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Research Paper

EFFECT OF FLUORIDE ON METABOLIC PATTERNS AND NITRATE REDUCTASE ACTIVITY IN *Ziziphus* SEEDLINGS

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Abstract

The objective of present study was to investigate the effect of sodium fluoride (NaF) on pigments, metabolic patterns and Nitrate reductase activity in *in-vitro* grown seedlings of *Ziziphus mauritiana* cv.Tikadi. Sodium fluoride treatments (1 and 10 mM) decreased pigment composition (chl a, b and carotenoids) and chlorophyll (a and b) is more reduced than carotenoids. F toxicity also decreased the soluble protein content whereas, NR activity and total soluble sugars were increased significantly with increased level of Fluoride. Lower concentration of fluoride increased proline content while it was decreased with higher concentration of NaF.

Key words: Fluoride, Nitrate reductase ,pigments , biochemicals , *Ziziphus mauritiana* cv. Tikadi.

INTRODUCTION

Fluoride is one of the most toxic atmospheric pollutants affecting germination and early seedling growth of plants (Gupta *et al.* 2009). It inhibits transport of water and minerals which are important for important for physiological and biochemical reactions of plants (Kamaluddin and Zwiazek, 2003; Abdallah *et al.,* 2006). It has been well documented that several physiological and biochemical reactions like photosynthesis, respiration, metabolism of enzymes activities,anti oxidant system, production of reactive oxygen species (ROS), photosynthetic pigments and yield are adversely affected by F toxicity in plants (Gadi *et al* ., 2012; Ram *et al.,* 2014; Bhutiyal *et al.,*2015). Response to F toxicity and mechanism for fluoride stress tolerance by changing metabolic patterns and enzyme activities in plants depends on dose, duration of exposure, age and genotypes of plants etc. (Datta *et al*.,2012; Bhutiyal *et al,* 2014;) .Moreover, fluoride causes diverse toxic effects in both humans and animals through drinking water and food chain. (Choubsia *et al* .,2012).

Ziziphus mauritiana cv.Tikadi is one of the most important …..fruit crops of arid region of Rajasthan .The fruits are rich source of minerals , carbohydrates, vitamins and amino acids. In this region,fluoride in groundwater and soil is high owing to natural and anthropogenic factors. Due to uncertainty and unequall distribution of rain fall , fluoride contaminated ground water is used for irrigation which adversely affects growth and physiology of crop specially at early stage of seedling growth.

The present investigation was undertaken to study the effect of NaF on photosynthetic pigments, metabolic changes (soluble protein, total soluble sugars and proline) and Nitrate reductase activity in *in vitro* grown seedlings of *Ziziphus mauritiana* cv.Tikadi.

MATERIALS AND METHODS

Seeds of *Ziziphus mauritiana* cv.Tikadi sterilized with 0.1% $HgCl_2$ for 5 minutes followed by through rising with distilled water. Seeds were germinated in petri plates containing whatman No. 1 filter paper and they were irrigated with distilled water (control), 1 and 10mM NaF treatments . These petri plates were kept at 28°±2°C in BOD incubator. After twenty four hours seed coats were peeled off and after five days old Seedlings were used for estimation of different biochemicals.

Photosynthetic pigments (chlorophyll a, b and carotenoids) were determined by following the method described by Arnon, (1949). Seedlings were homogenized in 80% acetone. The extract was centrifuged at 10,000 rpm for 15 minutes and absorbance of supernatant was read at 450, 645, 663 nm using spectrophotometer.

Total soluble sugars were estimated by McCready *et al*, (1950) method. It is based on the principle that in hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms green coloured product with anthrone Soluble proteins were estimated by the method of Bradford (1976). This involves the binding of Coomassie Brilliant Blue G-250 (CBBG-250) to proteins. CBBG-250 exists in two different colour forms red and blue. The red form is converted to blue form upon binding of the dye to protein. Bates *et al*,(1973) method was used for estimation of proline content. The extracted proline was made t react with ninhydrin in acidic condition to form the chromophore (red colour) which was read at 520 nm. Nitrate reductase (NR) activity was determined according to the method of Wray and Filner (1970). The data were statically analyzed and CD at 5% level of significant was calculated.

RESULTS AND DISCUSSION

The present study revealed that the NaF stress resulted in a decrease chlorophyll (chl-a, chl-b, total chlorophyll) and carotenoid content in *in vitro* grown seedlings of *Ziziphus mauritiana* cv.Tikadi as compared to control (Table-1). Higher concentration of NaF has more inhibitory effect than lower concentration. The possible causes for the decreasing of the pigment content may be due to inhibition of chlorophyll biosynthesis or breakdown of chlorophyll (Gupta *et al.*,2009). In addition, Abdallah *et al.,* (2006) also suggested that formation of MgF^{+} complex by binding of fluoride anion with Mg^{2+} may degrade the pigments.

Similar to the present study, earlier studies confirm that fluoride causes a reduction in the chlorophyll content in *Oryza sativa* (Gupta *et al.,* 2009), *Prosopis juliflora* (Saini *et al.,* 2013) and *Citrullus lanatus* (Ram *et al.,*2014). Chlorophyll content was decreased more than carotenoids in F stressed seedlings as it decreases the chl-a (41 %), Chl-b (45 %) and carotenids (25%) content over control. The lower reduction of carotenoids may be implicated for protection against oxidative stress because these are accessory pigments in photosynthetic systems and also function as non enzymatic antioxidants under stresses (Gomathi and Rakkiyapan,2011).

In the present study, it was also observed that increase in NaF levels significantly reduced soluble protein content. Similarly, a decrease in protein content was reported under F toxicity in *Cenchrus ciliaris* (Chaudhary and Bohra,1989). and *Vigna radiata* (Gadi *et al*.,2012).

Proline content increased by 27% over control with 1 mM NaF concentrations. On the other hand, it was slightly reduced in higher concentration of NaF treated seedlings. Proline accumulation induced by fluoride has been reported in *Vigna radiate (*Gadi *et al*. 2012). Reduced activity of proline catabolic enzymes and enhanced activity of its biosynthetic enzymes also increased proline accumulation in stress condition (Phutela *et al.,* 2000)**.**

Under adverse environmental conditions, accumulation of osmolytes (proline and sugars) play several protective roles including stabilization of membranes and proteins, protection against stresses, source of nitrogen and scavenger of ROS in plants (Couee *et al*., 2006;Szabados and Savoure,2010). NaF markedly increased the total soluble sugars content

with the higher concentration of NaF (10 mM) increasing the soluble sugars content by 55% compared to control seedlings (Table -2). Total soluble carbohydrates are important solutes which are synthesized and accumulated in cytosol under stress (Munns and Tester ,2008). Kamaluddin and Zwiazek (2003) reported that fluoride inhibits the water uptake and hydrolytic conductivity of plant at root level and creates water deficit for the plants. In the present study, the increased sugars may act as osmolytes which increase the water potential of seedlings for survival under water stress, scavenge the free radicals and thereby protection against oxidative damage.

Nitrate reductase, a key enzyme in the first step of nitrate assimilation, is negatively influenced by a wide range of environmental factors like salinity, oxidative stress ,temperature and osmotic stress in many plants (Flores *et al*., 2000; Godara and Gadi ,2015). NR activity is mainly decreased by an increase in the enzyme/protein breakdown by stress induced oxidative stress.or increased proteolytic activity that is increased stress induced free radicals (Lillo *et al.*, 2004; Saini *et al*.,2011).However, in the present study, the activities of NR increased with both concentration of NaF (Figure-2). With compared to control, the NR activity increased to 31% to 25% at 1 and 10m mM NaF respectively. It is in conformity with the earlier findings that NR activity is not decreased by NaF as reported in *Oryza sativa* L.(Srinivasan and Rama Rao ,1980) and *Cenchrus ciliaris* (Chaudhary and Bohra,1989).Viegas *et al* (1999) also suggested that, the *in vitro* leaf-NR activity was not changed in salt treated plants while it was decreased in *in vivo* grown plants.

It has been concluded that F toxicity inhibited pigments ,soluble protein content while increased the NR activity . Increase in sugars may play protective roles in osmotic adjustment and survival of *Ziziphus* seedlings under F toxicity.However, proline seems to play protective role under low concentration of fluoride only and it decreased at higher concentration of fluoride.

Table 1 : Effect of NaF on photosynthetic pigments (mg g^{-1} fw) in *in-vitro* grown seedlings of *Z. mauritiana* cv. Tikadi (± value of SD)

Treatment	Chl-a	Chl-b	Total chlorophyll	Carotenoides
Control	0.917±0.034	0.421±0.026	1.338±0.084	0.278±0.031
1 mM NaF	0.834±0.047	0.341±0.28*	1.175±0.063	0.253±0.026*
10 mM NaF	0.543±0.052*	0.231±0.023	0.774±0.092*	0.209±0.009
CD at 5 % level	0.047	0.026	0.074	0.036

*p≤0.05, Pesaran's cross-sectional dependence (CD) test.

Table 2 : Effect of NaF on protein Soluble protein ,proline, sugars and nitrate reductase activity in *in vitro* grown seedlings of *Z. mauritiana* cv. Tikadi (± value of SD)

Treatment	Protein (mg g^{-1} fw)	Proline (mmol g^{-1}fw)	Sugars (mg g^{-1} fw)	Nitrate Reductase (n mol mg^{-1} $Protein^{-1}$ min)
Control	54.909± 0.285	0.932± 0.017	45.271±0.338	3.658±0.025
1 mM NaF	52.718 ± 0.331*	1.186±0.009*	48.739±0.172*	4.445±0.009*
10 mM NaF	49.409 ± 0.412	0.861±0.010	57.915±0.541*	4.105±0.017
CD at 5 % level	0.052	0.028	0.081	0.027

*p≤0.05, Pesaran's cross-sectional dependence (CD) test.

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