Advances in Bioresearch
Adv. Biores., Vol 14 (1) January 2023: 257-261

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.1.257261

Advances in Bioresearch

ORIGINAL ARTICLE

Seed reserve hydrolysis under elevated fluoride levels during germination and early seedling growth in wheat (*Triticum aestivum* L.).

Basant Kumar Dadrwal✉, VijaiPandurangam and Jai Prakash Srivastava
Department of Plant Physiology, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, U.P, India
✉**Corresponding Author: Email:** dadrwal.basant@gmail.com

ABSTRACT
Hydrolysis of seed stored reserves under elevated fluoride (F) levels viz. 0 (T_1), 100 (T_2), 200 (T_3), and 300 (T_4) ppm was investigated in germinating seeds/seedlings of wheat (Triticum aestivum L.) variety HUW-234. Experiments were conducted on germination papers under laboratory conditions. The amylases, and seed reserve utilization were measured under different fluoride treatments. Seed germination and seedling growth reduced as F concentration in germination medium increased, and reduction in starch and increase in soluble sugars and free amino acids was also observed as F increased, Nevertheless, increased F in germination medium decreased amylases (α and β) activity. Overall, the results point to the possibility that elevated concentrations of fluoride might affect the hydrolysis of seed reserves and subsequent growth and development of wheat, which has significant repercussions for agricultural yield in fluoride-contaminated areas.
Keywords: *Amylases, fluoride, starch, sugars, wheat*

Received 02.01.2023 Revised 09.01.2023 Accepted 25.01.2023

How to cite this article:
B. K. Dadrwal, V.Pandurangam and J. P.: Seed reserve hydrolysis under elevated fluoride levels during germination and early seedling growth in wheat (Triticum aestivum L.). Adv. Biores. Vol 14 [1] January 2023. 257-261

INTRODUCTION

Fluorine is a halogen anion and a member of the halogen family (reduced form). It is a highly electronegative element. Because of its high reactivity, fluorine does not exist in its elemental form in nature (9). Soil fluoride concentrations, as high as 1000 to 5300 mg kg^{-1}, are reported in farms lands (13). F induces a variety of biochemical and physiological reactions in plants that affect plant growth and development, possibly leading in a substantial loss in economic output. F is also present in a variety of food samples including water, vegetation, and the atmosphere (9).

Hydrolysis of seed reserves is an important step during germination and early seedling development because it provides the energy and nutrients required for seedling establishment. Environmental stresses, like as high fluoride levels, may, nevertheless, have a major influence on this process (1). A decrease in amylase activity, which is essential for seed germination, is assumed to be the source of fluoride-induced germination reduction. Amylase activity is slowed down by F, but is partially restored when Ca^{2+} is introduced (16). As acid invertase is more active than neutral invertase, it is likely that the former plays a larger role in sucrose hydrolysis. F as well as the quantities of soluble protein and amino acids increased the activity of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase (2). It has been discovered that Triticum aestivum seedlings modify their nitrogen metabolism in response to the disruption of carbon metabolism caused by F. (2). Starch content in wheat genotypes' roots and shoots was significantly reduced as a consequence of fluoride ion treatment, as was the aggregation of reducing and non-reducing sugars. Moreover, it has been shown that fluoride inhibits the activity of the wheat enzymes soluble acid invertase and amylase (2).

Increased fluoride concentrations lowered total protein, carbohydrate content, and amylase activity in two sunflower cultivars (20). Reducing sugar was reported to increase with increasing NaF concentrations (8). Under fluoride stress, seedling germination %, seedling growth, development and

soluble protein content are all found to decrease. The addition of sodium fluoride resulted in a considerable increase in total soluble carbohydrates (6). NaF toxicity seems to be harmful to amino acids and protein content. Wheat seedlings treated with sodium fluoride accumulated higher soluble sugars and phenolics (11), however starch content in almond leaves fell dramatically as F concentrations increased (5).

Fluoride stress has been linked to a number of physiological responses in plants, including an increase in reactive oxygen species (ROS), which disturbs plant metabolism and causes an increase in MDA, protein denaturation, and DNA damage (17).

MATERIAL AND METHODS

The present research was carried out throughout the winter seasons of 2019-20 and 2020-21, utilizing the wheat variety HUW-234 (Malviya Wheat 234), which is extensively cultivated in India's North Eastern Plain Zone. Seeds were received from the Department of Genetics and Plant Breeding at Banaras Hindu University's Institute of Agricultural Sciences in Varanasi. The biochemical tests were carried out at the Department of Plant Physiology's Tissue Analysis Laboratory and Central Instrumentation Facility. The data collected throughout the course of the experiment's two years have been compiled and displayed here. Standard statistical techniques were used to evaluate the data for the factorial complete randomized design.

Germination Paper Roll Test:

Butter paper of the same size was placed on top of a sheet of (44 × 30 cm) germination paper such that the bottom 4 cm of the germination paper was not covered by the butter paper. Fluoride solutions were used to adequately saturate sheets of germination paper at concentrations of 0 (T1), 100 (T2), 200 (T3), or 300 (T4) ppm. A sheet covered in distilled water was used as a control. Seeds were evenly placed 15 cm above the bottom line on sheets of germination paper. Another saturated sheet of germination paper the same size was placed over it. The folded butter paper was placed on top of the germination paper. The same dose of fluoride solution was used to dip the bottom of each roll that wasn't wrapped with butter paper in a 500 mL beaker of rolls. Observations were obtained by unrolling the sheets 3, 6, and 9 days after the seeds had been allowed to germinate at room temperature.

Biochemical Parameters:

(a) Total amylase, α-amylase and β-amylase activity

For the estimation of the α-amylase (EC 3.2.1.1) and β-amylase activities, methods of Bernfeld (1955) and (7), respectively, were used. The unit activity was defined as the g maltose liberated in 3 minutes at 30°C by 1.00 mL enzyme extract.

(b) Soluble sugar content

Soluble sugar content was determined in germinating seeds at 3, 6 and 9 DAS under different treatment levels by anthrone method (4).

(c) Starch content

Starch content was determined in germinating seeds at 3, 6 and 9 days in different treatment levels by anthrone method (4).

(d) Amino acid content

Total free amino acids were determined in germinating seeds at 3, 6 and 9 days in different treatment levels by ninhydrin reagent (19).

RESULTS

α-amylase, *β-amylase* and *total amylase* activities (μM maltose released min^{-1} mL^{-1})

With an increased germination period, mean α-amylase, ***β-amylase*** and ***total amylase*** activities decreased significantly (Table 1; Fig. 1). Nevertheless, there was a significant reduction in mean α-amylase, ***β-amylase*** and ***total amylase*** activities when fluoride concentration in the germination medium increased. In comparison to T_1, seedling under T_3 and T_4 treatments showed a significant reduction in α-amylase activity. At all stages, seeds under T_1 (control) had considerably higher α-amylase, ***β-amylase*** and ***total amylase*** activities than seeds under other treatments.

Table 1: Effect of different concentrations of fluoride on *α-amylase* and *β-amylase* activities (µM maltose released min^{-1} mL^{-1}) in wheat genotype HUW-234 at 3, 6 and 9 days after sowing

S. No.	Treatment	*α-amylase* activity				*β-amylase* activity			
		Stage (days after sowing)			Mean	Stage (days after sowing)			Mean
		3	6	9		3	6	9	
1.	T_1	293.57	557.63	655.90	**502.36**	**143.97**	178.31	187.58	169.95
2.	T_2	253.58	481.38	588.90	**441.29**	**115.81**	148.31	155.75	139.95
3.	T_3	241.42	419.06	541.23	**400.56**	**111.14**	138.08	138.28	129.16
4.	T_4	218.89	364.16	461.33	**348.12**	**100.97**	127.75	124.28	117.66
	Mean	**251.86**	**455.55**	**561.84**		117.97	148.11	151.47	
		SEm±	CD (5%)			SEm±	CD (5%)		
Stage (S)		**0.34**	**1.01**			**0.44**	**1.28**		
Treatment (T)		**0.40**	**1.16**			**0.50**	**1.48**		
S × T		**0.68**	**2.01**			**0.87**	**2.56**		

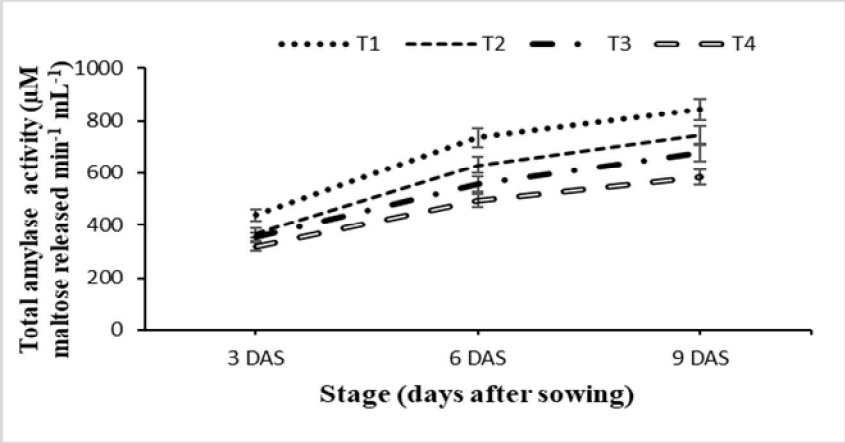

Fig 1: Effect of different concentrations of fluoride on ***total amylase*** activity (µM maltose released min^{-1} mL^{-1}) in wheat genotype HUW-234 at 3, 6 and 9 days after sowing

Soluble sugars (mg g^{-1} fresh weight)

The mean soluble sugar content increased significantly during the germination period. However, increased fluoride concentration in the germination medium resulted in a significant increase in mean soluble sugars. When compared to T_1, the T_3 and T_4 treatments contained higher soluble sugar. Seedlings treated with T_1 (control) had much less soluble sugar than those under other treatment at all stages (Table 2).

Starch content (mg g^{-1} fresh weight)

With an increased germination period, mean starch content decreased significantly. Considerable reduction in mean starch content was recorded with increased fluoride concentration in the germination medium. At all stages, seedlings under T_1 (control) had significantly higher starch content than those under other treatments (Table 2).

Table2: Effect of different concentrations of fluoride on soluble sugar and starch content (mg g^{-1} fresh weight) in wheat genotype HUW-234 at 3, 6 and 9 days after sowing

S. No.	Treatment	soluble sugar				starch content			
		Stage (days after sowing)			Mean	Stage (days after sowing)			Mean
		3	6	9		3	6	9	
1.	T_1	1.51	1.91	2.21	**1.88**	4.56	3.90	3.37	**3.94**
2.	T_2	1.66	2.06	2.36	**2.03**	3.99	3.36	2.55	**3.30**
3.	T_3	1.73	2.32	2.48	**2.17**	3.56	2.93	2.21	**2.90**
4.	T_4	1.95	2.40	2.63	**2.33**	3.28	2.44	2.12	**2.62**
	Mean	**1.72**	**2.17**	**2.42**		**3.85**	**3.16**	**2.57**	
		SEm±	CD (5%)			SEm±	CD (5%)		
Stage (S)		**0.06**	**0.19**			**0.07**	**0.19**		
Treatment (T)		**0.07**	**0.22**			**0.08**	**0.22**		
S × T		**0.12**	**0.38**			**0.13**	**0.40**		

Free amino acid content (mg g^{-1} fresh weight)

With the advancement in germination duration mean free amino acid content decreased significantly. However, there was a significant reduction in mean free amino acid content with increased fluoride

concentration in the germination medium. T_3 and T_4 treatments showed a significant reduction in comparison to T_1. At all stages, seedlings treated with T_1 (control) had considerably higher free amino acid content than those under other treatments (Fig. 2).

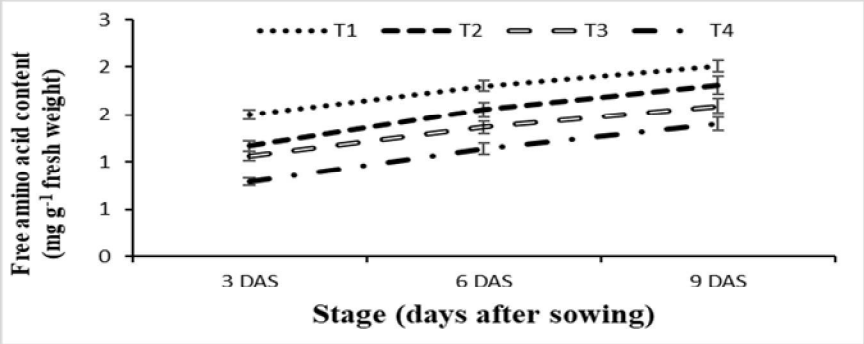

Fig. 2: Effect of different concentrations of fluoride on free amino acid content (mg g^{-1} fresh weight) in wheat genotype HUW-234 at 3, 6 and 9 days after sowing. T_1, T_2, T_3, and T_4, represent 0, 100, 200 and 300 ppm fluoride, respectively.

DISCUSSION

Starch is a significant storage element present in cereal grain endosperm tissue (12). According to studies, fluoride prevents seed germination by suppressing the enzyme amylase (16). Emerging seeds do not absorb enough carbon skeleton for growth and energy due to the delayed breakdown of starch in the presence of fluoride, resulting in germination suppression (16; 18). In F-treated seedlings, α-amylase, β-amylase, and total amylase activity decreased relative to the control. (Table 1and Fig. 1). This result confirms the observations of (16); (2). Amylase activity was correlated to starch content and the starch content of HUW-234 decreased as F concentrations increased. Amylase activity was reduced the most at 300 ppm F then at 200 ppm F. Amylase is involved in starch decomposition, therefore as F levels increase, the amount of starch in the system reduces even when amylases activity declined. Sugars have an essential role in the stabilization of membrane action, such as gene regulators and signaling molecules, as well as the synthesis of other substances and the creation of energy (8); (18). When compared to control seedlings, greater F concentrations resulted in an increased soluble sugar content in wheat seedling (Table 2). At 100 ppm, F had been proven to have very little effect on wheat seedlings. Higher F solution concentrations, i.e. 200-300 ppm F treatments, had the most significant effect. Sucrose levels in seedling may be higher in the presence of F due to increased sugar transformation from starch, as revealed by starch and sugar contents. With the addition of F, the quantity of soluble sugars increased, and starch and free amino acid decreased (Table 2 and Fig 2). This result confirms the observations by (5); (6); (16); (2). The F-induced starch breakdown was accompanied by increased sucrose production in developing potato tubers. (15) tests on *Pennisetum glaucm*, where sugar content was increased under F stress, back up their results. Carbon metabolism, which manifests itself in the breakdown of starch to soluble sugars, is a key disturbance of plant defense under F stress and its rate is absolutely dependent on F concentration. The usage of starch during F treatment also indicated that stress had induced amylase to stimulate starch decomposition in order to overcome stress (2).

Under normal growth circumstances, an appropriate supply of free amino acids is required to satisfy any fluctuations in protein synthesis and growth rates. Amino acid concentrations were highest in control, then quickly decreased with 200-300 ppm F treatment (Fig. 2). These results were also similar to those obtained in *Vigna* seedlings where addition of F increased proline levels (13).

Furthermore, increased fluoride concentration in the root zone causes a stressful environment for the plant, resulting in the accumulation of osmotic such as soluble sugars and proline in order to maintain cellular water status.

CONCLUSION

The hydrolysis of seed reserves is a significant procedure that supplies the energy and nutrients needed for the early seedling's growth and development. However, under fluoride stress, the hydrolysis of seed reserves is inhibited, leading to reduced growth and development of the seedling. Activities of α, β and total amylase reduced as fluoride concentrations increased in the germinating medium. Concentrations of soluble sugars in germinating seed increased as fluoride levels in the germination medium increased, but starch and free amino acids concentrations decreased.

The results of this study have important implications for agriculture, as fluoride pollution is becoming an increasingly significant problem in many areas of the world. Farmers and policymakers need to take

measures to reduce fluoride pollution to prevent the negative impact on crop production and food security. Future studies should focus on identifying the mechanisms by which fluoride inhibits seed reserve hydrolysis and developing strategies to mitigate the impact of fluoride pollution on crop growth and development.

ACKNOWLEDGEMENT

The current study is part of the first author's Doctoral dissertation. The first author gratefully acknowledges the financial help obtained throughout the study period in the form of a UGC-BHU fellowship.

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