FLUORIDE

Quarterly Journal of The International Society for Fluoride Research Inc.

Determination of Sodium Fluoride Effects on Germination, Seedling Growth, and Antioxidant Enzyme Activity of Two Buckwheat (*Fagopyrum esculentum*) Genotypes

Unique digital address (Digital object identifier [DOI] equivalent): https://www.fluorideresearch.online/epub/files/396.pdf

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Submitted: 2025 Jul 05
Accepted: 2025 Sep 20
Published as e396: 2025 Oct 08

ABSTRACT

Purpose: Stunted growth and reduced yield in buckwheat can be associated with nutrient absorption issues caused by sodium fluoride toxicity.

Methods: An investigation was conducted to examine the effects of sodium fluoride (NaF) at varying concentrations on the morphology and enzyme activity of catalase, peroxidase, and ascorbate during the germination and seedling stages of two buckwheat genotypes, G_7 and G_{14}. Seed germination was carefully observed in plastic petri dishes with different NaF concentrations, ranging from 0 (control) to 8.0 mM. Then, the antioxidant enzymes (catalase, peroxidase, and ascorbate peroxidase) and the level of hydrogen peroxide (H_2O_2), a marker of oxidative stress, were measured using the biochemical method.

Results: Results indicated that the NaF treatment significantly inhibited common buckwheat germination index, seedling growth, and altered oxidative stress markers. In G_7, shoot and root lengths decreased by 15.4%-80% and 46.55%-70.69%, respectively, while in G_{14}, reductions were 26.18%-81%.69% and 46.62%-74.12%. Antioxidant enzyme activities, catalase (CAT), and ascorbate peroxidase (APX), declined by 22.84%-60.33% and 2.73%-29.83% in G_7, and by 26.73%-61.33% and 1.70%-40.42% in G_{14}. Conversely, Peroxidase (POX) and hydrogen peroxide (H_2O_2) increased markedly by 2.34%-33.64% and 8.66%-52.43% in G_7 and 3.68%-50.52% and 10.78%-54.58% in G_{14}, indicating enhanced oxidative stress under sodium fluoride exposure.

Conclusions: This study highlights that NaF exposure significantly altered common buckwheat germination and seedling growth by inducing oxidative stress and disrupting the antioxidant defense mechanism.

Keywords: *Common buckwheat; Sodium fluoride; Oxidative stress; Antioxidant enzymes*

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum*) is an outcrossing pseudo-cereal crop belonging to the Polygonaceae family, with significant potential in the food processing industry.[1,2] Its grains are nutritionally rich, containing all essential amino acids, thiamin-binding proteins, essential fatty acids (linoleic and linolenic acids), dietary fiber, minerals, vitamins, and a range of bioactive compounds including flavonoids (rutin, flavonol, quercetin, and vitexin) and fagopyrins.[3,4] This crop is employed both as a food source and in traditional medicine for bloating, excess blood pressure, elevated cholesterol levels, cardiac issues, diabetes, cancer, edema, skin disorders, and other major medical disorders.[5,6]

Sodium fluoride is a toxic substance widely used in industry and various human activities. Due to its high electronegativity and reactivity, it is rarely found in pure form in nature**.**[7] Elevated fluoride levels are often associated with crustal elements, industrial pollutants, and other inorganic substances.[8] The underground water fluoride danger map shows regions in the middle of Australia, Northwestern America, eastern Brazil, and various sections of Africa and Asia. Globally, around 180 million people are at risk, mainly in Asia (51-59%) and Africa (37-46%), with the latter group making up 6.5% of the continent's population.[9] While Bangladesh generally has low fluoride in freshwater, some remote and coastal regions exceed safe limits.[10]

Fluoride is beneficial in small amounts for teeth, dental, and bone mineralization,[7] but becomes a health threat under chronic high-level exposure, leading to dental, skeletal, and non-skeletal fluorosis in both humans[11-13] and animals.[14-16] Animal studies have documented that long-term ingestion of excess fluoride causes oxidative stress, mitochondrial dysfunction, and inflammation in organs such as the liver, kidney, and testes.[17] In contaminated areas, fluoride exposure also affects crops by disrupting chlorophyll levels, plant metabolism, and causing oxidative damage. [18,19] It can lead to visible injury, reduced stem/root size, and lower yields, though in some cases, higher doses increased biomass.[20,21] Sodium fluoride (NaF) or fluoride also alters pigment content, biochemical parameters, and growth, causing morphological changes.[22]

Seed germination is a vital and complex process that determines seed quality and significantly influences the nutritional and antioxidant properties of plants.[23] It marks the onset of a plant's life cycle, initiating seedling development.[24] Biochemical analyses are frequently employed to observe the germination, with particular attention to the final stage, where visible seedling growth occurs, and the initial stage, imbibition, which exhibits notable physiological changes.[25] During the major imbibition, water uptake triggers spatial expansion within the seed, leading to radicle emergence and subsequent root and shoot development.[26]

Reactive oxygen species (ROSs) are produced from the normal oxidation of oxygen, play a dual role in promoting germination by breaking dormancy through oxidation modification of cellular components, and regulating physiological and hormonal signaling pathways.[27] Among them, hydrogen peroxide (H_2O_2) is an essential ROS agent since it may penetrate easily through diverse cell membranes.[28] Catalase, peroxidase, and ascorbate peroxidase are potential antioxidant enzymes that scavenge ROS activity and H_2O_2.[29] The purpose of this study was to investigate the effects of various NaF concentrations on common buckwheat germination and seedling growth, as well as to evaluate the activity of antioxidant enzymes during the stages of seed germination and seedling development.

MATERIAL AND METHODS

Experimental Design

The study was conducted under controlled laboratory conditions using two genotypes of common buckwheat seeds, G_7 and G_{14}, employing the Randomized Complete Block Design (RCBD) model. The seeds were selected and obtained from the buckwheat germplasm center of the Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. After that, petri dishes were sterilized with the 70% ethanol solution and washed with distilled water. Fifty chosen seeds were set up on plastic petri dishes that were prepared with two sheets of filter paper. Following this, the control group was moistened with 50 ml of distilled water, while the treated group was moistened with NaF solution at concentrations of 2 mM, 4.0 mM, 6 mM, and 8 mM NaF, with three biological replicates. All the petri dishes were placed in a dark place at a constant room temperature. Treatment of the above concentrations was applied at 50 ml/day for 12 days. The number of germinating seeds, germination percentages, germination vigor, vigor index, germination speed, germination index, and coefficient of speed germination were calculated during the germination stage. To evaluate the oxidative stress response during early development, the activities of the antioxidant enzymes catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), as well as the hydrogen peroxide (H_2O_2) accumulation, were measured in the seedling stage following the methodology of Pelc et al.[24]

Determination of germination profile

Germination percentage (%)

The seed germination (%) is the proportion of seeds that germinate out of the total number of seeds planted.[30] The number of germinated seeds was counted between 3 and 8 days following the beginning of germination. The germination percentage was calculated according to the following equation.

$$\text{Germination percentage} = \frac{\text{Numbers of normally germinated seedlings}}{\text{Total number of seedlings}} \times 100$$

Germination vigor refers to the number of seeds that successfully germinate when provided with ideal conditions of 25^{o}C, along with appropriate moisture and lighting, during the initial count of germination relative to the total sample size.[30] Germination vigor was calculated as per the following equation.

$$\text{Germination vigor} = \frac{\text{Number of germinated seeds in the first count}}{\text{Total numbers of seeds}} \times 100$$

The seed vigor index is the summation of the root and shoot length of the plant divided by the germination percentage.[31] The following equation was used for this calculation.

$$\text{Vigor index} = \left(\text{Root length} + \text{Shoot length}\right) \times (\text{Germination }\%)$$

Germination Speed

The speed of germination quantifies the rate at which seeds germinate compared to the total seed number germinating in a time interval. Germination speed was estimated using the following equation.

$$\text{Germination speed} = \frac{\text{N1} \times \text{T1} + \cdots + \text{Nn} \times \text{Tn}}{\text{N1} + \text{N2} + \cdots + \text{Nn}}$$

Germination Index

The germination index measures both the percentage and number of germinated seeds.[32] The calculation of the germination index was performed using the following formula.

$$\text{Germination index} = \frac{\text{N1}}{\text{T1}} + \frac{\text{N2}}{\text{T2}} + \frac{\text{N3}}{\text{T3}} + \cdots + \frac{\text{Nn}}{\text{Tn}}$$

Coefficient of Speed of Germination

The coefficient of speed of germination refers to the average measure of seeds that have germinated across three replications, measured under controlled laboratory conditions over 8 days from the initiation of germination. The coefficient of speed of germination was calculated according to the following equation.

$$\text{Coefficient of speed germination} = \frac{\text{Average number of seedlings}}{\text{Number of days}}$$

Root Length

The root length was measured using a scale at 6 and 12 days after germination.

Shoot Length

The shoot length was measured using a scale at 6 and 12 days after germination.

Determination of Antioxidant Enzyme Assay

Extraction of plant enzymes from buckwheat shoot samples

Shoot samples were collected from the control and treated groups. About 50 mg of shoot samples were homogenized with 3 ml of 50 mM potassium phosphate buffer (pH 8.0) at 4°C. Subsequently, the homogenized mixture underwent centrifugation at 15,000 × g for 20 minutes, after which the supernatant was stored for the antioxidant enzyme assay.

Catalase (CAT) activity was determined according to the procedure of Siddiqui et al.[33] This method involves detecting the decrease in absorbance produced by H_2O_2 breakdown in samples at 240 nm. The enzyme activity was calculated by the following formula-

$$\text{CAT} \ (\text{mMoleg}^{-1}\text{FW}) = \frac{(\text{Absorbance difference /min}) \times \text{Dilution factor (DF)}}{40}$$

The measurement of peroxidase (POD) activity was conducted via spectroscopy, utilizing the oxidative degradation of guaiacol in the presence of H_2O_2. The following formula calculates the enzyme activity-

$$\text{POD} \ (\mu\text{mol g}^{-1}\text{FW}) = \frac{(\text{Absorbance difference /min}) \times \text{DF}}{26.6}$$

Ascorbate peroxidase (APX) activity was assessed through spectroscopy, focusing on the scavenging of H2O2 in the presence of APX.[34] The following formula was used to determine the enzyme activity-

$$\text{APX} \ (\text{Ug}^{-1}\text{FW}) = \frac{(\text{Absorbance difference /min}) \times \text{DF}}{2.8}$$

Hydrogen peroxide (H_2O_2) activity was measured using the method mentioned in Rasel et al.[35] Briefly, 0.1 g of the buckwheat shoot sample was taken, to which 1 ml of 0.1% trichloroacetic acid was added. The sample underwent grinding followed by centrifugation at 10,000 × g for a duration of 15 minutes. Subsequently, equal volumes of potassium phosphate buffer (0.5 ml) and potassium iodide solution (0.5 ml) were mixed with 1 ml of extract and incubated in the shade for a single hour. Finally, the absorbance of the mixture was recorded by spectroscopy at 390 nm.

Statistical data analysis

The mean and standard deviation were calculated using the data of two morphological traits, six germination profiles, and three antioxidant enzymes by R Studio v.4.2.3.[36]

Table 1. Effects of different levels of NaF (0, 2, 4, 6, and 8 mM) on the germination (%), vigour germination (%), vigour index, speed of germination day/seed, germination index, and coefficient of speed germination in the early germination stages of two common buckwheat genotypes

Buckwheat genotypes	NaF (mM)	Germination (%)	Vigor Germination (%)	Vigor index	Speed germination	Germination index	Coefficient of the speed of germination
G_7	0	98.00 ± 2.00^{a}	36.66 ± 3.05^{ab}	2544.53 ± 122.07^{a}	2.23 ± 0.15^{abc}	12.50 ± 0.51^{ab}	6.96 ± 0.15^{a}
	2	92.66 ± 3.05^{ab}	35.33 ± 3.05^{ab}	1951.66 ± 103.46^{ab}	2.20 ± 0.10^{abc}	11.83 ± 0.51^{bcd}	6.56 ± 0.20^{ab}
	4	90.66 ± 3.05^{bc}	32.66 ± 2.30^{ab}	2131.06 ± 186.39^{ab}	2.36 ± 0.15^{ab}	11.36 ± 0.20^{cde}	6.43 ± 0.25^{bc}
	6	84.00 ± 2.00^{cd}	29.33 ± 3.05^{b}	2165.00 ± 277.36^{ab}	2.30 ± 0.17^{abc}	10.63 ± 0.20^{ef}	5.96 ± 0.15^{cde}
	8	82.00 ± 2.00^{d}	32.00 ± 2.00^{b}	1684.66 ± 434.72^{b}	2.30 ± 0.17^{abc}	10.46 ± 0.15^{ef}	5.83 ± 0.15^{de}
G_{14}	0	99.33 ± 1.15^{a}	40.00 ± 2.00^{a}	2450.00 ± 200.00^{ab}	2.03 ± 0.05^{c}	13.13 ± 0.23^{a}	7.06 ± 0.05^{a}
	2	94.00 ± 2.00^{ab}	34.66 ± 1.15^{ab}	2009.33 ± 338.40^{ab}	2.13 ± 0.05^{bc}	12.10 ± 0.36^{bc}	6.66 ± 0.15^{ab}
	4	90.00 ± 2.00^{bc}	30.00 ± 2.00^{b}	2298.80 ± 166.06^{ab}	2.46 ± 0.05^{a}	11.03 ± 0.35^{def}	6.30 ± 0.17^{bcd}
	6	84.00 ± 2.00^{cd}	32.00 ± 2.00^{b}	1916.26 ± 113.96^{ab}	2.20 ± 0.00^{abc}	10.83 ± 0.20^{ef}	5.96 ± 0.15^{cde}
	8	80.00 ± 2.00^{d}	29.33 ± 3.05^{b}	1841.80 ± 366.18^{ab}	2.23 ± 0.15^{abc}	10.23 ± 0.15^{f}	5.66 ± 0.15^{e}

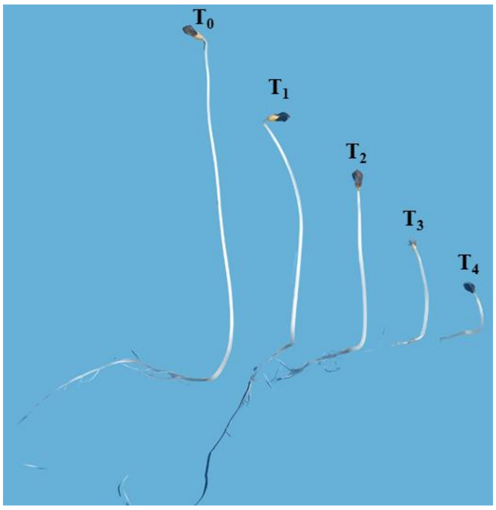

Figure 1. Shoot and root lengths gradually decreased as NaF concentrations increased

RESULTS

Physiological Parameters Based on NaF Stress

Table 1 presents the germination profile data of the two different buckwheat genotypes. Research has shown that NaF has negative effects on seed germination. The control treatment displayed the highest performance, with the maximum percentage of germination observed. The G_{14} genotype showed an impressive germination rate of 99.33%, while the G_7 genotype achieved a slightly lower rate of 98.00%. Two different buckwheat genotypes were treated with varying concentrations of NaF (2, 4, 6, and 8 mM) to investigate their germination rates across various ranges, as shown in Table 1. As the concentrations of NaF increased, germination rates were negatively affected. After treating the seeds with 2 mM NaF, the G_7 genotype exhibited a germination rate of 92.66%, while the G14 genotype showed an impressive 94% germination rate. Interestingly, the germination rates of both seeds showed similarity when exposed to NaF at concentrations of 4 mM and 6 mM, resulting in rates of 90% and 84%, respectively.

When subjected to sodium fluoride treatment, the vulnerable buckwheat genotypes G_7 and G_{14} exhibited detrimental effects and a notable decrease in multiple germination-related traits (such as the vigor index, average vigor germination, index germination, and coefficient of germination). The control treatment of the two genotypes yielded the highest vigor index results; however, as the NaF treatment level increased, the germination scale decreased significantly. In the case of Genotype 14, there was a notable increase in the vigor index when treated with 4 mM NaF, reaching a value of 2298.80. This contrasts with the vigor index 2009.33 observed for the 2 mM NaF treatment. On the other hand, the speed of germination per day showed improvement as the treatment increased. The G_7 and G14 genotypes showed the highest result at the 4 mM treatment, with values of 2.36 and 2.46, respectively.

This study found that excess NaF concentration affects the morphological features of buckwheat. In the case of the G_7 genotype, when treated with 4 mM NaF, the shoot length measured 7.06 cm, indicating a reduction of approximately 29.4% compared to the control group (0 mM NaF) (Table 2). Subsequently, at 6 mM, the length measured 3.53 cm, which showed a notable decrease of 64.7%. Subsequently, when exposed to 8 mM NaF, the reduction in shoot height was estimated to be 80% lower than that of the control. Contrastingly, the shoot length of the G14 exhibited a greater decrease than that of the first genotype. At a concentration of 4 mM, the length decreased by nearly 50%, specifically 5.20 cm, in comparison to the control.

At the concentration of 8mM NaF, the length measured 1.86 cm, which was significantly reduced in comparison to the control. In the case of G_7 genotypes of buckwheat, there was a significant decrease in root length when exposed to NaF concentrations of 2 mM and 6 mM. The reduction was 27% and 34.01%, respectively, compared to the controls, with root lengths of 3.10 cm and 2.40 cm. Lastly, a 40.01% decrease in root length was observed at the 8 mM concentration. In contrast, the length of G_{14} genotypes decreased by 30.17% when treated with a 2 mM NaF concentration compared to the control. A noticeable decrease was observed as the concentration increased. But, in the highest concentration of NaF (8 mM), the root length experienced a significant reduction of 50.04% compared to the root length of the control plant, measuring 1.76 cm. With an increase in NaF content, shoot and root lengths steadily reduced (Figure 1). Furthermore, these effects were most noticeable in genotype 14 between G_7 (Figures 2 and 3).

Table 2. Effects of different concentrations of NaF (mM) on root length and shoot length in early germination stages of two common buckwheat genotypes

Buckwheat genotypes	NaF (mM)	Shoot length (cm)	Root length (cm)
G_7	0	10.00 ± 0.50^{ab}	5.80 ± 1.11^{a}
	2	8.46 ± 0.45^{abc}	3.10 ± 0.26^{bc}
	4	7.06 ± 0.97^{cd}	2.70 ± 0.26^{bc}
	6	3.53 ± 2.02^{ef}	2.40 ± 0.65^{bc}
	8	2.00 ± 0.50^{f}	1.70 ± 0.95^{c}
G_{14}	0	10.16 ± 1.04^{a}	6.80 ± 1.11^{a}
	2	7.50 ± 0.50^{bcd}	3.63 ± 0.47^{b}
	4	5.20 ± 0.55^{de}	2.10 ± 0.17^{bc}
	6	2.50 ± 0.40^{f}	2.13 ± 0.40^{bc}
	8	1.86 ± 0.15^{f}	1.76 ± 0.76^{c}

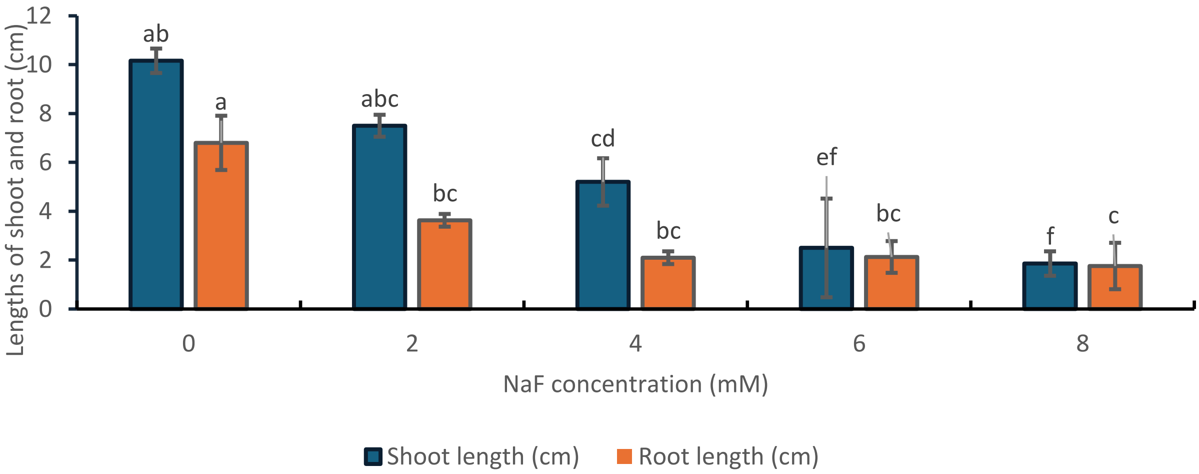

Figure 2. Effects of different concentrations (0, 2, 4, 6, and 8 mM) of NaF on root length and shoot length in the seedling stage of common buckwheat (G_7)

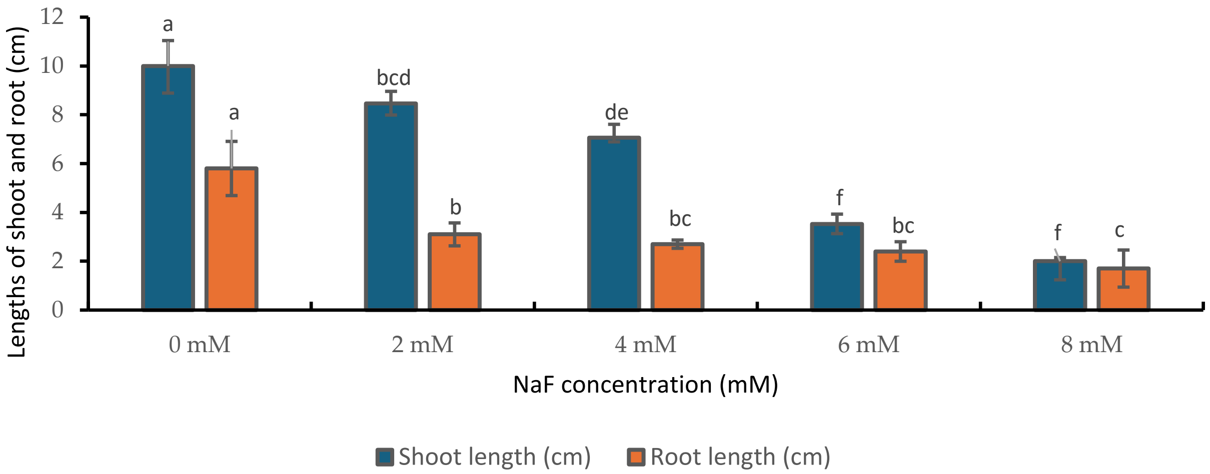

Figure 3. Effects of different concentrations (0, 2, 4, 6, and 8 mM) of NaF on root length and shoot length in the seedling stage of common buckwheat (G_{14})

Antioxidant enzyme activity

Sodium fluoride has a detrimental effect on plants by raising the concentration of ROS species. To investigate the salinity level, buckwheat was subjected to sodium fluoride stress. This study highlights a significant reduction in growth levels and antioxidant enzyme content (POX, CAT, and APX) and ROS content (H_2O_2) in seedlings exposed to NaF when compared with the control group that was tested using a concentration-dependent analysis of antioxidant enzyme activity (0, 2, 4, 6, and 8 mM NaF). For both genotypes, CAT and APX activity significantly decreased at 8 mM of sodium fluoride (Table 3). The concentration of NaF caused the greatest inhibition of enzyme activity when compared to the control; this was 11.11% for the G_7 cultivar and 11.24% for the G_{14} cultivar. This is because high fluoride levels can damage the globular structures of enzymatic proteins like APX and CAT. Nonetheless, the G_7 genotype demonstrated a similar outcome to the 8 mM concentration, which was 19.35, at a 4 mM NaF concentration. In the end, ROS exceeded the threshold, leading to the emergence of injury symptoms. A progressive rise in POX and H_2O_2 activity was noted in both varieties, with a peak observed at 8 mM. The concentration of NaF caused the largest increase in POX activity, 2.86 and 2.46, which was 72% and 80% higher than the control for G_7 and G_{14}, respectively.

However, the G_7 cultivar showed a significant stimulation of POX activity for concentrations of 4 mM and 6 mM, which were 32% and 65% higher than the control, respectively. In contrast, the G_{14} cultivar showed a significant increase in activity for the 6 mM concentration, which was 45% higher than the control. Like this, the greatest increase in H_2O_2 activity was observed for 8 mM NaF concentrations of 11.75 and 12.02, which were 65.6% and 61.6% higher than the control for G_7 and G_{14}, respectively. However, both genotypes displayed the same result at 2 mM concentration, which was 6.12. Meanwhile, for G_7 and G_{14}, the levels were 9.05 and 10.30 at 6 mM concentration, indicating 34.6% and 48.4%, respectively.

Table 3. Effects of different NaF concentrations on antioxidant enzymes (POX, CAT, APX) and H_2O_2 activities in the seedling stage of the two common buckwheat genotypes

Buckwheat genotypes	NaF (mM)	Peroxidase (µmol g^{-1} FW)	Catalase (mMoleg^{-1} FW)	Ascorbate peroxidase (Ug^{-1} FW)	H_2O_2 (µmol/g FW)
G_7	0	2.14 ± 0.18^{abc}	1.84 ± 0.04^{b}	22.69 ± 7.72^{ab}	5.59 ± 0.28^{f}
	2	2.19 ± 0.17^{abc}	1.42 ± 0.06^{c}	22.07 ± 3.40^{ab}	6.12 ± 0.13^{ef}
	4	2.46 ± 0.04^{ab}	1.17 ± 0.02^{de}	19.35 ± 3.33^{abc}	7.29 ± 0.35^{de}
	6	2.79 ± 0.14^{a}	0.96 ± 0.03^{f}	17.30 ± 2.54^{ab}	9.05 ± 0.38^{bc}
	8	2.86 ± 0.25^{a}	0.73 ± 0.11^{g}	14.52 ± 1.33^{abc}	11.75 ± 0.55^{a}
G_{14}	0	1.66 ± 0.32^{c}	2.02 ± 0.07^{a}	27.61 ± 1.79^{bc}	5.46 ± 0.50^{f}
	2	1.63 ± 0.34^{c}	1.48 ± 0.05^{c}	27.14 ± 13.38^{a}	6.12 ± 0.22^{ef}
	4	1.71 ± 0.55^{bc}	1.34 ± 0.08^{cd}	22.14 ± 11.6^{bc}	7.70 ± 0.39^{cd}
	6	2.11 ± 0.18^{abc}	1.07 ± 0.07^{ef}	20.57 ± 2.62^{bc}	10.30 ± 1.14^{b}
	8	2.46 ± 0.12^{ab}	0.78 ± 0.09^{g}	16.45 ± 1.5^{c}	12.02 ± 0.75^{a}

DISCUSSION

There is increasing concern about the adverse effects of high NaF concentrations on plant health and development. NaF has been widely used as a model for assessing crop tolerance to abiotic stress, particularly during germination.[37-39] Numerous studies have reported that NaF negatively impacts seed germination and seedling growth by disrupting chloroplast and mitochondrial structures, impairing membrane permeability, and reducing photosynthetic efficiency.[40-45] Our findings are consistent with this, as we observed significant reductions in both shoot and root lengths in buckwheat seedlings exposed to increasing concentrations of NaF, suggesting inhibited cellular elongation and nutrient transport under fluoride stress.

Previous studies have also shown that NaF acts as a metabolic inhibitor during early development, decreasing germination rate, vigor index, and tolerance index.[46,47] We observed similar trends, with NaF-treated seedlings displaying reduced germination index and seedling growth, particularly at higher concentrations. Such reductions are frequently attributed to the accumulation of reactive oxygen species (ROS), which cause oxidative damage to cellular structures.[48-51]

In the present study, NaF exposure led to a notable increase in H_2O_2 content, confirming enhanced oxidative stress during the seedling stage. These findings align with previous studies that demonstrated that NaF elevates ROS production in stressed plants.[52,53] Despite the upregulation of H_2O_2, the activity of key antioxidant enzymes, catalase (CAT) and ascorbate peroxidase (APX), significantly decreased in NaF-treated plants. These findings support previous research indicating that fluoride stress suppresses primary antioxidant enzymes, weakening the plant's defense system.[54] Reduced CAT activity, in particular, may impair H_2O_2 detoxification, resulting in cellular damage and lipid peroxidation.[55.56]

Interestingly, peroxidase (POX) activity increased with NaF concentration, which may reflect a compensatory response aimed at mitigating ROS accumulation. Similar observations were made in *Cicer arietinum* and soybean (*Glycine max*) under NaF stress, where POX activity rose as part of the plant's adaptive mechanism.[57-59] Our results suggest that while buckwheat activates secondary antioxidant pathways like POX under stress, its primary ROS-scavenging system (CAT and APX) becomes compromised, leading to imbalanced redox homeostasis and stunted growth.

Overall, this study indicates that NaF induces oxidative stress in common buckwheat seedlings by disrupting antioxidant enzyme activities, altering the germination profile, and inhibiting shoot and root development. These responses highlight the significance of genotype-specific defensive mechanisms, which may be investigated further to find cultivars that are resistant to NaF.

CONCLUSIONS

The present study determined that the common buckwheat (G_7 and G_{14}) showed sensitivity to NaF-stress, as evident from reduced germination and morphological growth, along with imbalanced antioxidant enzyme activity, indicating limited adaptive response under NaF exposure. Among the two genotypes, G_{14} showed the best tolerance to NaF stress. Higher concentrations reduced root and shoot length, biomass, and significantly affected ROS-scavenging enzyme activities. While antioxidant enzymes such as APX, CAT, and POX were activated in response to oxidative stress, these defenses were not sufficient to prevent growth inhibition, indicating that NaF exposure adversely affects overall plant development. This study, conducted under laboratory conditions with limited NaF concentrations and two common buckwheat genotypes, G_7 and G_{14}, may not fully represent field responses. Future studies should include field trials, a wider range of NaF levels, more genotypes, and additional stress-related markers. Studying yield, grain quality, and mitigation strategies will also improve the understanding of fluoride stress.

ACKNOWLEDGEMENTS

Each writer contributed equally to this work. Thank you to the co-authors for their valuable assistance, financial support, editing, and writing efforts, which contributed significantly to the study's success.

FUNDING

Not applicable.

CONFLICT OF INTERESTS

None.

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