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Effect of sodium fluoride on certain enzymes and proline content in *Cenchrus* leaves

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Abstract. Fluoride has long been known to act as an enzyme inhibitor and several studies have been conducted attempting to explain how fluorides affect basic enzyme reactions. The present investigation was initiated to monitor the effect of sodium fluoride on the activities of some of the key enzymes and proline content in the leave of *Cenchrus ciliaris.* NaF, in general, promoted the activity of alanine aminotransferase (GPT). Lower concentrations of fluoride used, slightly increased the activity of nitrate reductase. On the other hand, NaF inhibited the activity of aspartate aminotransferase (GOT), glutamate dehydrogenase (GLDH) and malate dehydrogenase (MDH). As far as, the level of proline content is concerned, concentration of NaF used, worked quite favourably.

Key words: Aminotransferases; *Cenchrus ciliaris;* Glutamate dehydrogenase; Malate dehydrogenase; Nitrate reductase; Proline content; Sodium fluoride.

Introduction

Fluoride has been postulated to affect most fundamentally the activities of enzymes essential to such plant processes as respiration, photosynthesis, carbohydrate metabolism, protein synthesis, cell wall formation energy balance, nucleotide and nucleic acid synthesis (Treshow, 1970).

Fluoride inhibition of both enolase and catalase was demonstrated by Mc Cune *et al.* (1965). Fluoride also combines with the ferric ion in the heme groups of peroxidase, catalase and cytochrome oxidase, inhibiting the activities of these enzymes. Hewitt and Nicholas (1963) and McNulty and Newman (1957) reported that fluoride stimulated oxygen uptake in leaf tissue before any visible injury appeared. Effect of fluoride on photosynthesis has also been demonstrated. As proposed by Thomas (1958), that a threshold of fluoride concentration and exposure time exists and that above it there is a reduction in photosynthesis and presumably growth rate, in excess of that which can be accumulated for by necrosis or chlorosis alone. Fluoride treatment exhibited a change in the activity of certain enzymes (Chang, 1967; Chang and Thompson, 1966; Chang, 1975). As there is a paucity of information as regards the role of fluoride on the Indian desert crops, experiments were designed to evaluate the role of sodium

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fluoride on the activities of certain enzymes and proline content in *Cenchrus ciliaris* which is an important valuable fodder and pasture grass of arid and semi-arid region of India.

Materials and Methods

For the present study *Cenchrus ciliaris* Linn. served as the experimental material. Plants were raised in earthenware pots during the month of May 1987. After thinning, 2 to 4 plants of uniform size were maintained in each pot. The first spray was given to five month old plants. Subsequently second, third and fourth spray were made with an interval of 7 days. For each spray, 500 ml solution of NaF of varied concentrations was used per pot. The concentrations used were 10 ppm, 50 ppm, 100 ppm and 200 ppm. The control plants received an equal amount of water.

Enzyme activities were assayed in crude extracts of leaves of *Cenchrus* prepared essentially as described by Huber and Sankhla (1974). The activities of glutamate dehydrogenase (GLDH) and malate dehydrogenase (MDH) were measured by following reduction of absorption at 340 nm due to oxidation of NADH. The activities of GPT and GOT were assayed as described by Bergmeyer (1970). For nitrate reductase (NR) (*In vivo* assay), the enzyme was extracted using the method given by Jaworski (1971). Proline content was measured by the method described by Bates *et al.* (1973). The enzyme activities have been represented as percent of control value on protein basis.

Results

Results relating to the effect of sodium fluoride on the activity of GPT and GOT have been represented in Fig. 1. It is clear that with an increase in the concentration of NaF, there is a slight increase in the activity of GPT, while lower concentration of NaF, i.e., 10 ppm and 50 ppm, are inhibiting the activity of GOT and a decrease of about 3 and 17% was observed. Only

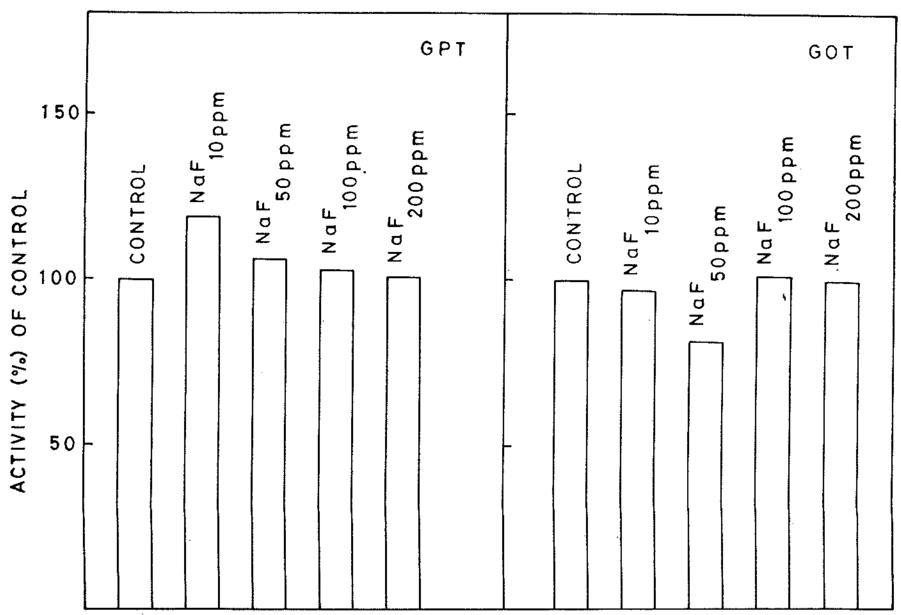

Fig. 1. Effect of NaF on the activities of alanine aminotransferase (GPT) and aspartate aminotransferase (GOT). Enzyme activity is expressed as (%) of control.

one concentration of NaF (100 ppm) is slightly favourable and an increase of only 2% was noticed. On the other hand, NaF inhibited the activity of glutamate dehydrogenase (GLDH) and malate dehydrogenase (MDH). As much as 40, 50, 66 and 71 percent decrease was observed in GLDH activity following treatment with NaF (10 ppm, 50 ppm, 100 ppm and 200 ppm). With an increase in the concentration of NaF, a gradual decline in the activity of MDH was noticed, except the lowest concentration of NaF (10 ppm) slightly enhanced the activity of MDH and an increase of 3% was observed (Fig. 2).

Results relating to the effect of NaF on the activity of NR are shown in Fig. 3. In comparison to the control value, the activity of NR increased by about 4 and 20 percent in the presence of 10 ppm and 50 ppm NaF, respectively. But the higher concentrations of NaF used (100 ppm and 200 ppm) made enzyme activity to decline by 12 and 24 percent, respectively.

Plants treated with NaF indicated high proline content. With an increase in the concentration of NaF a corresponding increase in proline was evident in *Cenchrus* leaves. Although the highest concentration of NaF used (200 ppm) was not that much of favourable for proline content, but an increase of about 10, 20 and 40 percent was clearly evident in *Cenchrus* plants treated with NaF (10 ppm, 50 ppm and 100 ppm), respectively.

Discussion

The activity of aminotransferase is prone to be modified with several factors including plant growth regulators, fluorides, (Sankhla *et al.*, 1983; Weinstein, 1977), and salinity (Joshi *et al.*, 1962; Huber and Sankhla, 1973; Upadhyaya, 1982). In the present investigation NaF used, favourably increased the activity of alanine aminotransferase (GPT) as compared to aspartate aminotransferase (GOT) where NaF has no significant effect on its activity of GPT, since GPT is an important regulatory enzyme (Hedley and Stoddart, 1971a,b), it is logical that its activity be affected much more than GOT (Ahmed and Sankhla, 1979; Ahmed, 1983;

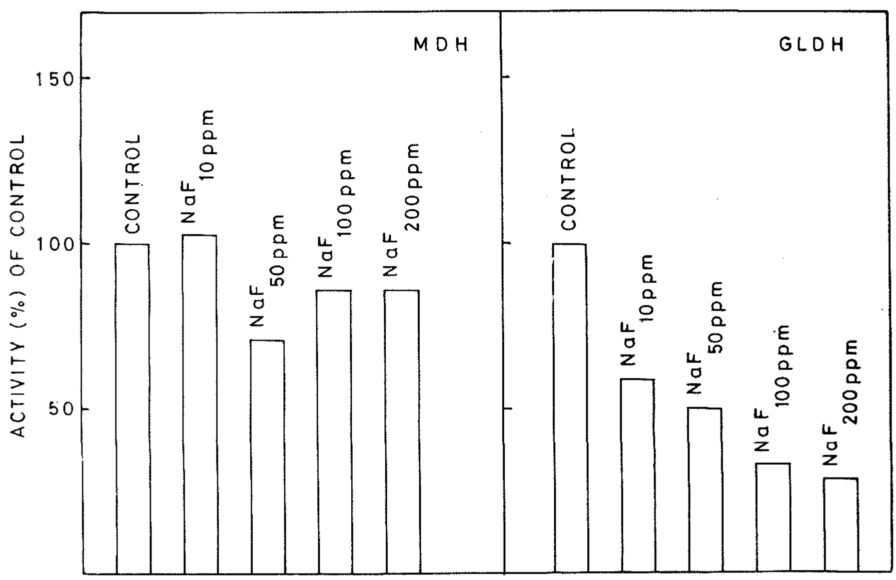

Fig. 2. Effect of NaF on the activities of malate dehydrogenase (MDH) and glutamate dehydrogenase (GLDH). Enzyme activity is expressed as (%) of control.

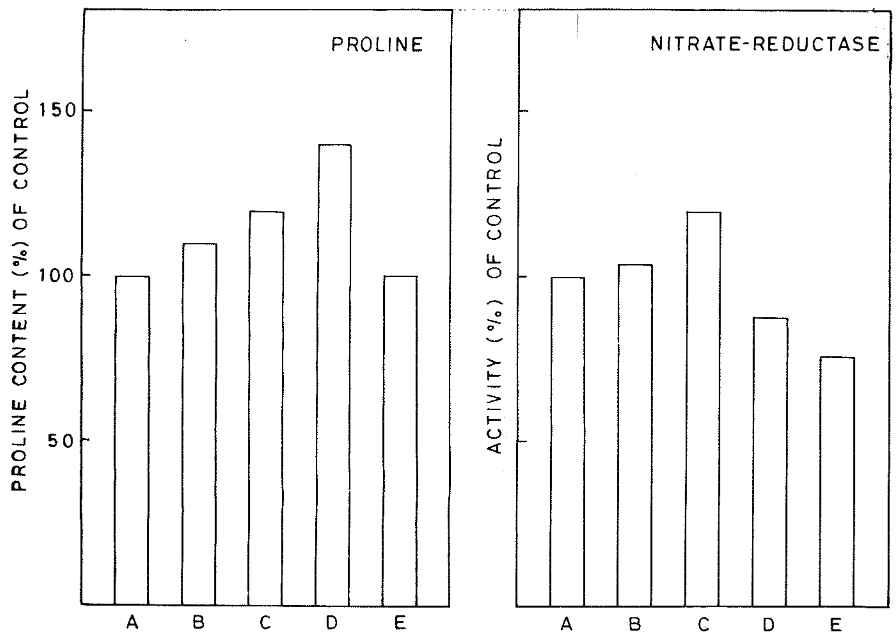

Fig. 3. Effect of NaF on the proline content and Nitrate Reductase activity. The concentrations were used as follows: A: Control; B: NaF 10 ppm; C: NaF 50 ppm; D: NaF 100 ppm; E: NaF 200 ppm. Values expressed as (%) of control.

Huber and Sankhla, 1973). Since the activity of transaminases is stimulated under conditions deleterious to protein synthesis, it is postulated that these enzymes play an important role in photosynthesis of C_4 plants as well as adaptation of plants under conditions of stress (Hatch and Mau, 1973; Huber and Sankhla, 1973).

It is interesting to note that during present investigation NaF inhibits protein synthesis and the activities of GLDH and MDH. Although there are reports that the activities of GLDH and MDH favourably affected following treatment with salt and CEPA in *Lasiurus, Cenchrus, Pennisetum* (Bohra, 1980; Sankhla and Huber, 1974). Glutamate dehydrogenase catalyses reductive ammination of α-ketoglutarate to glutamic acid, which is believed to be major reaction by which ammonia is assimilated by higher plants (Webster, 1959). Since NaF inhibits the activity of both GLDH and MDH, this may indicate an inhibitory action on protein synthesis.

Nitrate reductase is substrate inducible enzyme, its activity depends upon the several factors including nitrate supply, bio-regulants, and salt (Bohra, 1980; Sankhla *et al.*, 1983). In the present investigation NaF used differentially modulate the activity of this key enzyme of nitrogen assimilations.

A characteristic feature of present investigation is the accumulation of proline under the influence of NaF. In many plants the level of free proline is known to rise under stress (Singh *et al.*, 1972; Eder *et al.*, 1677; Ahmed and Sankhla, 1979). The results of the present investigations are in accordance with these findings. The capacity to accumulate proline was found to be closely related to drought tolerance of the plants (Stewart and Lee, 1974). The present investigation suggests that NaF used may induce some changes in metabolic activities on metabolic compounds, which may play a decisive role in the adaptation forwards stress.

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氟化鈉對非洲狼尾草葉內某些酵素活性與脯氨酸含量之影響

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很久以來即知氟化物可作爲酵素抑制劑，而一些研究也曾試圖瞭解氟化物如何影響基本酵素反應。本研究即從觀察氟化鈉對非洲狼尾草（*Cenchrus ciliaris*）葉內一些主要氮代謝酵素活性及脯氨酸（proline）含量之反應著手。一般而言，氟化鈉促進丙氨酸轉氨酶（alanine aminotransferase）之活性，且低濃度之氟化物可稍微增加硝酸還原酶的活性。另一方面，氟化鈉可抑制天冬氨酸轉氨酶（aspartate aminotransferase）、穀氨酸去氫酶（glutamate dehydrogenase）及蘋果酸去氫酶（malate dehydrogenase）的活性。而就脯氨酸含量而言，使用不同濃度之氟化鈉，可有很好的結果。