



Germination and morphological responses of *Triticum aestivum* L. to different concentrations of fluoride

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ARTICLE INFO	ABSTRACT
<p>Received : 16 June 2021 Revised : 11 August 2021 Accepted : 20 August 2021</p> <p>Available online: 19 November 2021</p> <p>Key Words: Fluoride Germination Relative Growth rate <i>Triticum aestivum</i></p>	<p>A laboratory experiment was conducted on germination papers to study the effect of fluoride (F) at 0 (T₁), 50 (T₂), 100 (T₃), 200 (T₄), 250 (T₅) and 300 (T₆) ppm on germination and morphological parameters in wheat (<i>Triticum aestivum</i> L.) variety, HUW-234 at 2, 4 and 6 days after initiation of germination process. Fluoride toxicity caused reduction in germination per cent, germination index, coefficient of velocity of germination and germination energy (%) while mean germination time increased with fluoride concentration. Root and shoot lengths and dry matters decreased with increased concentrations of fluoride. Ratio of root: shoot weight increased with increased concentration of fluoride. Elongation of seminal roots was adversely affected by increased fluoride level. Increased fluoride level in the germination medium decreased RGR of seedlings progressively. Present study revealed that enhanced fluoride concentration in germination medium caused reduction in germination and germination related parameters.</p>

Introduction

Wheat is the second most important cereal crop next to rice. Fluorides are well known prevalent, non-biodegradable and dangerous non-metal pollutant (Agalakova and Gusev, 2012). Soil pollution caused by fluoride non-metal is one of the main problems worldwide (Chaudhary and Khan, 2014). Fluoride in smaller amount is essential for normal plant growth but at higher concentration it causes potential damage to plant as well as to the environment (Gao *et al.*, 2012). Fluoride-encouraged inhibition of germination is testified to be due to reduction in amylase activity; which is essential for seed germination. Amylase activity is lowered by fluoride which is partially restored by Ca²⁺ ions. It has been concluded that the inhibitory effect of fluoride on amylase might be through elimination of Ca⁺² by fluoride (Sethy and Ghosh, 2013). In wheat fluoride toxicity is reported to retard seed germination and related parameters

(Bhargava and Bhardwaj 2010; Kumar *et al.*, 2013; Arshi and Khan 2016). Increased fluoride level in root zone reduced shoot length, and root and shoot dry weight per plant reported by Singh *et al.*, 2017. The significance of seed germination in plant growth is commonly documented and its study has been used as a model for examining elemental toxicity. Hence, present investigation was undertaken to visualize response of fluoride toxicity on germination, germination related and morphological parameters of wheat under germination paper technology.

Material and Methods

Present investigation was carried out taking wheat variety HUW-234 in *rabi* (winter season) 2016-17 and 2017-18. Seeds were procured from the Department of Genetics & Plant Breeding; the experiment was performed in the Tissue Analysis

Lab of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Germinating seeds on germination paper

A germination paper was placed on butter paper sheet in such a way that lower 4 cm portion of germination paper was not covered by the butter paper. Papers were fully saturated with sodium fluoride solution containing 0 (T₁), 50 (T₂), 100 (T₃), 200 (T₄), 250 (T₅) and 300 (T₆) ppm fluoride, prepared by dissolving required amounts of NaF in distilled water. Seeds were placed on germination paper sheets 15 cm above bottom line at equal distance. Another germination paper sheet of same size was again put over it and moistened with NaF solution of the respective concentration. Butter paper extended on the top of the germination paper was folded inside and rolled. Rolls were placed in a 500 mL beaker containing same concentration of fluoride solution in such a way that bottom portion of roll, which was not covered by butter paper, remained inside solution. Seeds were allowed to germinate at room temperature. Observations were recorded at 2, 4 and 6 days after initiation of germination process by unrolling germination papers.

Germination parameters

Germination percentage was calculated by Association of Official Seed Analysis (1983) and germination index (GI) was calculated according to the formulae of Ranal *et al.*, (2009)

Germination index (GI) = $(G_1 \times N_1) + (G_2 \times N_2) + (G_3 \times N_3)$

Where N₁, N₂ and N₃ are the number of germinated seeds on second, fourth and sixth days, and G₁ is the total period of germination, i. e., 6 days, G₂ is 6 - 2 = 4 and G₃ is 6 - 4 = 2 as total period of germination was six days and observations were recorded at an interval of two days. Coefficient of velocity of germination (CVG) was calculated according to the formulae of Nichols and Heydecker (1968), mean germination time (days) was calculated by the procedure of Ellis and Roberts (1981) and germination energy was calculated at 6th day after sowing of seeds by Ruan *et al.* (2002)

Morphological parameters

Root and shoot length (cm) were measured with the help of scale from base to tip. Root and shoot were excised, placed separately in oven (NSW-142) at

105°C for 1 hour then at 65°C till constant weight. Dry weight (mg five seedlings⁻¹) was taken at 2, 4 and 6 days after initiation of germination by electrical balance (ADGR-200). Relative growth rate (RGR) of seedlings was calculated using the formula given by Blackman (1919).

Statistical analysis

Mean values were taken of three independent replication from each treatment. Analysis of variance for CRD was performed by SPSS version 20.0 software. A significant difference among treatments was determined by Duncan's multiple range test.

Results and Discussion

Germination parameters

The effect of different concentration of fluoride on germination parameters are summarized in Table 1. The data of Table 1 clearly indicate that various concentration of fluoride showed a reduction in germination percentage, germination index, coefficient of velocity of germination and germination energy. Differences in germination parameters of wheat due to fluoride stress were significant with respect to treatment (P < 0.05). SPSS analysis showed significant variance between treatments are showed alphabetically in Table 1. Germination is a critical stage in the life of plants (Ahmad *et al.*, 2009). Derangement in germination and seedling growth results in poor crop stand and reduction in crop yield. Presence of ions in excess amounts in germination medium may also result in reduction of seed germination and such evidence is well documented for fluoride. It is reported that excess amount of fluoride in germination medium affects seed germination and related parameters viz. germination per cent, germination index, germination energy, coefficient of velocity and mean emergence time and of seedlings. Such results are available for wheat (Bhargava and Bhardwaj 2010; Kumar *et al.*, 2013; Kumar and Iqbal 2014; Alim *et al.*, 2017) and other crops such as gram (Datta *et al.*, (2012); okra (Arshi and Khan 2016); grasses (Gulzar and Khan 2001; Siddhu *et al.*, 2008). Per cent germination under T₁ (control) treatment was higher and at different treatments it decreased progressively with increased fluoride concentration in the germination medium at all the stages of observation (P < 0.05). These observation germination percentage is in conformity with

Table 1: Effect of different concentrations of fluoride on germination percentage at 2, 4 and 6 days after initiation of germination, germination index, Coefficient of velocity of germination (%), Mean emergence time (days) and Germination energy (%) of wheat genotype HUW-234 (mean data of two years)

Treatments	Germination percentage (%)			Germination Index	Coefficient of velocity of germination (%)	Mean emergence time (days)	Germination energy (%)
	2	4	6				
T ₁	43.08 ± 1.93 ^a	76.72 ± 0.00 ^a	86.93 ± 3.08 ^a	41.67 ± 0.88 ^a	32.30 ± 0.85 ^a	3.52 ± 0.16 ^d	17.39 ± 0.62 ^a
T ₂	41.15 ± 1.93 ^a	72.29 ± 8.87 ^{ab}	68.11 ± 2.89 ^b	38.33 ± 2.34 ^a	29.52 ± 0.28 ^b	3.71 ± 0.07 ^d	14.39 ± 0.89 ^b
T ₃	35.01 ± 2.11 ^b	59.21 ± 4.23 ^{bc}	58.11 ± 2.84 ^c	31.67 ± 1.45 ^b	26.29 ± 0.35 ^c	4.08 ± 0.10 ^c	12.04 ± 0.64 ^c
T ₄	30.79 ± 2.11 ^{bc}	51.14 ± 6.15 ^c	51.96 ± 2.69 ^{cd}	25.67 ± 1.77 ^c	24.08 ± 0.25 ^d	4.21 ± 0.08 ^{bc}	10.59 ± 0.53 ^{cd}
T ₅	28.68 ± 2.11 ^c	46.92 ± 3.85 ^c	47.88 ± 2.89 ^{dc}	24.00 ± 3.06 ^{cd}	23.42 ± 0.19 ^d	4.43 ± 0.07 ^{ab}	9.78 ± 0.19 ^{dc}
T ₆	26.57 ± 0.00 ^c	43.08 ± 3.85 ^c	42.12 ± 2.89 ^c	18.67 ± 0.33 ^d	21.71 ± 0.16 ^c	4.68 ± 0.06 ^a	8.62 ± 0.51 ^c

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P < 0.05

finding of Bhargava and Bhardwaj, 2010 in wheat. As compared to T₁ (control) treatment, each increment in fluoride level in germination medium caused parallel reduction in germination index under T₂, T₃, T₄, T₅ and T₆ treatments. The maximum coefficient of velocity of germination was recorded under T₁ (control) treatment (32.30) which under T₂, T₃, T₄, T₅ and T₆ treatments decreased to 29.52, 26.29, 24.08, 23.42 and 21.71, respectively. Increased fluoride concentration in germination medium resulted in increased mean emergence time significantly (P < 0.05). Under T₁ (control) treatment mean emergence time was 3.52 days which under T₂, T₃, T₄, T₅ and T₆ treatments increased to 5.39, 15.90, 19.60, 25.85 and 32.95 per cent, respectively. Data pertaining to germination energy was recorded at 6th day after germination as level of fluoride in germination medium increased germination energy decreased. Under T₁ (control) treatment germination energy was 17.39 % while under T₂, T₃, T₄, T₅ and T₆ treatments it decreased to 14.39, 12.04, 10.59, 9.78 and 8.62%, respectively. Results are in accordance with the earlier finding. It is observed that at all stages fluoride caused significant reduction in germination and germination related parameters (P < 0.05), therefore, it is concluded that presence of fluoride in germination medium is toxic to germination and present study indicated that even 50 ppm fluoride in germination medium has toxic effect on germination and germination related parameters. Kumar *et al.* (2013) while working with different genotypes of wheat reported that toxicity of fluoride on germination of seed at 100 ppm and

higher doses. 200 ppm fluoride has been lethal. While Bhargava and Bhardwaj (2010) reported 20 ppm fluoride to be lethal to the germination of wheat seeds var. Raj. 4083. Inhibition in seed germination might, therefore, be due to injurious effect of fluoride ions. It is documented that fluoride binds with divalent cations like Ca²⁺ and Mg²⁺ (Sethy and Ghosh, 2013). It is also reported that fluoride inhibits seed germination by inhibiting activity of α -amylase (Sethy and Ghosh, 2013). α -Amylase requires calcium for its activity. In the presence of fluoride activity of α -amylase declines due to binding of calcium with fluoride making calcium non-available. It results in suppressed breakdown of starch as a result germinating seeds in the presence of fluoride, do not get sufficient carbon skeleton for growth and energy and cause inhibition of germination. Such observation has also been reported by Sethy and Ghosh (2013). Reduction in seed germination was proportional to the fluoride concentration in the germination medium.

Morphological parameters

The effect of various levels of fluoride on morphological parameters are summarized in Table 2, 3 and 4. Differences in morphological parameters of wheat due to fluoride stress were significant with respect to treatment (P < 0.05). SPSS analysis showed significant variance between treatments are showed alphabetically in Table 2, 3 and 4. Fluoride not only retarded germination percentage and other germination related parameters, but also reduced root length and shoot length (Table 2) their dry weights (Table 3).

Table 2: Effect of different concentrations of fluoride on root length and shoot length (cm) of wheat genotype HUW-234 at 2, 4 and 6 days after initiation of germination (mean data of two years)

Treatments	Root Dry Weight (mg 5 seedlings ⁻¹)			Shoot Dry Weight (mg 5 seedlings ⁻¹)		
	2	4	6	2	4	6
T ₁	5.37 ± 0.09 ^a	12.33 ± 0.21 ^a	35.98 ± 0.57 ^a	4.37 ± 0.09 ^a	11.25 ± 0.51 ^a	38.05 ± 1.49 ^a
T ₂	4.72 ± 0.15 ^b	11.07 ± 0.09 ^b	31.07 ± 0.54 ^b	3.88 ± 0.10 ^b	8.78 ± 0.16 ^b	33.07 ± 0.61 ^b
T ₃	4.25 ± 0.08 ^c	10.08 ± 0.06 ^c	23.55 ± 0.64 ^c	3.43 ± 0.03 ^c	7.05 ± 0.13 ^c	32.12 ± 0.43 ^b
T ₄	4.05 ± 0.13 ^c	9.53 ± 0.24 ^c	20.70 ± 0.78 ^d	3.12 ± 0.06 ^d	6.23 ± 0.23 ^d	30.73 ± 0.97 ^b
T ₅	3.62 ± 0.09 ^d	7.87 ± 0.07 ^d	18.05 ± 0.28 ^c	2.63 ± 0.07 ^c	5.57 ± 0.08 ^{de}	27.70 ± 1.13 ^c
T ₆	3.25 ± 0.05 ^e	7.32 ± 0.34 ^d	16.10 ± 0.45 ^f	2.10 ± 0.09 ^f	5.20 ± 0.13 ^e	21.45 ± 0.48 ^d

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P <0.05

Table 3: Effect of different concentrations of fluoride on root dry weight and shoot dry weight (mg 5 seedlings⁻¹) of wheat genotype HUW-234 at 2, 4 and 6 days after initiation of germination (mean data of two years).

Treatments	Root length (cm)			Shoot length (cm)		
	2	4	6	2	4	6
T ₁	1.70 ± 0.00 ^a	6.33 ± 0.09 ^a	12.22 ± 0.09 ^a	0.90 ± 0.03 ^a	1.87 ± 0.03 ^a	5.37 ± 0.09 ^a
T ₂	1.33 ± 0.02 ^b	5.52 ± 0.19 ^b	10.47 ± 0.32 ^b	0.73 ± 0.02 ^b	1.65 ± 0.12 ^b	5.03 ± 0.19 ^{ab}
T ₃	1.17 ± 0.03 ^c	5.35 ± 0.13 ^b	9.25 ± 0.30 ^c	0.53 ± 0.03 ^c	1.45 ± 0.05 ^c	5.10 ± 0.06 ^{ab}
T ₄	0.72 ± 0.07 ^d	4.70 ± 0.06 ^c	7.85 ± 0.34 ^d	0.35 ± 0.05 ^d	1.37 ± 0.06 ^c	4.95 ± 0.08 ^b
T ₅	0.47 ± 0.03 ^e	4.18 ± 0.02 ^d	6.57 ± 0.36 ^e	0.25 ± 0.03 ^{de}	1.33 ± 0.06 ^c	4.57 ± 0.12 ^c
T ₆	0.33 ± 0.07 ^e	3.98 ± 0.14 ^d	5.95 ± 0.23 ^e	0.17 ± 0.03 ^e	1.12 ± 0.02 ^d	4.27 ± 0.15 ^c

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P <0.05

Table 4: Effect of different concentrations of fluoride on relative growth rate (mg mg⁻¹ day⁻¹) and root: shoot ratio (dry weight basis) of wheat genotype HUW-234 at 2, 4 and 6 days after initiation of germination (mean data of two years).

Treatments	RGR (mg mg ⁻¹ day ⁻¹)			R:S (dry weight basis)		
	2	4	6	2	4	6
T ₁	1.14 ± 0.01 ^a	0.44 ± 0.01 ^a	0.57 ± 0.02 ^{ab}	1.24 ± 0.02 ^b	1.10 ± 0.06 ^d	0.95 ± 0.04 ^a
T ₂	1.08 ± 0.00 ^b	0.42 ± 0.01 ^{abc}	0.59 ± 0.01 ^{ab}	1.22 ± 0.07 ^b	1.26 ± 0.02 ^c	0.94 ± 0.00 ^a
T ₃	1.02 ± 0.00 ^c	0.40 ± 0.00 ^{bc}	0.59 ± 0.00 ^{ab}	1.24 ± 0.03 ^b	1.43 ± 0.03 ^{ab}	0.73 ± 0.03 ^{bc}
T ₄	0.98 ± 0.01 ^d	0.39 ± 0.01 ^{bc}	0.59 ± 0.01 ^{ab}	1.30 ± 0.07 ^b	1.53 ± 0.04 ^a	0.67 ± 0.03 ^{bc}
T ₅	0.92 ± 0.01 ^e	0.38 ± 0.01 ^c	0.61 ± 0.01 ^a	1.37 ± 0.03 ^b	1.41 ± 0.02 ^{ab}	0.65 ± 0.02 ^c
T ₆	0.84 ± 0.01 ^f	0.42 ± 0.02 ^{ab}	0.55 ± 0.03 ^b	1.55 ± 0.07 ^a	1.41 ± 0.03 ^b	0.75 ± 0.02 ^b

Presented data are means of three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P <0.05

Reduction in seedling growth in the presence of fluoride has been reported by Sabal *et al.* (2006); Pant *et al.* (2008); Bhargava and Bhardwaj, (2010) and Singh *et al.* (2017). In the initial germination stage germinating seedlings are totally dependent on the reserves present in the seeds. Reduction in above-mentioned parameters with increased level of fluoride indicated that supply of carbon skeleton to actively growing regions of seeds is hampered by fluoride. Even 50 ppm fluoride was sufficient to cause significant reduction in growth parameters of

seedling while 300 ppm appeared to be very toxic. Data indicated that mean values for root and shoot length increased significantly with increased germination time, while mean values for treatments decreased significantly (P <0.05) with increased fluoride concentration in the germination medium (Table 2). The data on root and shoot dry weight followed same trend as observed in length parameters (Table 3). These findings are conformity with study of Bhargava and Bhardwaj, 2010 in wheat. As root : shoot ratio marginally

improved under 50 to 300 ppm fluoride levels (Table 4). 300 ppm fluoride caused relatively higher allocation of dry matter toward roots. Regulation of partitioning of assimilate between different sinks by fluoride has already been reported (Arya 1971), therefore, present observations are in accordance with this work. When relative growth rate (RGR) of growing seedlings was calculated; it was significantly higher between 0-2 days, declined sharply between 2-4 days and then increased marginally between 4-6 DAS (Table 4). With increased fluoride level in germination medium relative growth rate declined it further indicated that efficiency of seedlings to accumulate dry matter per unit of existing dry matter declined in the presence of fluoride. Literature is available to indicate effect of fluoride on RGR by Sodani *et al.* (2018), but this investigation indicated that fluoride tends to retard efficiency of accumulation of dry matter per unit of existing dry matter in wheat seedlings. On the basis of present investigation it is, therefore, concluded that fluoride has toxic effect on seed germination, utilisation of reserve seed materials by developing seedlings and apportioning of dry matter between

root and shoot of germinating seedlings. Lesser availability of carbon skeleton in fluoride treated seedlings is the major cause for reduced germinating seedlings strength and other parameters related with germination.

Conclusion

In the present investigation concluded that increase concentration of fluoride in germination medium from 0 to 300 ppm reduced germination per cent, germination index, coefficient of velocity of germination, germination energy, root shoot length and their dry weight and relative growth rate while mean emergence time and root shoot ratio increased with increased concentrations of fluoride. Nevertheless, mechanism by which fluoride alters dry matter apportioning between root and shoot and regulates germination related parameters are to be studied.

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