

## Accumulation of Natural Antioxidants in Ferns Exposed to Mutagenic Stress

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### ABSTRACT

Different species of ferns were analyzed for the modulations in the pool of non-enzymatic antioxidants in response to the maleic hydrazide treatments. Treatments at very low doses were found to trigger the accumulation of both ascorbate and proline contents. Total amount of protein and chlorophyll contents showed varying degree of sensitivity in all cultivars of ferns. Proline accumulation was found to be high in treated plants compared with control. Proline, ascorbate and flavonoid contents were found to be accumulated in all plants exposed to high doses of maleic hydrazide. All the three species showed high proneness towards the mutagen. Improved tolerance in treated plants might be explained on the basis of the elevated level of enzymatic and non-enzymatic antioxidants.

**Key Words:** Mutagen, Ascorbate, Proline, protein, Ferns

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### INTRODUCTION

Ferns are found abundantly in many different habitats of the world. They were the dominant part of the vegetation during the Carboniferous Period which is called as 'Age of Ferns'. Most of the ferns of the Carboniferous became extinct but some later evolved into our modern ferns. There are about 12,000 species in the world today [2]. Three fern species were selected for the present study: *Cheilanthes farinose*, *Lygodium scandens* and *Adiantum caudatum*. The plants prefer light (sandy), medium (loamy) and heavy (clay) soils [12, 13]. The plants are dominant in soils of acidic, neutral and alkaline nature.

Plants possess many antioxidants, usually classified in two broader categories. They are: enzymatic antioxidants and non-enzymatic antioxidants. The alterations in the activities of antioxidants is observed in the plants exposed to different environmental stresses such as drought, heavy metals, pesticides, ultraviolet radiations etc. Since human activities are increasing the level of pollutants in the environment day by day, it has become an interesting area of research to observe their effects on plant communities (Producers of the Ecosystems). The damage to the biological ecosystems may be measured in terms of the morphological and biochemical alterations in primary producers. Numerous studies have been conducted on photosynthetic enzymes, pigments, proteins, seed patterns and antioxidant compound contents in plants. Maleic hydrazide is one of the agents that have been found to bring heritable alterations in the genes, chemical mutagens are undoubtedly very potent ones which can induce genetic or physical alterations in dormant seed or spore. This is a strong mutagen that induces mitotic inhibition and cytological abnormalities in a number of higher plants [4, 8, 16]. It possesses growth regulating properties [19, 22]. It is a known depressor of auxin transport in plant. Stem growth, root growth and seed germination can be regulated by its treatment.

The presence/absence and increasing/decreasing property of antioxidant compounds, production of free radicals and the amount of lipid peroxidation in terms of elevated level of antioxidants might provide significant clues to assess and evaluate the antioxidant potential of various *Cheilanthes* species against environmental stresses. Plants are able to develop special mechanisms for adjusting the changed environment. Many groups of stresses like heavy metals, ultraviolet radiations etc are shown to generate singlet oxygen and other active oxygen species at various sites of photosynthetic electron transport chain [9] and affect the growth of plant. Many studies have been done with emphasis on morphological, biochemical and genetic characteristics of *Cheilanthes rufa*, *Lygodium scandens* and *Adiantum caudatum* with respect to ultraviolet radiations, gamma radiations and various light qualities like red light,

blue light etc. The present study was done with setting forth the objective of studying the effect of Maleic hydrazide on the accumulation of non-enzymatic antioxidants in different fern species.

## MATERIALS AND METHODS

### Organisms and culture conditions

Spores of *Cheilanthes rufa*, *Lygodium scandens* and *Adiantum caudatum* were collected from plants growing in the kushmi forest of Gorakhpur (a tarai area of north India). The spores were surface sterilized with 2% sodium hypochlorite solution and then sown uniformly on 25 ml of autoclave sterilized (15 lb/in<sup>2</sup>) inorganic medium at pH 5.4 in petri dishes [20]. Sowing of spores was done in an inoculation chamber fitted with germicidal UV lamp (USA). Then the plates were maintained at  $24 \pm 2^{\circ}\text{C}$  under continuous white fluorescent illumination at the intensity of 2700 lux. Spores for treatments were placed in liquid nutrient media for 72 hours and then subjected to various concentrations of maleic hydrazide. Each experiment was conducted in triplicates.

### Extraction and estimation of total Protein, Malondialdehyde and peroxide contents

Growth determination was done by estimating total protein contents after 12 days of treatments. Protein contents were determined using Folins-Lowry method with lysozyme as the standard [14]. Total amount of hydrogen peroxide radicals was estimated by using ferrithiocyanate method as described by Sagisaka (1976) [18]. Standardization of H<sub>2</sub>O<sub>2</sub> was performed to minimize the interference of catalase. The level of lipid peroxidation was measured in terms of total malondialdehyde (MDA) contents. The reaction reagent consisted of 0.4 N TCA + 19.68 ml of distilled water + 0.4 ml of HCl + 100mg TBA [11]. Prepared leaf extract (in phosphate buffer) was added to the reaction reagent and absorbance was taken at 532 nm.

### Flavonoids: Extraction and Estimation

Flavonoids were extracted in mutagen-treated and untreated fern leaflets by using the method of Mirecki and Teramura (1984). Extraction mixture consisted of acidified methanol (methanol: water: HCl, 78: 20: 2, v/v) + leaflets, incubated for for 24h at 4<sup>o</sup>C. The filtered extract was then used for measuring the absorbance at 320 nm, which is indicative of relative concentration of UVB absorbing pigments [15]. Flavonoid contents were expressed as absorbance g<sup>-1</sup> fresh mass of tissue at 320 nm.

### Extraction and estimation of Proline contents

Proline contents in leaf homogenate of mutagen- treated and untreated cultures were estimated according to the standard method [3]. Proline contents in unknown samples were calculated by comparing with standard curve of L-proline. Amount of proline is represented in terms of  $\mu\text{g g}^{-1}$  FW.

### Ascorbic acid estimation

Ascorbic acid was extracted by dehydrating ascorbic acid by shaking it with acid washed NORIT\* in the presence of acetic acid. After coupling with 2, 4-Dinitrophenyl hydrazine, the solution is treated with sulfuric acid to produce the red color whose absorbance was measured at 540 nm.

#### \*Acid washed NORIT preparation:

200 gram NORIT (charcoal) is suspended in 1000 ml of 10% HCl, heated upto boiling point and filtered under suction. The cake is removed and stirred with 1000 ml water and filtered. This procedure is repeated until the washing give a negative test for Fe<sup>3+</sup> ions. The NORIT is then dried overnight at 110-120<sup>o</sup>C.

## RESULTS AND DISCUSSIONS

Results observed were found to be variable with different fern species. Overall growth and accumulation of ascorbate, proline and flavonoid contents showed modulations in vales in response to the mutagenic chemical– Maleic hydrazide. Growth measured in terms of protein contents showed initial increase at low doses of mutagen (% control increase = 16-22% in all three ferns). But the high doses were found to reduce the total protein contents speedily (5-60% at 125 ppm as compared to the control) (Figure 1a). The declining trend in protein contents continued with rising concentration of maleic hydrazide. Initial recovery in protein contents might be explained on the basis of increase in the pool of enzymatic antioxidants that help plants in overcoming the oxidative stress [6, 7] The present study might give us clues for the impacts of mutagens on total biomass yield.

Cells contain important non-enzymatic antioxidants such as carotenoids, ascorbic acid, proline, glutathione,  $\alpha$ -tocopherol etc., for mitigating the toxic effects of free radicals and AOS (active oxygen species) under oxidative stress. In the present work, ascorbate contents showed increase in the amounts (% control increase= 10-45% in all plants) with mutagen exposure up to 16-62 ppm and the decrease at high dose (6-15% after 125 ppm exposure of maleic hydrazide), compared to untreated samples. (figure 1 b). There are two possibilities regarding increase in ascorbic acid contents; either its synthesis has increased or its regeneration rate through the Asada-Halliwell pathway has increased (as observed in *Ulva fasciata*) [21].

The chemical evolution and significance of flavonoids has been assumed to play an important role in overcoming the oxidative stress in cells [17]. Evidences suggest that the presence of flavonoids in UV-B irradiated leaves could alter the perception or response of other defense mechanisms. Presently, flavonoid contents showed enhanced synthesis in maleic hydrazide treated fern species- *C. rufa*, *Lygodium scandens* and *Adiantum caudatum*. A % control increase of 5-40% was observed at the doses of 16-62 ppm but at the high dose (125 ppm), the values decreased to 25%, 20% and 30% in *C. rufa*, *Lygodium scandens* and *Adiantum caudatum*, respectively (Figure 1 b). Earlier findings showed remarkable increase in the Flavonoids contents of the stressed soybean cultivars. Since flavonoids inhibit the enzymes responsible for superoxide anion production thus the increase in their values may be attributed to the protection from free radical induced damage. Similarly proline contents were found to be accumulated at all the doses of maleic hydrazide in all the fern cultivars- *C. rufa*, *Lygodium scandens* and *Adiantum caudatum*. A high accumulation (10-60%) of proline contents were observed at the highest dose of mutagen (125 ppm). The accumulation and protective effect of proline has been observed in many higher plants and bacteria as well as protozoa, algae, and marine invertebrates [5].

Mutagen induced lipid peroxidation of the cellular components in plants were studied by estimating the level of MDA in treated and untreated plantlets and the related data are depicted in the figure 2 a. The lipid peroxidation in non- stressed *C. Rufa* was observed as 1.4609 nmol MDA (mg fresh mass)<sup>-1</sup>, whereas it was found to be 1.351 and 1.6125 nmol MDA (mg fresh mass)<sup>-1</sup>. Treated plantlets showed 10-45% increase in total Malondialdehyde contents as compared to the untreated plants showing high level of lipid peroxidation in maleic hydrazide treated plants. Similarly the increase in peroxide radical contents was observed to be linearly related with the level of lipid peroxidation (Figure 2 b). MDA is an intermediate compound produced due to lipid peroxidation, the measurements of its contents can be used as an index for the injury caused by free radicals produced during oxidative stress. The results obtained here are in agreement with other authors [1, 10].

## CONCLUSION

According to the results obtained, it may be concluded that the mutagen-maleic hydrazide affected the overall growth of all fern species, severely. Decrease in total protein contents and high increase in the level of lipid peroxidation proved the oxidative damage caused by free radicals formed in response to the mutagen. Increase in non-enzymatic antioxidants may be attributed to the elevation of natural antioxidant defense system. Initial increase in the enzymatic activities might be due to the increased activities of stress relief genes and their gene products. Ferns are good Phytoremediator and can be used to remove heavy metals and other pollutants from the polluted soil. The study might provide suitable keys for studying the interrelationships of the chemical treatments and plant defense systems.

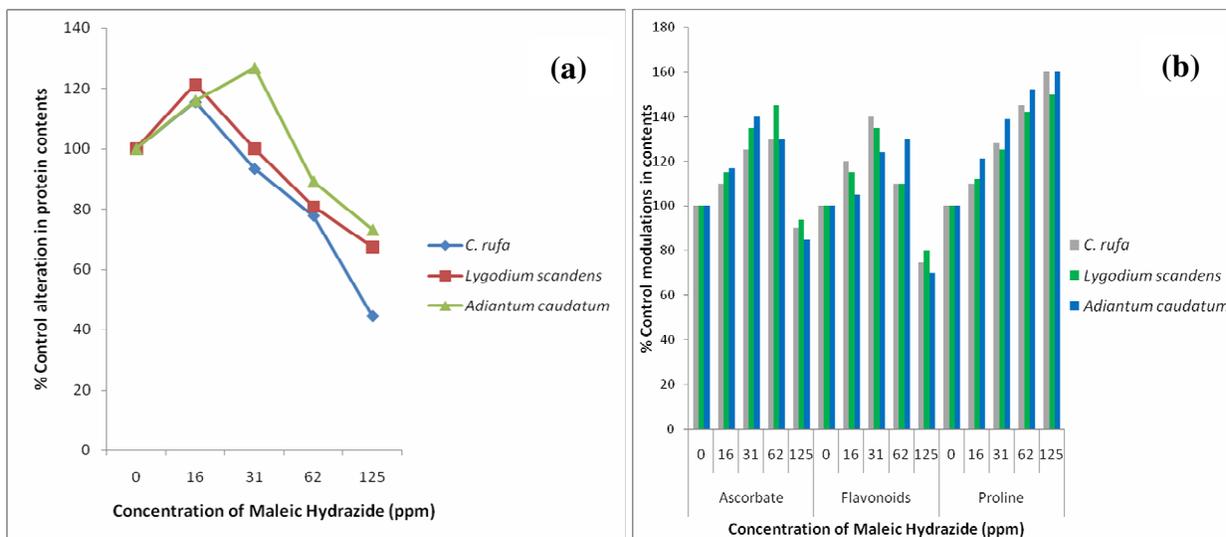


Fig.-1: Effect of Maleic hydrazide on protein (a) and non-enzymatic antioxidants (b) in ferns.

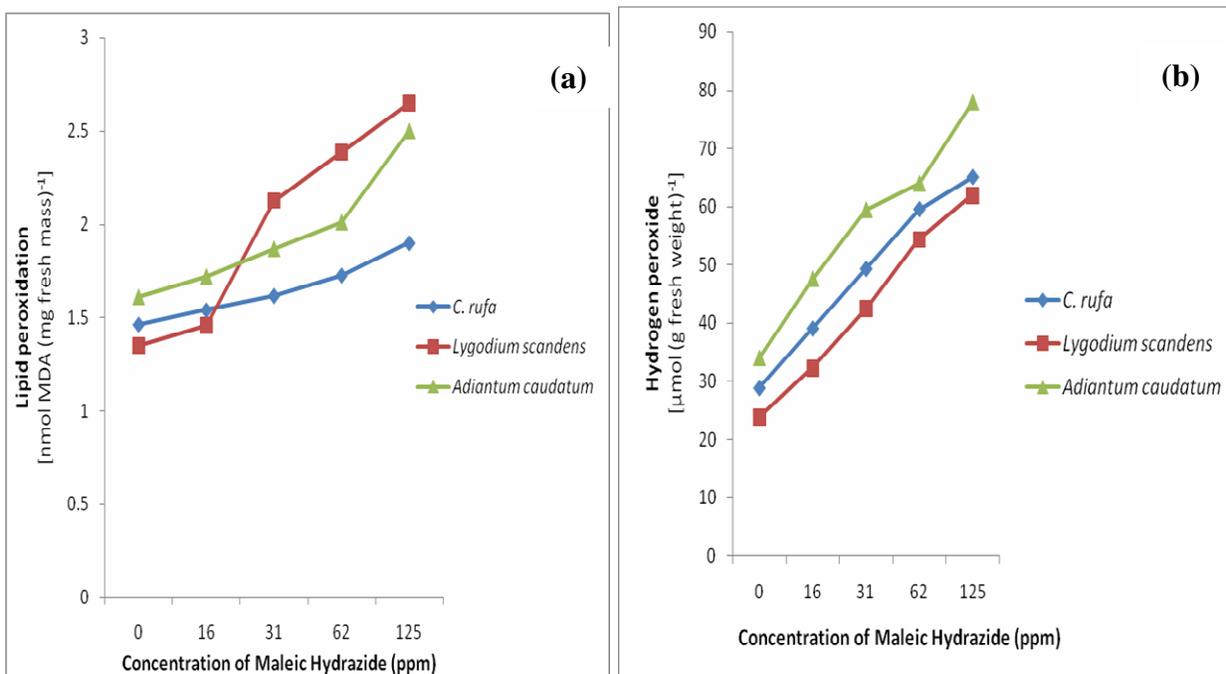


Fig.-2: Effect of Maleic hydrazide on lipid peroxidation (a) and hydrogen peroxide radicals (b) in ferns.

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