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Research Article

Sodium fluoride (NaF) induced changes in growth and DNA profile of *Vigna radiata*

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Abstract

Fluoride (F) is a natural, ubiquitous, non-biodegradable and hazardous pollutant. To investigate its effect, *Vigna radiata* seeds were treated with NaF (0, 25, 50, 75, 100 mg NaF kg-1) and its effect was studied on germination, growth and RAPD profile. The variation in DNA profile in response to NaF treatment was detected by RAPD-PCR technique. The result of RAPD was observed in terms of GTS% (Genomic Template Stability). Results showed that there was gradual decrease in GTS% i.e. increase in DNA damage from 25 to 75 mg NaF kg-1 concentration. These data demonstrate that RAPD is a reliable tool and permits greater insights into the genetic alteration of *V. radiata*.

Keywords
Fluoride; GTS; RAPD; *Vigna radiata*

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1. Introduction

Fluorides (F) are well recognized widespread, non biodegradable and hazardous nonmetal pollutant (Agalakova and Gusev, 2012). Soil pollution by F non-metal is one of the main worldwide problems (Chaudhary and Khan, 2014). F is necessary for normal plant growth in smaller amount (Gao *et al.*, 2012) at high concentration it causes potential damage to it as well as the environment. F is absorbed by plant roots from the soil and then transported via xylematic flow to the transpiratory organs like leaves, where it can accumulate with adverse effects (Elloumi *et al.*, 2005).

Adverse effects of F are due to a range of interactions of it at the cellular and molecular level. At molecular level F cause DNA damage which affects gene expression and further responsible for particular amino acid formation and leads to change in growth parameters. At cellular level binding of F to sulphydryl groups in proteins, leading to an inhibition of activity or disruption of structure, or from the displacing of an essential element resulting in deficiency effects (Baunthiyal *et al.*, 2014). Inhibition of protein synthesis and secretion interrupts the signaling pathways involved in cell proliferation and apoptosis. In addition the excess concentration of F may induce the formation of free radicals and reactive oxygen species (ROS), possibly resulting in increased oxidative stress (Dietz *et al.*, 1999).

Vigna radiata belonging to the family Fabaceae, is an important pulse crop commonly known as mung bean or green gram, grown principally for its protein rich edible seeds. These beans have worldwide productivity and commonly cultivated in Asia. The major *Vigna* producing states in India are Madhya Pradesh, Orissa, Rajasthan, Maharashtra and Andhra Pradesh which accounts about 70% of the total production of it.

From available reports, it is found that *Vigna* is sensitive to F (Yu, 1996), there is a marked decrease in root elongation. Molecular marker such as random

amplified polymorphic DNA (RAPD), is a simple and fast technique than the other multilocus markers used for genotoxic analysis tools. It is reliable and capable to detect the point mutation as well as temporary DNA alteration (Grassi *et al.*, 2003) and allows detection of very low concentration of pollutants (Theodorakis *et al.*, 2006; Liu *et al.*, 2009).

Fluoride stress is one of the major abiotic stresses. It is a well known fact that fluoride stress negatively affects crop productivity and growth rate. The main aim of this proposed study is to find out the process of plant damage at morphological and genetic level. Because, fluoride stress may affect plant growth through DNA damage at the molecular level. So, to confirm this hypothesis, morphological and molecular analysis through RAPD was performed under fluoride induced stress.

The present study was undertaken to detect the DNA damage induced by F in *Vigna radiata* seedling using RAPD and to compare the changes in RAPD profiles with certain morphological parameters.

2. Materials and Method

The seeds of *Vigna radiata* var. SML668 were obtained for experimental purpose from Krishi Vigyan Kendra of Banasthali Vidyapith, Rajasthan India. Seeds were surface sterilized with 0.1% $HgCl_2$ for 2 minutes. Sterilized seeds were placed on filter paper presoaked with distilled water in the petriplates for 24 hours in the dark. After emergence of plumule, ten young seedlings were transferred to each plastic pot containing soilrite. Soilrite was mixed with different concentration of NaF (0, 25, 50, 75 and 100 mg NaF kg^{-1}). Pots containing pretreated soilrite were placed under control conditions with a photoperiod of 14 hours light and 10 hours dark with the 35±2˚C temperature in the greenhouse chamber. After thirty days triplicates plants were taken and their root-shoot length and vigor index were calculated for all the treatments (0, 25, 50, 75, 100 mg NaF kg^{-1}).

The total genomic DNA was isolated from fresh leaves of *Vigna radiata* by a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method of (Khan *et al.*, 2010).

A total of 10 random polymorphic primers (custom synthesized by Bangalore Genei Pvt. Ltd., India) were screened randomly (Table 1). The primers with more than 60% GC content were used for RAPD analysis.

Table 1 Sequence of 10 RAPD markers used in this experiment

#	Primer Code	Sequence of primer (5'→ 3')
1	Primer 1	TTCCGAACCC
2	Primer 2	GTGAGGCGTC
3	Primer 3	GTCCACACGG
4	Primer 4	GACCGCTTGT
5	Primer 5	GTTGCGATCC
6	Primer 6	TCGGCGATAG
7	Primer 7	TGTCTGGGTG
8	Primer 8	GCGCGTGGAG
9	Primer 9	GTAGAGCAGC
10	Primer 10	GTGCGAGAAC

The changes in RAPD profiles due to the genotoxic effects of F are reflected as genomic template stability (GTS) values, which is a qualitative method used for the detection of genotoxic effects of any stress. Genomic template stability (GTS, %) was calculated as follows, according to (Liu *et al.*, 2009).

$$GTS\ (\%) = (1 - a/n) \times 100$$

Where "a" is the average number of polymorphic bands, detected in each treated sample and "n" the number of total bands in the control.

Statistical analysis

Standard deviation (SD) and spearman's correlation were calculated by statistical software package (SPSS 16.0).

3. Result and Discussion

3.1. Germination percentage

From the present study it is clear that the F treatment resulted in a decrease in germination percentage of seeds. With the increase in NaF concentration (100 mg NaF kg^{-1}) the germination percentage reduced by (26.33%) as compared to control seedlings which was followed by 75 mg NaF kg^{-1} (23%), 50 mg NaF kg^{-1} (16.33%) and 25 mg NaF kg^{-1} (9.66%) (Figure 1a). F cause alteration in gene expression which directly links to proteins and enzyme activity and due to inhibition of a particular enzyme (amylase) activity, the germination percentage of seedlings also get decreased (Wilde *et al.*, 1998).

From the various studies, it has been reported that the F stress reduces the germination percentage in cluster bean (Gupta *et al.*, 2009), *Oryza sativa* and *Cicer arietinum* (Tang *et al.*, 1999).

3.2. Vigour index

The properties which determine the potential activity of the embryo and seed performance during germination is defined as vigour index (Tang *et al.*, 1999). The high vigour index of the seeds allows rapid embryo growth and in stressed condition it gets reduced. (Figure 1b) shows that vigour index was decreased (87%) as the concentration of F increases. Similarly, a reduction in vigour index has also been reported in *Cicer arietinum* (Tang *et al.*, 1999).

3.3. Root and Shoot length

Shoot and root length get decreased and were decreased regularly with an increase in NaF concentration. It was also found that the highest NaF concentration (100 mg NaF kg^{-1}) shoot length decreased maximum about 46% and root length 35% (Figure 1c) indicating the differential sensitivity. Reduction in root length and shoot length has also been reported in previous studies in Cluster bean (Gupta *et al.*, 2009), *Oryza sativa* and *Cicer arietinum* (Tang *et al.*, 1999). Root and shoot length reduction by F stress is due to unbalanced nutrient uptake by seedlings (Pant *et al.*, 2008).

3.4. Molecular detection of F genotoxicity

The RAPD assay carried out with a small amount of control genomic DNA and yielded total 4-7 bands.

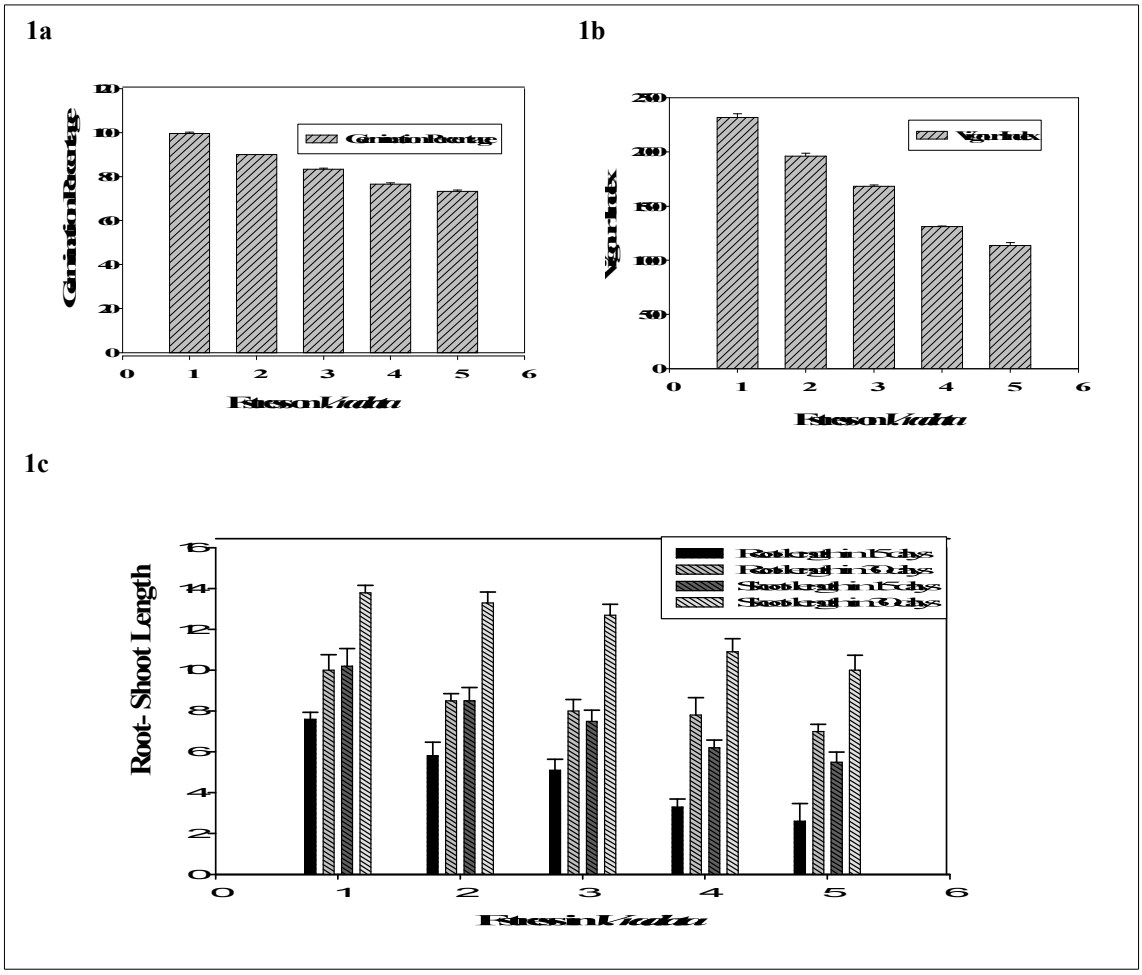

Figure 1 Result showing germination percentage, vigour index and root- shoot length in F stressed *V. radiata plant* samples. Values are mean ± Standard Deviation (SD): according to ANOVA and DMRT for each column.

Number of bands varied from 3-7, primer no. 1 showed 3 bands, primer no. 2 showed 7 bands and primer no. 3 showed 6 bands for treated sample. The result is supported by previous studies (Kernodle *et al.*,1993). They reported that the variation in the number of bands amplified by different primers is influenced by variable factors such as template quantity, primer structure and less number of annealing sites in the plant genome. Similar findings were also observed by Mahmood *et al.* (2009), in *Gossypium* spp.

Although some other changes in RAPD profiles also found at different concentration of fluoride stress e.g. bands in all the 3 primers have changed in intensity, appearance of new bands, and some minor modifications also occurred (Figure 2). In addition, some bands were disappearing, it was a major event generated by the treated sample with F from control to 100 mg NaF kg^{-1} concentration (Table 2).

Different types of DNA damage may cause structural rearrangements in DNA. Although the genomic structure of plant is very stable, but its DNA could be damaged from the exposure of different kinds of stress condition. DNA damage may cause some morphological and physiological effects, which in turn affects whole organism's growth and development (Cambier *et al.*, 2010). Recent advances in the field of molecular biology have led to the development of various sensitive assays for the analysis of DNA, which could be further applied in the field of genetic toxicology.

The variation in band intensity and the disappearance of some bands (Figure 2; Table 2) is due to the formation of some photoproducts in DNA templates by stressing condition. These photo products may result in reduction of binding sites for *Taq* polymerase. Some structural changes in DNA like breaks, transpositions and deletions cause appearance of some new bands. Different concentration of NaF produces different types of alteration in plant samples. These different types of DNA damages must be detected by changes in RAPD profiles.

Genomic template stability (GTS), is defined as the method for detection of genotoxic effects of stress condition on plant sample. The banding pattern changes were due to these genotoxic effects. GTS was more sensitive than other parameters (F accumulation and antioxidant enzyme activities). In the study, GTS was expressed as a reduction at some concentration like at 25 mg NaF kg^{-1} GTS value was 76.5%, at 50 mg NaF kg^{-1} 64.7%, 75 mg NaF kg^{-1} 41.2%

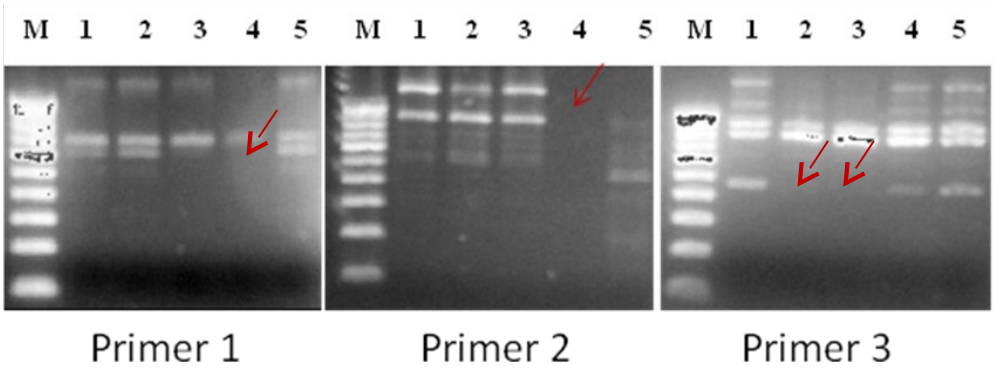

Figure 2 RAPD profiles of genomic DNA from leaves of *V. radiata* plantlets exposed to different F concentration for 60 days. Lane *1* = control; *2* =25 mg NaF kg^{-1}; *3* =50 mg NaF kg^{-1}; *4* =75 mg NaF kg^{-1} ; 5 =100 mg NaF kg^{-1}, M = DNA molecular size marker (3000, 2500, 2000, 1500, 1000, 600, 300, 200 and 100 bp from top to bottom) and arrows represent deletion of bands.

Table 2 Changes of total bands in control and of polymorphic bands and varied bands in the seedlings of F contaminated *V. radiata* for 60 days.

No. of primers	**F concentration (mg NaF kg^{-1})**																
	C	**25**				**50**				**75**				**100**			
		a	b	c	d	A	b	c	d	A	b	c	d	A	b	C	d
Primer 1	4	0	0	0	0	0	1	1	0	0	3	1	0	0	0	0	4
Primer 2	7	0	1	1	4	0	1	1	6	0	7	0	0	3	3	4	0
Primer 3	6	2	1	4	1	0	4	1	1	0	0	3	3	0	0	3	0
Total bands	17	2	2	5	5	0	6	3	7	0	10	4	3	3	3	7	4
a + b	-	4	-	-	-	6	-	-	-	10	-	-	-	6	-	-	-
a + b + c + d		14	-	-	-	16	-	-	-	17	-	-	-	17	-	-	-

Note: a = number of new bands appeared, b = number of bands disappeared, c = decrease in band intensities, d = increase in band intensities, a + b = number of polymorphic bands, and a + b + c + d = number of varied band

and at 100 mg NaF kg^{-1} was 64.5%, due to changes in RAPD profiles. From the results maximum DNA damage occurred at NaF concentration (75 mg NaF kg^{-1}), GTS value was found to be minimum (41.2%) as compared to 100 mg NaF kg^{-1} which was 64.5%. F is negatively charged and is biochemically very active. Thus F at 75 mg NaF kg^{-1} concentration could have a severe effect on DNA stability because of its affinity for uracil and amide bonds by sodium-fluoride (Na-F) interactions. This particular interaction can rupture of hydrogen bonds of the base pairs (adenine and thiamine), resulting in alteration in DNA synthesis and cause linkage between basic groups in DNA replication process (Li *et al.*, 1987).

DNA fingerprinting offers a useful biomarker assay for the soil toxicology study, changes in RAPD profiles induced by pollutants can be regarded as changes in GTS and this genotoxic effect can be directly compared with alterations in other parameters (Atienzar *et al.*, 2000; Liu *et al.*, 2009).

4. Conclusion

Our data suggest that higher concentrations of F adversely affect the germination activity of *Vigna radiata* and cause the alteration in DNA sequences. Genotoxicity was effectively evaluated by RAPD analysis. Molecular characterization of these markers would be able to indicate whether they could amply F stress induce changes in DNA and thus have a wide applicability in toxicological study. Identification of DNA damage through RAPD analysis in conjunction with other biomarker would prove a powerful ecotoxicological tool. Further work on the molecular mechanism of the F toxicity and its associated genes could be mapped in the plants.

5. Competing Interests

The authors declare that they have no competing interests.

6. Author' Contribution

Swati Agarwal: Performed the experiments.

Suphiya Khan: Designed experiment.

7. Acknowledgements

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