Effect of Fluoride on Protein Profiles in Two Cultivars of Mulberry Leaves

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Abstract: Senescence contributes to decline in productivity of many economically important plants include Mulberry. Thus understanding and controlling senescence could be of great economic value. Fluoride and its effects on the physiology and metabolism of plants has been the subject of various reviews slow accumulation of fluoride over days or weeks leads to symptoms of chlorosis at leaf tips and margins. To compare the rate of protein content in senescing leaves of mulberry in control and fluoride treated. The total protein content declined progressively in tissues and was registered in both cultivars on exposure to fluoride. SDS-PAGE gel electrophoresis also clearly, demonstrated the variability in protein profiles in M and V mulberry strains on exposure to different concentrations of fluoride over period of exposure which indicates that v is sensitive than M in senescing mulberry leaves on exposure to fluoride. The decreased protein content can be explained by decrease in protein synthesis and enhanced protein degradation.

Key words: Senescence • Fluoride • Protein Profiles • SDS PAGE

INTRODUCTION

Proteins are important in all biological systems playing a wide variety of structural and functional roles. All of the enzymes the catalysts in biochemical transformations are protein in the nature. Proteins form the frame work of cells and can also be broken-down for the release of energy. However, tissue proteins represent the last source of energy that is only used when there are no carbohydrates or fats available. Proteins also function as regulators in that they control the chemical reactions and metabolic processes which occur in organisms. The protein budget of the cell can be considered as an important analyte in evaluating the physiological standards of the cell [1].

The reaction of plants to environmental stresses are complex and involved many kinds of physiological and biochemical responses. Since proteins are important organic nitrogenous constituents of plants, they play a great role in the compensatory metabolism of a plant species during fluoride stress conditions. Fluoride could evoke compensatory metabolic changes through modification and modulation of the quantity and quality of proteins. Various workers observed in decrease in total proteins in different organs of plants exposed to fluoride concentrations [2] Further increasing fluoride concentration led to a significant increase in amino acid content and the increase in amino acid content was time dependent and positively correlated with fluoride concentrations [2,3]. Significant correlation was found between soil fluoride content and foliar fluoride concentration [4].

MATERIELS AND METHODS

Mulberry varieties of V, and M, were procured 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

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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. 100, 200 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 wm² 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.

**Isolation of Proteins and Data Analysis:** The concentration of proteins was estimated according to Lowry et al. [5]. Separation of proteins on linear discontinuous SDS-Polyacrylamide gel electrophoresis SDS-PAGE was carried out by the method described by Laemmli [6]. Data analysis involved the use of Duncan’s Multiple Range Test [7].

**RESULTS AND DISCUSSION**

From the data presented in Table 1 and Fig. 1, it is observed that relative to controls, the total protein significantly decreased in the V1 and M5. Further the results indicated that the leaf protein content in both cultivars declined and the magnitude of decrease was found to be depended on concentration and period of exposure. It was also observed that there exists a marked difference between the V1 and M5 cultivars.

The polypeptide profiles of total proteins of mulberry leaves treated with different concentrations of fluoride and periods were revealed by SDS-PAGE. About 12 clearly detectable mulberry protein bands over a wide range of molecular weight 14 KDa to 92 KDa were recognized (plates I and II). Fluoride caused a gradual decrease in total protein content as judged by the decreasing intensity of all bands in the polypeptide spectrum compared with controls and especially, in the quantity of the polypeptides migrating in the upper (30-60 K Da) and low molecular weight zones (below 15 KDa) plates I and II, lane 1 compared to 2, 3 and

![Fig. 1: Percent decrease over control in the Proteins in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2, 4, 6 and 8 days.](image)

![Plate I: SDS-PAGE.Polypeptide profiles of total proteins in the leaves of 2 mulberry verities(M5 and V1) of control and on exposure to different concentrations of fluoride (100 ppm ; 200ppm and 300ppm ) at 2nd and 4th days](image)
Table 1: Proteins (mg gm\(^{-1}\) fresh wt) in the leaves of mulberry verities of control and on exposure to different concentrations of fluoride at 2\(^{a}\), 4\(^{b}\), 6\(^{c}\) and 8\(^{d}\) days

<table>
<thead>
<tr>
<th>Day</th>
<th>V1 M5</th>
<th>Control</th>
<th>100ppm</th>
<th>200ppm</th>
<th>300ppm</th>
<th>Control</th>
<th>100ppm</th>
<th>200ppm</th>
<th>300ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>V1</td>
<td>1.723(^a)</td>
<td>1.543(^b) (-0.446)</td>
<td>1.391(^c) (-9.268)</td>
<td>1.279(^d) (-5.769)</td>
<td>1.302(^a)</td>
<td>1.289(^b) (-.998)</td>
<td>1.214(^c) (-8.758)</td>
<td>1.128(^d) (-3.364)</td>
</tr>
<tr>
<td>4</td>
<td>V1</td>
<td>1.774(^a)</td>
<td>1.438(^b) (-2.50)</td>
<td>1.352(^c) (-3.788)</td>
<td>1.229(^d) (-8.940)</td>
<td>1.448(^a)</td>
<td>1.267(^b) (-7.21)</td>
<td>1.144(^c) (-9.994)</td>
<td>1.094(^d) (-4.447)</td>
</tr>
<tr>
<td>6</td>
<td>V1</td>
<td>1.878(^a)</td>
<td>1.394(^b) (-5.772)</td>
<td>1.325(^c) (-9.446)</td>
<td>1.129(^d) (-9.882)</td>
<td>1.583(^a)</td>
<td>1.213(^b) (-3.373)</td>
<td>1.124(^c) (-8.995)</td>
<td>1.098(^d) (-6.323)</td>
</tr>
<tr>
<td>8</td>
<td>V1</td>
<td>1.966(^a)</td>
<td>1.280(^b) (-4.893)</td>
<td>1.122(^c) (-2.929)</td>
<td>1.167(^d) (-0.640)</td>
<td>1.647(^a)</td>
<td>1.157(^b) (-9.751)</td>
<td>1.157(^c) (-8.433)</td>
<td>0.874(^d) (-6.933)</td>
</tr>
</tbody>
</table>

* 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.

Plate II: SDS-PAGE. Polypeptide profiles of total proteins in the leaves of 2 mulberry cultivars of control and on exposure to different concentrations of fluoride (100 ppm F; 200ppm and 300ppm) at 6\(^{a}\) and 8\(^{b}\) days

Fluoride treatments decreased the total protein contents in the both cultivars of mulberry. It suggests the suppression of protein synthesis and / or utilization of proteins for energy purposes. This could be attributed to the ability of fluoride to modify the ratio of free nucleotides and that of RNA, to decrease of the rate of RNA synthesis and / or to enhance ribonuclease activity. At the subcellular level nuclear DNA is either decreased [8] or remains unchanged [9], but a decrease was reported in intracellular m RNA [10,11]. Polyribosomes are partially disintegrated [12,13] whereas r-RNA is reduced and ribonucleases are increased [11]. Thymidine uptake by DNA and Uridine uptake by RNA are lowered [14]. Ribosome translation, which stimulates Polypeptide Synthesis, is altered. Reduction of mitochondrial ATP could inhibit the activation of hyalo plasmic amino acids and the functional value of the aminoacyl t RNA protein synthesis might be depressed by fluoride at different levels including nucleous, ribosomes and mitochondria [15] as a result, it affects protein synthesis negatively. Earlier workers Bhatnagar and Bhatnagar [16] and Asthir et al. [17] reported that wheat grains respond to fluoride mediated disruption of carbon metabolism by a compensatory effect on nitrogen metabolism. SDS-PAGE gel electrophoresis also clearly, demonstrated the variability in protein profiles in M5 and V1 mulberry strains on exposure to different concentrations of fluoride over a period of exposure. In other studies, Mulberry cultivar, M5 exhibited fluoride tolerance than V1 cultivar [18].

According to the proteomic analysis, it is suggested that total protein content declined progressively in mulberry leaves in both cultivars on exposure to fluoride. SDS-PAGE gel electrophoresis also clearly, demonstrated the variability in protein profiles in M5 and V1 cultivars at different concentrations of fluoride over period of exposure which indicates that V1 is sensitive than M5 in senescing mulberry leaves on exposure to fluoride.
REFERENCES