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EFFECTS OF FLUORIDE ON GERMINATION, EARLY GROWTH AND ANTIOXIDANT ENZYME ACTIVITIES OF *Cicer arientium*

P. SAINI[a1], S. KHAN[b] AND V. SHARMA[c]

[abc]Rakesh P.G. College Pilani, Banasthali, Rajasthan, India

ABSTARCT

The present study was undertaken to determine the influence of NaF phytotoxicity grown in soil (0, 25, 50 and 75 ppm) and in water hydroponically (0, 25, 50 and 75 ppm) on the germination together with some biochemical parameters viz, antioxidant enzyme activities (peroxidase and catalase) in *Cicer Arientium* seedlings. After 15 days of treatment, germination, ($r = -0.54$, $p<0.01$), moisture percentage ($r = -0.72$, $p<0.01$) root growth ($r = -0.28$, $p<0.01$), shoot growth ($r = -0.976$, $p<0.01$) and vigor index ($r = -0.384$, $p<0.01$) were in decreasing trend with increasing concentration of NaF. Activity of both catalase (2.2 folds) and peroxidase (1.7 folds) enzymes increased with increase in NaF concentration. There was a significant correlation ($r = 0.31$, $p<0.05$) between increase in the enzyme activities and increasing NaF concentration. F adversely effects on growth parameters and several kinds of enzyme activities, so reduced the plant general fitness prior to or in the absence of the appearance of any visible injury. No visible injuries reported after 15 days of NaF treatment. Therefore, this legume species may be used as suitable bioindicator species for potentially F affected areas in absence of necrosis. Further, higher antioxidant enzymes activities suggesting a role of in imparting tolerance to fluoride stress.

KEYWORDS: Catalase, Fluoride, Germination, Peroxidase

The phytotoxicity of fluoride depends on its concentration in the atmosphere, duration of exposure and sensitivity of the plant species (Weinstein and Davison, 2004). Fluorine and many fluorides, viz sodium and potassium fluorides (NaF/KF) are extremely poisonous, high concentrations of these compounds can occur in acid soils, which are contaminated with pollutants coming from the atmosphere (Supharungsun and Wainwright, 1982). The importance of seed germination in plant growth is widely recognized, and the effects of F toxicity on it have been studied by various researchers (Miller, 1993) (Sabal *et al.,* 2006). Certain physiological processes are known to be markedly affected by F including seed germination, early root and shoot growth, chlorosis, leaf tip burn and leaf necrosis (Weinstein and Davison, 2004) (Mcnulty and Newman, 1961). Under optimal conditions, many metabolic processes occurring in plants produce active oxygen species which, besides their deleterious potential may act as signaling molecules (Cristina *et al.,* 2008). Soil salinity increases the catalase and peroxidase activity among tolerant and sensitive varieties of plant (Gossett *et al.,* 1994). The relationship between salinity and antioxidants was reported that O_2 - radical and H_2O_2 could play an important role in the mechanism of adaptation (Swamy and Reddy, 1991). Peroxidase (POX) is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalysing its decomposition through oxidation of phenolic substrates (Dionisio-Seseand Tobita, 1998) (Sudhakar *et al.,* 2001) (Lin and Kao, 2002). Catalase (CAT) is present in the peroxisomes of nearly all aerobic cells and is virtually absent from chloroplast (Dionisio-Seseand Tobita, 1998). It can protect the cell from H_2O_2 by catalyzing its decomposition into O_2 and H_2O.[12] The expression of catalase genes is not only developmentally regulated but it is also sensitive to various environmental signals (Havir and McHale, 1989) (Matters and Scandalios, 1986) (Scandalios *et al.,* 1997). Although they are involved in scavenging, their turnover being continuous, and their steady-state level can be rapidly lowered under any stress conditions in which translation is inhibited or degradation enhanced (Feierabend *et al.,* 1996).

The present study is done to assess the effect of NaF on the germination, early root and shoot growth, moisture percentage, vigor index and antioxidant enzyme activities (peroxidase and catalase) in *Cicer Arientium* seedlings.

MATERIALS AND METHODS

Plant materials and their cultivation: Seeds of *Cicer Arientium* were procured from IARI Delhi. Surface sterilized and imbibed seeds were germinated on wet filter paper in petri plates and then transferred to petridishes. The germinated seeds were transferred and grown hydroponically in Hoagland's nutrient solution at different

[1]Corresponding author

NaF concentrations ranging from 10-50 ppm viz. 10, 20, 30, 40, 50 ppm. The experiments were carried out after 15 days. Control experiments were also set up in identical manner with deionised water devoid of NaF. Throughout, the germinating seeds were maintained in a growth chamber at a temperature 30±2^{0}C, light intensity of 1000 lux with 14 hrs photoperiod and 70% relative humidity. Vigor index was calculated as per equation by Anderson (Anderson and Abdul-Baki, 1973).

Vigor Index = (Root length + Shoot length) X Germination Percentage

Antioxidant Enzyme Activities

Enzyme Extraction: 1 g of fresh mass of plantlets was ground with a pestle in an ice-cold mortar with 10 ml of 0.05 M sodium phosphate buffer (pH = 7.0). The homogenates were filtered through 4 layers of cheese cloth and then centrifuged at 4°C for 10 min at 10000 rpm. The supernatants were used for the assays of enzyme activities.

CAT activity (EC 1.11.1.6) was determined by measuring the change of absorbance at 240 nm that accompanied the consumption of H_2O_2 (Luck, 1974). The 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and 100–150 µl of enzyme extract. The absorbance at 240 nm was measured. The activity was calculated using the extinction coefficient = 0.0369 per µmole per mL. One unit of CAT activity was defined as the amount required to decompose 1 nmol H_2O_2 min^{-1} mg^{-1}fresh weight.

POD activity (EC 1.11.1.7) was determined as oxidation of guaiacol by H_2O_2 (Putter, 1974). The reaction mixture contained 3 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.1 ml of 3% H_2O_2, 0.05 ml of 1% guaiacol and 100 µl of enzyme extract. The absorbance at 420 nm was measured. One unit of POD activity was defined as the amount required to decompose 1 µmol guaiacol min^{-1} mg^{-1} fresh weight.

After 15 days of treatment, roots/shoots and plantlets were harvested for various morphological parameters such as percent germination, root length, shoot length, and biochemical parameter such as antioxidant enzyme activities were measured. Standard deviation and spearman's correlation were calculated by statistical software package (SPSS 14.0) to examine the differences between each treatment and the level of statistical significance was set at $P \leq 0.05$ and $P \leq 0.01$ of the experimental data.

RESULTS AND DISCUSSION

Effects on Morphological Parameters

Percent Germination and Moisture Percentage

Seed germination, moisture percentage and vigor index (Table 1) showed a decreasing trend in germination percentage with increasing concentration of sodium fluoride. As showed in Table 1, under control conditions 76% germination had occurred, whereas at 50 ppm NaF, the germination decreased to 73%. The difference observed in percentage germination was due to effect of varied concentration of NaF. However moisture percentage and vigor index also showed a decreasing trend with increasing fluoride concentration. Activity of both catalase (2.2 folds) and peroxidase (1.7 folds) enzymes increased with increase in NaF concentration.

Table 1: Germiantion, Moisture and Vigor Index of *C. arientium* seedlings after 15 days after exposure to different concentration of NaF (mean ± SD)

Concentration of NaF (ppm)	Germination (%)	Moisture (%)	Vigor Index
Control	74.2 ± 0.14	76.7 ± 0.09	1066.8
10	73.8 ± 0.24	75.2 ± 0.04	985.40
20	73.2 ± 0.31	74.6 ± 0.12	970.08
30	73.1 ± 0.54	73.1 ± 0.14	931.24
40	72.4 ± 0.21	72.6 ± 0.06	892.80
50	72.0 ± 0.26	71.4 ± 0.08	854.10

In addition, at 50 ppm the moisture content in the leaves was found to be 71.4% which results that increasing level of NaF shows the reduction in elongation of root and shoot length and thus reduction in their moisture percentage and vigor index. Correlations with increasing concentrations of NaF were significant in case of germination percentage ($r = -0.54$, $p<0.01$) moisture percentage ($r = -0.72$ $p<0.01$) and vigor index ($r = -0.28$, $p<0.01$) *Cicer Arientium.*

Root and Shoot growth: In present study, after 15 days of F treatment, decreasing trend in the growth of roots and shoots with increasing concentration of NaF were found. The correlation analysis has confirmed a decreasing trend in growth of roots and shoots with increasing concentration of NaF in all the seedlings. Correlations with increasing concentrations of NaF were significant in case of root ($r = -0.28$, $p<0.01$) and shoot ($r = -0.976$, $p<0.01$) in *C. arientium* plant. As seen in Table 2, the average root and shoot length decreased with increasing F concentration. In this study the shoots system was affected more by higher concentrations of NaF in comparison to roots. A similar observation was found in the previous study done for wheat, mustard and cluster bean (Sharma, 1985) (Sabal *et al.,* 2006). Similar to present study which was conducted on another legume species *Vicia faba* (Davies *et al.,* 1998), confirmed the ability of roots to accumulate higher amount of F than that of the shoot system that suggests roots were relatively more tolerant to F than that of shoots at higher concentration of NaF in our study.

Table 2: Root and shoot lengths of *C. arientium* seedlings after 15 days after exposure to different concentration of NaF (mean ± SD)

Concentration of NaF (ppm)	Root Length (cm)	Shoot Length (cm)
Control	7.8 ± 0.12	6.2 ± 0.21
10	7.2 ± 0.64	5.8 ± 0.34
20	7.4 ± 0.34	5.5 ± 0.41
30	7.1 ± 0.76	5.3 ± 0.23
40	6.8 ± 0.03	5.2 ± 0.17
50	6.7 ± 0.18	5.0 ± 0.25

EFFECTS ON ANTIOXIDANT ENZYME ACTIVITIES

Peroxidase Activity

The hydrogen peroxide is toxic for plants as it acts both as an oxidant as well as a reductant. The hyperactivity of POD under F stress indicated the scavenging activity of H_2O_2 generated through activity of photo respiration in plant cells. Therefore, an increase in POD activity prevents plants from toxic effects of H_2O_2. During present study enhancement of POD activity suggests its role in constant detoxification of H_2O_2 in F toxicity. The hyper activity of antioxidant enzymes might be consequences of the strategies adapted by plant for its survival under stress imposed by F. Peroxidase activity increased with increase in F concentration. However, highest increase was observed at 40 ppm F concentration where a maximum of 2.3 fold increase in activity was seen. Therefore there was a direct correlation between the F concentration & peroxidase activity ($r = 0.67$, $p<0.05$).

Catalase Activity

Catalase scavenges H_2O_2 by breaking it down directly to form water and oxygen. Catalase activity increased steadily with increase in fluoride concentration, at 50 ppm, the increase was 3.2 folds. There was a significant correlation ($r = 0.72$, $p<0.05$) between increase in catalase activity and NaF concentration. Similar trends in increase in CAT activity were also obtained with increase in salinity levels in *Triticum aestivum* (Heidari, 2009). The higher CAT activity indicates that fluoride tolerant lines had better ability to scavenge H_2O_2.

From the result it is very clear that *Cicer arientium* treated with different concentration of F showed an increase in catalase and peroxidase activites. As seen from the data, the increase in catalase and peroxidase is strongly correlated with F ion concentration. This can be compared with earlier report on cadmium effect on enzyme activity in *Colocassia esculentum* (Mandakini *et al.,* 2005) (El-baky *et al.,* 2003) reported that salt stress increased the enzyme activities such as catalase, guiacol peroxidase, ascorbic acid oxidase, polyphynol oxidase. Antioxidants are well known to play a prominent role in defense against free radicals in plants.

Overall, our study concludes that NaF has significant impact on seed germination and seedling growth of *C. arientium* The ability to accumulate metals from soil in plants are in following order roots>stem>leaves (Senthilkumar *et al.,* 2005). In present case, root length was less affected in comparison to shoot length despite of high accumulation by F, that showed roots are more tolerant to F stress. F adversely effects on growth parameters and several kinds of enzyme activities, so reduced the plant general fitness prior to or in the absence of the appearance of any visible injury. No visible injuries reported after 15 days of NaF treatment. Therefore, this legume species may be used as suitable bioindicator species for potentially F affected areas, in absence of necrosis. Higher antioxidant activities with increase in F concentrations suggest a role in imparting tolerance to fluoride stress in *C. arientium*. Therefore, further study on this plant will suggest a view of its ability to hyper accumulate F and its worth uses in phytoremediation purposes.

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