Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 2245-2248
Received: 10-09-2017
Accepted: 11-10-2017

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Effect of elevated fluoride levels on morph-physiological parameters of wheat and barley

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Abstract
Experiment to evaluate effect of elevated fluoride levels on morph-physiological parameters of wheat and barley was carried out during *Rabi* 2016-17. Wheat genotype HUW-234 and barley genotype EMBSM were grown hydoponically for 40 days and then exposed to 100 and 200 ppm fluoride. Observations were recorded after 40 days of imposing fluoride stress. Increased F level in root zone reduced shoot length, and root and shoot dry weight per plant. Chlorophyll and carotenoid contents also decreased but both the crops showed differential patterns. On the basis of per cent reduction in dry weights of root, shoot as well as photosynthetic pigments, it is observed that as compared to barley wheat is relatively more tolerant at 100 ppm fluoride treatment, whereas, at 200 ppm fluoride level both crops are almost equally susceptible to fluoride toxicity.

Keywords: Fluoride toxicity, morphological parameters, tolerant, photosynthetic pigments

1. Introduction

Fluoride is an anion of halogen family. Gaseous fluoride enters in plant through stomata and migrates towards tip of the leaves (Kamaluddin *et al.* 2003) [1]. Fluoride present in soil enters into plant through root and after absorption, it moves into shoot (Pant *et al*. 2008) [2]. In plants fluoride concentration is reported to be the maximum in root than in shoot and the minimum in grains (Mendoza-Schulz *et al*. 2009) [3]. The initial visual symptoms in fluoride toxicity in plants are development of necrotic spots at the tip and margins of leaves and after continues and prolonged exposure to fluoride; leaves become necrotic (Daines *et.al*. 1980) [4]. Increased levels of fluoride in plants are reported to inhibit root length, shoot length, plant height, leaf size, number of leaves per plant, fruit and seed setting and test weight (Singh *et al*. 2014) [5]. Fluoride in higher concentration is also injurious to animals and human beings. Earlier it was thought that the major source to fluoride toxicity in human beings is through drinking water, but now it is well documented that plants contribute significantly to fluoride toxicity in organisms (Gautam *et al.* 2010) [6]. Crop and crop varieties are reported to respond differently to increased fluoride concentration in soil and accumulate differential amounts of fluoride in their vegetative and reproductive parts. Barley is reported to be more sensitive to fluoride toxicity than wheat (Arya *et al*.1978 [7], Agrawal 1979) [8]. Toxicity of fluoride is expected to intensify with increased atmospheric pollution and depleting soil water table. It is, therefore, expected that in days to come hazard of fluoride will intensify.

2. Materials and methods

A hydroponic experiment was carried out in the Net House of the Agricultural Farm of the Institute of Agricultural Sciences, Banaras Hindu University, during *rabi* (winter season) 2016-17Varanasi taking wheat and barley genotypes consisting of two treatments and three replications. Surface sterilized seeds of wheat (HUW-234) and barley (EMBSN-34) were placed in *Petri* dishes lined with moist filter paper and kept in an incubator for germination for 3 days at 28±1ºC. Plastic containers (21×18× 9 cm) were taken and filled with 21 L distilled water. Containers were covered with steriofoam sheet in such a way that it was 1 cm above the upper level of water in the container. Steriofoam sheets were having holes (2 cm diameter) at a distance of 5 cm. Three days old seedlings of uniform growth were taken out carefully from *Petri* dishes and planted in each hole on steriofoam sheet with the support of sterilized cotton. For three days seedlings were grown on distilled water and then supplied with N/2 Hoagland's solution [9] for the next 3 days. Thereafter, it was replaced by N-Hoagland's solution. Plants were allowed to grow in N-Hoagland solution up to the age of 40-days. During this period Hoagland solution was replaced at an interval of every three days. Trays were grouped into 3 sets and after 40 days plants were supplemented with N-Hoagland (T_0), N-Hoagland+100 ppm fluoride (T_1) and N-Hoagland + 200 ppm fluoride (T_2). Fluoride was provided in the form of NaF. Observations pertaining to root length, shoot length, dry weight, number of tillers, number of leaves, total chlorophyll and carotenoid contents [10] were recorded after 25 days of imposing NaF treatment.

2.1 Statistical analysis

All observations were recorded in three replications and mean values were calculated. Data were analyzed following completely randomized design (Panse and Sukhatme (1967) [11]. Critical difference (C.D) values were calculated at 1%t level.

3. Result and discussion

Effect of 100 ppm and 200 ppm fluoride levels was studies in wheat (HUM-234) and barley (EMBSM) during Rabi 2016-17. In wheat when plants were expose to fluoride treatments, it is observed that tiller number per plant decreased significantly (Table1). In control plants tillers per plant were the maximum i.e. six; which reduce to four in plants under T_1 and T_2 treatments. Number of leaves per plant was the maximum in control (T_0) plants, and reduced in plants under T_1treatment, but increased marginally in plants under T_2 treatment (Table 12). Root length, as compare to control plant, decreasing in plant under T_1 treatment, but increased in plants under T_2 treatment (Table 1). Root length in plants under T_2 treatment was significantly higher than in plant under T_1 treatment and at par with those under control treatment. There was a significant and progressive decline in shoot length, root dry weight and shoot dry weight per plant as the concentration of fluoride increased in root zone solution (Table1). When effect of different leaves of fluoride was examined in barley on morphological parameters it is observed that tillers per plant, leaves per plant, root length, shoot length, root dry weight and shoot dry weight per plant decreased progressively as the level of sodium fluoride increased in nutrient solution. Treatment differences were found to be significant (Table 2).

Table 1: Effect of different levels of fluorides on tiller plant^{-1}, leaves plant^{-1}, root length (cm), shoot length (cm), root dry weight (g plant^{-1}) shoot dry weight (g plant^{-1}) in wheat (HUW-234).

S. No.	Treatments*	Tiller	Leaves	Root length	Shoot length	Root dry weight	Shoot dry weight
1.	T_0	6.0	21.6	24.00	60.40	0.755	4.464
2.	T_1	4.0	13.0	18.20	58.80	0.609	3.987
3.	T_2	4.0	18.0	26.00	51.50	0.399	1.142
	SEm±	0.7	1.5	1.22	1.67	0.064	0.446
	C.D at1%	1.6	5.3	4.22	5.79	0.222	1.544

***T_0** = Normal Hoagland (Control), T_1 = Normal Hoagland + 100 ppm fluoride, T_2 = Normal Hoagland + 200ppm fluoride. Plants were imposed with above treatments after 40 days of growth. Observation were taken after 25 days of imposing fluoride treatments (at observation total plant age was 65 days).

Table 2: Effect of different levels of fluorides on tiller plant^{-1}, leaves plant^{-1}, root length (cm), shoot length (cm), root dry weight (g plant^{-1}) and shoot dry weight (g plant^{-1}) in barley (EMBSM).

S. No.	Treatments*	Tiller	Leaves	Root length	Shoot length	Root dry weight	Shoot dry weight
1.	T_0	5.6	18.4	23.90	56.00	0.789	3.646
2.	T_1	6.3	18.4	24.00	34.10	0.709	1.300
3.	T_2	3.0	16.0	19.34	44.90	0.399	1.203
	SEm±	0.6	0.5	1.14	4.27	0.089	0.446
	C.D at 1%	2.2	1.9	3.95	14.78	0.308	1.545

*T_0 = Normal Hoagland (Control) T_1 = Normal Hoagland + 100 ppm fluoride, T_2 = Normal Hoagland + 200ppm fluoride. Plants were imposed with above treatments after 40 days of growth. Observation were taken after 25 days of imposing fluoride treatments (at observation total plant age 65 days)

Effect of different levels of fluoride treatments on chlorophyll a, chlorophyll b, chlorophyll a to chlorophyll b ratio, total chlorophylls and carotenoid contents were examined in wheat and barley crops. Total chlorophyll, chlorophyll a, chlorophyll b, and chlorophyll a to chlorophyll b ratio changed under the influence of fluoride treatment in both crops (Table 3). In case of wheat chlorophyll a did not vary significantly between treatments T_0 and T_1 but at 200 ppm fluoride (T_2) it decreased significantly (Table 3). Similar was the effect on chlorophyll b and total chlorophyll contents. Chlorophyll a to chlorophyll b ratio did not vary significant under different treatments. Carotenoid content in wheat also decreased at higher levels of fluoride treatments (Table 3). Differences in carotenoids content were not significant between T_0 and T_1 treatments, but it decreased significantly in plants treated with 200 ppm fluoride.

When effects of different level of fluoride on chlorophyll and carotenoids contents were observed in barley, it is noted that in this crop there was significant effect (Table 4). As compared to control, chlorophyll a and b decreased marginally in plants under T_1 treatment and further increase marginally in plants treated with 200 ppm fluoride. Chlorophyll a and b ratio did not differ much in this crop, however, level of carotenoids decreased in T_1 treatment and it further increased in plants under 200 ppm fluoride treatment (Table 4).

Table 3: Effect of different levels of fluoride treatments on the contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids (mg g-[1] fresh weight) in wheat (HUW-234).

S. No.	Treatment*	chl a	chl b	chl a and chl b ratio	Total chlorophyll	Carotenoids
1.	T_0	0.863	0.093	9.279	0.956	0.545
2.	T_1	1.132	0.133	8.511	1.265	0.627
3.	T_2	0.620	0.066	9.401	0.686	0.345
	SEm±	0.089	0.012		0.101	0.497
	C.D at1%	0.310	0.042		0.352	0.172

***** T_0 = Normal Hoagland (Control) T_1 = Normal Hoagland + 100 ppm fluoride, T_2 = Normal Hoagland + 200ppm fluoride. Plants were imposed with above treatments after 40 days of growth. Observation were taken after 25 days of imposing fluoride treatments (at observation total plant age 65 days).

Table 4: Effect of different levels of sodium fluoride treatments on the contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids (mg g-[1] fresh weight) in barley (EMBSM).

S. No	Treatment*	chl. A	chl. b	chl. a and b ratio	Total chlorophyll	Carotenoids
1.	T_0	0.799	0.095	8.410	0.895	0.423
2.	T_1	0.691	0.072	9.597	0.764	0.326
3.	T_2	0.843	0.103	8.184	0.946	0.404
	SEm±	0.036	0.010		0.044	0.022
	C.D at 1%	0.125	0.035		0.154	0.077

*T_0 = Normal Hoagland (Control), T_1 = Normal Hoagland + 100 ppm fluoride, T_2 = Normal Hoagland + 200ppm fluoride. Plants were imposed with above treatments after 40 days of growth. Observation were taken after 25 days of imposing fluoride treatments (at observation total plant age 65 days).

Effects of fluoride on morphological parameters of crops are well documented. It is reported that high level of fluoride causes reduction in leaves per plant, leaf size, root dry weight, shoot dry weight and plant dry weight (Kumar 2008) [12]. In wheat and barley similar effects on the morphological parameters have been reported by Neetu and Devendra (2014) [13]. They also reported that barley is relatively susceptible to NaF than other crop like pea, corn and tomato.

Present investigation indicated that increased fluoride level in root zone generally caused reduction in number of tillers per plant, leaves per plant, shoot length per plant, root length per plant, shoot dry weight and root dry weight. As compare to control, reduction in tillers per plant in wheat was 33.33% at both levels of fluoride treatments while in barley at T_1 level tiller increased but at T_2 level reduced to 46.42%.

Per cent reduction in various morphological parameters further indicated that both shoot weight and root weight per plant are more sensitive to fluoride. As per cent reduction in shoot weight as compare to control in T_1 of wheat was lesser than that in barley under same treatment, while root dry weights reduced to almost similar extended (in term of percentage) in both crops, therefore, it is inferred that 100 ppm fluoride is deleterious to barley but not to wheat, however, 200 ppm fluoride has almost equal toxic effects on shoot and root dry weights in studied genotypes of wheat and barley. Effect of fluoride on chlorophyll a chlorophyll b and total chlorophyll contents are well studied in crop plant. It is reported that concentrations of these photosynthetic pigments decline under the influence of F toxicity (Yamazoe 1962) [14]. In the present investigation, almost similar observations were recorded. When extent of damage in term of percentage under different F treatments as compared to control was calculated, it has been observed that per cent reduction in these parameters was to a greater magnitude in wheat than in barley. In wheat100 ppm fluoride did not cause any changes in chlorophyll a chlorophyll b and total chlorophyll contents while opposite trend was observed in barley. At 200 ppm fluoride per cent reduction in the levels of these pigments was more in wheat, while in barley under this treatment there was marginal improvement in chlorophyll a chlorophyll b, total chlorophyll and carotenoid contents. No report is available on effect of fluoride on carotenoids content in plants. Nevertheless, no definite trend was observed in change in carotenoid content in studied crops under different levels of sodium fluoride.

4. Conclusion

Morphological and physiological attribute in both crops were studies after 25 days of expose to NaF. At the time of observation, total plant age was 65 days. Following conclusion can be sighted.

In wheat and barley under the influence of sodium fluoride treatments there were reduction in numbers of tillers per plant, leaves per plant, shoot length, root dry weight and shoot dry weight per plant. Shoot dry weight reduced more in barley at T_1 level than in wheat, but at T_2 level reduction was almost to the same extent in both crops. In case of wheat chlorophyll a did not vary significantly between treatments T_0 and T_1 but at 200 ppm fluoride it decreased significantly. Similar was the effect on chlorophyll b and total chlorophyll content. Chlorophyll a to chlorophyll b ratio did not vary significantly under different treatments. Carotenoid content also decreased but differences were not significant between T_0 and T_1 treatments, however, it decreased significantly in plant treated with 200 ppm fluoride. In barley, as compared to control, chlorophyll a and b contents decreased marginally in plants under T_1 treatment and further increased marginally in plants treated with 200 ppm fluoride. Chlorophyll a and b ratio did not differ much, however, level of carotenoids decrease in T_1 treatment and it further increased in plants under treatment with 200 ppm fluoride. On the basis of per cent reduction in dry weights of root, shoot, and photosynthetic pigments it is concluded that at 100 ppm fluoride wheat is more tolerant than barley, but at 200 ppm fluoride level both crops are almost equally susceptible to fluoride toxicity.

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