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THE EFFECTS OF FLUORIDE STRESS ON OKRA (*ABELMOSCHUS ESCULENTUS* L.)

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ABSTRACT: The influence of sodium fluoride on two cultivars of okra (Nirali and Arka Anamika) was evaluated during the seedling and growth stages, by applying different concentrations of NaF (50, 100, 150, 200, 250, and 300 ppm). All the parameters studied showed a greater reduction with the higher concentrations of sodium fluoride (250 and 300 ppm) than with the lower concentrations (50 and 100 ppm). In cultivar Nirali, the plants treated with 300 ppm NaF showed reduction percentages in the shoot and root fresh weight, of 84% and 90%, respectively, while the corresponding reductions in Arka Anamika were 82% and 91%. Increased concentrations of fluoride can significantly decrease plant pigment levels (chlorophyll a & b, total chlorophyll, and total carotenoids), the total soluble sugar, and the protein content. In contrast, increasing concentrations of sodium fluoride, increased the proline content significantly.

Keywords: *Abelmoschus esculentus;* Growth; Okra; Pakistan; Sodium fluoride; Yield.

INTRODUCTION

Okra (*Abelmoschus esculentus*) is a vegetable crop belonging to the family Malvaceae. Cultivated crops of okra are currently grown in many countries and it is dispersed extensively in Asia, Africa, America, and Europe. It is a crop which likes a tropical to subtropical environment and grows well in warm conditions.[1] In Pakistan, okra is usually grown in the provinces of Punjab and Sindh and the total area under cultivation with the crop is to be estimated 2.21×10^5 hectares yielding about 2.86×10^6 tons of green pods.

In nature, fluorine appears in natural minerals, especially fluorite, apatite, muscovite, and biotite. The accumulation of the fluoride ion (F) in soil leads to lower crop yields.[2] Fluoride is well recognized as a widespread, non-biodegradable, and hazardous nonmetallic pollutant.[3] Soil pollution by F is a major problem worldwide.[4] F is not essential for normal plant growth and at high concentrations it may cause damage to both plants and the environment.[5]

The entry point in leaves for gaseous fluoride is via the stomata and the leaves are usually most sensitive when a plant is young and expanding.[6] The rate of the development of the symptoms of F toxicity appears to depend on the weather and the duration of the exposure to the fluoride stress. It has been reported that increased levels of F in acid soils reduce crop yield due to increasing aluminum and decreasing the uptake of phosphorus.[2] The objective of the present study was to identify plant growth parameters for okra that could be used as selection criteria for the early evaluation of different cultivars for fluoride stress.

MATERIALS AND METHODS

Seeds of *Abelmoschus esculentus,* cultivar Arka Anamika, were obtained from the Punjab Seed Corporation, Lahore, and of cultivar Nirali from Pride seeds, Lahore

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Pakistan. The Botanical Garden, Quaid-e-Azam campus, University of the Punjab, Lahore was selected as the location for the experiment (31º35-00-N, 74º21-00-E). The duration of experiment was from March to May 2016. A wire-netting enclosure was used to carry out the experiment and normal agricultural practices were used to prepare a mixture of sandy loamy soil.

The average temperature during the experiment was between 27±3ºC to 44±4ºC. The range of the relative humidity between 16±3% to 38±4% and the rainfall ranged from 22.4±2 mm to 41.2±3 mm. This data was obtained from the Pakistan Meteorological Department, Lahore.

Various dilutions of sodium fluoride were prepared by mixing appropriate amounts of solutes in distilled water. Different concentrations of sodium fluoride were applied to determine their effect on the okra cultivars Nirali and Arka Anamika over time during the 2016 growth season (Table 1).

Table 1. Different concentrations of sodium fluoride

Concentration (ppm)	Amount of solute (mg/L)	Amount of solute (g/L)
Control	0.00	0.00
NaF-50	50	0.05
NaF-100	100	0.10
NaF-150	150	0.15
NaF-200	200	0.20
NaF-250	250	0.25
NaF-300	300	0.30

The first treatment of sodium fluoride was applied 31 days after sowing (DAS). The treatments were applied twice a week throughout the season till the final harvest. Two destructive harvests were taken at 45 DAS and 70 DAS. The first and second harvests were taken on April 25 and May 20, 2016 at the reproductive and maturity phases, respectively (Table 2).

Table 2. Summary of the events during the experiment

Major event	Date	Days after sowing (DAS)
Soaking of seeds	March 9, 2016	0
Sowing of seeds	March 10, 2016	0
Start of germination	March 15, 2016	5
Completion of germination	March 28, 2016	18
Thinning of seedlings	April 7, 2016	27
Stress application	April 11, 2016	31
1st harvest	April 25, 2016	45
2nd harvest	May 20, 2016	70

The plant pigments of okra plant were measured as the chlorophyll and carotenoids content at 70 DAS. The chlorophyll a, chlorophyll b, and carotenoids were measured according to procedures of Aron[7] and Davies,[8] respectively.

$$\text{Chlorophyll a (mg/g FW)} = (0.0127 \times \text{OD } 663) + (0.00269 \times \text{OD } 645)$$

$$\text{Chlorophyll b (mg/g FW)} = (0.0229 \times \text{OD } 645) + (0.00468 \times \text{OD } 663)$$

$$\text{Total chlorophyll (mg/g FW)} = (0.0202 \times \text{OD } 645) + (0.0082 \times \text{OD } 663)$$

$$\text{Carotenoids (g/mL)} = \frac{A^{car}}{E^{m}} \times 100$$

where: FW=fresh weight
OD= optical density
A^{car}= OD 480 + 0.114 (OD 663) – 0.638 (OD 645)
$E^{100\%}$ (cm) = 2500

The total soluble sugars were estimated with the method of McCready et al.[9] The soluble protein contents were estimated by the Bradford method for the estimation of soluble protein concentration.[10] The free proline contents were determined spectrophotometrically by adapting the ninhydrin method.[11] The statistical analysis was made by using the Costats software package (version 1.01).[12]

RESULTS

The crop growth of okra (*Abelmoschus esculentus* L.) was recorded for the two cultivars. In cultivar Nirali, the plants treated with 300 ppm NaF showed reduction percentages in the shoot and root fresh weights, of 84% and 90%, respectively, while the corresponding reductions in Arka Anamika were 82% and 91%. In the cultivar Nirali, the control group showed the maximum lengths of shoot and root and the minimum lengths occurred in 300 the ppm sodium fluoride group. Compared to the control group, at 45 DAS, the fractional decreases in the lengths of the shoot and root treated with NaF-50 were 4.38% and 4%, respectively. The plants treated with 100, 150, 200, 250, and 300 ppm NaF had reductions in their shoot length (15.57%, 20.96%, 32.74%, 36%, and 51%, respectively) and root length (7%, 10%, 14%, 33%, and 73.6% respectively). At 75 DAS, the decrease in the percentage of shoot and root length in the group treated with NaF-50 was 5.42% and 25.28%, respectively, as compared to the control. A trend was present for the shoot and root lengths to decrease at 45 DAS and 70 DAS.

In the cultivar Arka Anamika, at 45 DAS, the percentage decreases in the shoot and root lengths of the plants treated with NaF-50 ppm were 1.3% and 1.5%, respectively, as compared to the control group. The plants treated with 100, 150, 200, 250, and 300 ppm sodium fluoride showed reductions in their shoot lengths (6.4%, 21%, 39%, 43%, and 53%, respectively) and root length (2.02%, 7.98%, 8.67%, 32.59%, and 69.6%, respectively) as compared to the control group. At 70 DAS, the percentage decreases in the length of the shoot and root in the plants treated with NaF-50 were 4.23% and 3.82%, respectively.

In the cultivar Nirali, the minimum leaf area was observed with the treatment with NaF-300 ppm, as compared to the plants treated with lower concentrations of sodium fluoride. A similar decreasing trend was observed in the cultivar Arka Anamika at both the reproductive (45 DAS) and maturity (70 DAS) stages. In the cultivar Nirali, the number of leaves decreased with increasing sodium fluoride concentrations. In both the cultivars, Nirali and Arka Anamika, the control plants treated with water had the highest number of leaves while the plants treated with NaF-300 had lowest number. The plants treated with 150, 200, 250, and 300 ppm sodium fluoride showed reductions in the number of leaves (7.3%, 7.3%, 21%, and 21%, respectively) at 45 DAS. The same trend was observed at 70 DAS. In the plants treated with 50 ppm and 100 ppm NaF, no effect was seen in number of leaves.

The photosynthetic pigments were highest in the control group (Table 3). They showed a trend of decreasing with increasing concentrations of sodium fluoride. The reductions in chlorophyll a and b were least with treatment with 50 ppm NaF and greatest with the treatment with 300 ppm sodium fluoride. In the Nirali cultivar, the percentage decreases of in chlorophyll a relative to the control group with treatment with 50, 100, 150, 200, 250, and 300 ppm NaF were 4.6%, 11.05%, 16.27%, 23.33%, 27.33%, and 34.3%, respectively. The corresponding reductions for chlorophyll b, were 14.81%, 35.19%, 51.85%, 62.96%, 66.66%, and 77.77%, respectively. The percentage reductions for the carotenoids and the total chlorophyll had their maximum values with treatment with the highest NaF concentration (300 ppm), 76.38% and 30.19%. respectively. The amounts of chlorophyll a, chlorophyll b, and carotenoids were highest in the control group and lowest in the NaF-300 for both cultivars (Table 3).

For the Nirali cultivar, there was a gradual reduction in the protein content as the sodium fluoride concentration increased (Table 4). The reduction in the protein weight was at its maximum (75%) in the group treated with 300 ppm NaF, while the minimum was observed in the group treated with NaF-50 (18.86%). Similarly, the cultivar Arka Anamika also showed a decrease in the protein content with increasing concentrations of sodium fluoride (Table 4). The reduction in the protein content was greater in the Nirali cultivar than in the Arka Anamika cultivar.

In cultivar Nirali, a decrease in total soluble sugars was observed in present studies following the NaF treatments (Table 4). The percentage reductions in the total soluble sugar with treatment with 50, 100, 150, 200, 250, and 300 ppm NaF, were 2.88%, 10.79%, 17.99%, 21.58%, 27.34% and 29.5%, respectively. In cultivar Arka Anamika, a similar reduction in the total soluble sugar content also occurred with fluoride stress. The total soluble sugar content was highest (1.47 mg/g) in the control group and lowest (1.04 mg/g) in the NaF-300 and the percentage reductions with treatment with 50, 100, 150, 200, 250, and 300 ppm NaF, were 3.40%, 9.52%, 14.97%, 19.73%, 24.49% and 29.25%, respectively (Table 4).

An increase in the free proline content was observed in the plants of okra following the NaF treatments. Compared to the control group, the proline content increased as the sodium fluoride concentration increased from 50 to 300 ppm. The proline content minimum value (0.5 mg/g) occurred in the control group and maximum value (0.88mg/g) in the NaF-300 group (Table 4).

Table 3. Plant pigments of *Abelmoschus esculentus* cv. Nirali & cv. Arka Anamika at 45 days after sowing (DAS). (Chlor a=chlorophyll a; Chlor b=chlorophyll b; Total chlor =total chlorophyll; Carot=carotenoids; FW=fresh weight)

Treatment (ppm of NaF)	Plant pigments							
	Cultivar Nirali				Cultivar Arka Anamika			
	Chlor a (mg/g FW)	Chlor b (mg/g FW)	Total chlor (mg/g FW)	Carot (mg/g FW)	Chlor a (mg/g FW)	Chlor b (mg/g FW)	Total chlor (mg/g FW)	Carot (mg/g FW)
Control	1.72a ±0.01	0.54a ±0.01	0.56a ±0.01	1.66a ±0.01	1.60a ±0.03	0.40a ±0.01	0.55a ±0.02	1.59a ±0.01
NaF-50	1.64b ±0.01	0.46b ±0.01	0.41b ±0.01	1.59b ±0.01	1.57a ±0.01	0.35b ±0.01	0.48b ±0.02	1.45b ±0.01
NaF-100	1.53c ±0.01	0.35c ±0.01	0.35c ±0.01	1.43c ±0.01	1.47b ±0.01	0.28c ±0.01	0.40c ±0.02	1.30c ±0.01
NaF-150	1.44d ±0.01	0.26d ±0.01	0.30d ±0.01	1.40d ±0.01	1.31c ±0.01	0.25d ±0.01	0.32d ±0.02	1.27d ±0.01
NaF-200	1.32e ±0.01	0.20e ±0.01	0.23e ±0.01	1.33e ±0.01	1.29c ±0.01	0.20e ±0.01	0.24e ±0.02	1.20e ±0.01
NaF-250	1.25f ±0.01	0.18f ±0.01	0.20f ±0.01	1.26f ±0.01	1.25d ±0.01	0.15f ±0.01	0.21e ±0.02	1.16f ±0.01
NaF-300	1.13g ±0.01	0.12g ±0.01	0.18g ±0.01	1.16g ±0.01	1.15e ±0.01	0.10g ±0.01	0.13f ±0.02	1.11g ±0.01

Each treatment mean is the sum of three replicates and ± represents the standard error (SE). Within each parameter, values not followed by the same letter are significantly different with Duncan's multiple range test.

Table 4. Proline content, protein content, and total soluble sugars of *Abelmoschus esculentus* cultivar Nirali at 45 days after sowing (DAS). (FW=fresh weight)

Treatment (ppm)	Biochemical attributes					
	Cultivar Nirali			Cultivar Arka Anamika		
	Free proline (mg/ g FW)	Protein (mg/ g FW)	Total soluble sugars (mg/ g FW)	Free proline (mg/ g FW)	Protein (mg/ g FW)	Total soluble sugars (mg/ g FW)
Control	0.29a ±0.01	0.28a ±0.01	1.39a ±0.01	0.50a ±0.01	0.37a ±0.01	1.47a ±0.01
NaF-50	0.37b ±0.0	0.23b ±0.01	1.35b ±0.01	0.56ab ±0.02	0.31b ±0.01	1.42b ±0.01
NaF-100	0.44c ±0.01	0.21c ±0.01	1.24c ±0.01	0.62bc ±0.02	0.25c ±0.01	1.33c ±0.02
NaF-150	0.48d ±0.01	0.17d ±0.01	1.14d ±0.01	0.70c ±0.01	0.19d ±0.01	1.25d ±0.03
NaF-200	0.55e ±0.01	0.12e ±0.01	1.09e ±0.01	0.76d ±0.02	0.16e ±0.01	1.18e ±0.01
NaF-250	0.62f ±0.01	0.10f ±0.01	1.01f ±0.01	0.82de ±0.02	0.12f ±0.01	1.11f ±0.01
NaF-300	0.74g ±0.01	0.07g ±0.01	0.98g ±0.01	0.88e ±0.02	0.10f ±0.01	1.04g ±0.01

Each treatment mean is the sum of three replicates and ± represents the standard error (SE). Within each parameter values not followed by the same letter are significantly different with Duncan's multiple range test.

DISCUSSION

Globally, an increase fluoride contamination of water, soil, and crops is a current problem.[13] In plants like okra, certain metabolic processes are known to be distinctly disturbed by fluoride resulting in decreased plant growth, chlorosis, leaf tip burn and necrosis, and a decrease in the content of chlorophyll, total soluble sugar, and protein.[14] The results of the present study clearly indicate that high concentrations of sodium fluoride significantly reduced plant growth of okra. The maximum reduction was observed in the plants treated with 300 ppm of NaF. Plants treated with 100, 150, 200, 250, and 300 ppm sodium fluoride showed progressive and successive reductions in their shoot length with every increase in the sodium fluoride concentration, as compared to the control group.[14]

Sodium fluoride stress caused a reduction in the chlorophyll a, chlorophyll b, and carotenoid content which in turn resulted in necrosis and chlorosis. The results are in agreement with earlier findings on the inhibition of photosynthetic pigments as an indirect toxic effect of the fluoride ion in Mg deficiency where it reduces Mg absorption by plants.[15] It has been reported that fluoride effects photosynthesis by acting on the membranes and stomatal enzymes associated with carbon dioxide fixation.

In the present study, the protein content was decreased in both cultivars of okra subjected to fluoride stress as compared to control plants. It was found that NaF at 300 ppm concentration caused the maximum decrease in the total protein content, while at 50 ppm sodium fluoride the concentration decreased minimally, compared to the control group. In previous studies fluoride was found to reduce the protein content in *Cicer arietinum*.[16] The present study demonstrated that the total soluble sugar decreased with increasing fluoride stress in both cultivars. The reduction in the total soluble sugar content was highest in the plants treated with 300 ppm NaF. According to Agarwal and Kahn,[17] the total soluble sugar decreased in *Vigna radiata* with increased fluoride stress. In the present study, fluoride stress increased the proline content in okra. This is consistent with the finding by Saleh and Abdel-Kader of an increase in he proline content in *Helianthus annus* cultivars with increased fluoride stress.[18]

CONCLUSION

Fluoride stress in the Nirali and Arka Anamika cultivars of okra ((Abelmoschus esculentus) may reduce shoot and root length and weight, decrease the protein and total soluble sugars, and increase the proline content.

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