13-15] concluded that iron atoms of the catalase have hydroxyl groups that may be replaced by low molecular weight anions, in sufficient concentrations, such that catalase is correspondingly inhibited. However, the fluoride in vivo may be causing a reduction in the amount of catalase erase and not just a simple inhibition of the catalase system as measured in the homogenates.

The present study indicated that an enhancement in the activity of guiacol peroxidase suggesting that this enzyme serves as an intrinsic defiance tool to resist fluoride induced oxidative damage in mulberry leaves guiacol peroxides is widely accepted as stress enzymes [16]. These reports which provide evidence that fluoride stress increased the enzyme activities such as catalase, guiacol peroxidase, ascorbic acid oxidase, polyphynol oxidase [17]. Observed increased activity of peroxidase, in drought tolerant as well as in drought sensitive rice cultivars. The activity of peroxidase increased in water stressed seed leaves [18]. In this study, a stress intensity dependent increase in guiacol peroxidase activity in both genotypes was observed. However the activity was relatively greater in M_5 compared to V_5. The higher activity of guiacol peroxidase was observed in M_5 under fluoride exposure.

Similar to guiacol peroxidase, the activity of ascorbate peroxidase showed a concomitant increase in mulberry leaves with increase in fluoride exposure levels and under and fluoride treatments however M_5 showed higher levels of enzyme activity than V_1 cultivars. Ascorbate peroxidase (APX) and glutathione reductase (G.R) are indispensable components of ascorbate glutathione pathway, required to scavenge H_2O_2 produced mainly in chloroplasts and other cell organelles and to maintain the redox state of the cell 1[4] Ascorbate peroxidase utilizes the reducing power of ascorbic acid to eliminate potentially harmful H_2O_2. in the present study the results indicate an enhancement in the activity of ascorbic acid peroxidase in response to fluoride stress. Similar induction was reported in response to mild water stress [19] chilling [20] drought [21]. And heavy metal toxicity [17] Ascorbic peroxidase along with catalase and super oxide dismutases are considered as key enzymes within the antioxidative defense mechanism, which directly determine the cellular concentration of O_2 and H_2O_2 [7]. The present study, suggest that fluoride toxicity causes oxidative stress in mulberry leaves and the enzymes guiacol peroxidase appear to play a key role in counteracting the oxidative stress in plants. As, unlike iron, fluoride is not an oxido-reducing metal, the oxidative stress induced by fluoride in the leaves of mulberry appears to be an indirect effect of fluoride toxicity leading to production of reactive oxygen species with simultaneous increase in tissue levels of peroxidases.

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