applied sciences

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Effect of Fluoride on Germination, Early Growth and Antioxidant Enzymes Activity of Three Winter Wheat (*Triticum aestivum* L.) Cultivars

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Received: 30 August 2020; Accepted: 29 September 2020; Published: 5 October 2020

Abstract: This paper assesses the impact of sodium fluoride on the morphological parameters and activity of catalase and peroxidase during the germination and root growth phases of three winter wheat cultivars: Tobak, Dalewar, and Arkadia. During examination, the seeds were placed on plastic Petri dishes with an NaF solution at concentrations of 0 (control), 2.5, 5.0, 8.0, and 10.0 mmol dm^{-3}. The obtained results have shown a decrease in germination, inhibition of root growth, and inhibition of catalase activity, both in the embryos and roots of all tested winter wheat cultivars. The observed effects have been strengthened with the increase of the fluoride concentration. However, the effect of NaF on the peroxidase activity has been dependent on the wheat cultivar. It is difficult to state unequivocally which of the tested winter wheat cultivars has been characterized by the highest sensitivity to fluoride. An η^2 analysis has confirmed that the NaF concentration has a greater effect than the winter wheat cultivar on the activity of the determined antioxidant enzymes.

Keywords: antioxidant enzymes; fluoride; germination; imbibition; winter wheat cultivars

1. Introduction

Fluoride (F) is commonly found in the environment, and its total content in the Earth's crust is estimated at a level of 0.077% [1]. This element is a non-metal from the group of halogens characterized by the highest electronegativity, which makes it one of the most reactive elements, meaning it practically does not exist in nature in a free form [2]. Natural sources of fluoride are mainly volcanic eruptions and minerals such as apatite $Ca_5(PO_4)_3F$, cryolite Na_3AlF_6, fluorite CaF_2, and topaz $Al_2(SiO_4)F_2$, which lead to accumulations of fluoride in soil as a result of their weathering and leaching [3,4]. High fluoride content is reported in most of the waters in the Middle East, East and Central Asia, and Africa (above 1.5 mg·dm^{-3}, also expressed as mg/L), which is the result of leaching from fluorine-rich minerals. Hence, this amount of F in water from high fluoride content area is reported over 10 mg F·dm^{-3} to even 2800 mg·dm^{-3}. In soil from the high F polluted regions the concentration is usually highter than 300 mg F/kg of soil dry mass [5,6].

Fluoride is often mentioned as one of the pollutants among compounds that create pollution in the environment, as it is ranked fifth in the hierarchy of environmental poisons [7]. The problem of environmental pollution with fluoride compounds has been noticed relatively recently and is mainly related to industrial activity, production of artificial fertilizers (superphosphates), and the emission of fluoride compounds into the atmosphere in the form of dust and gas from aluminum smelters [8]. Currently, about 25% of herbicides contain at least one fluoride atom, or fluoride in the form of difluoromethyl and trifluoromethyl groups, which has significantly contributed to the development

of agrochemical industry products [9]. The introduction of fluoride into organic molecules increases the lipophilic properties of herbicides, the rate of penetration of plant cells, and the blocking of active enzyme sites. This phenomenon is used to enhance the fungicidal properties of herbicides [10].

This element is one of the most toxic elements for plants and, so far, it has not been reported to have a positive effect on these organisms. Fluoride reduces agricultural harvests by up to 50 percent because most plants are highly sensitive to fluoride. F^- compounds have been shown to be highly toxic to cereals and, in particular, wheat [11,12].

Wheat (*Tricitum aestivum* L.) is the world's second-most important crop after rice, with a harvest of about 761.5 million tons during 2019 [13]. Wheat produces a high yield and is one of the most important food crops for the majority of the world's population. Wheat is also a good source of protein, fiber, carbohydrates, minerals, and B-group vitamins [14]. However, for the first time ever in 2018, the total wheat yield did not deliver the previously predicted increase, and global wheat production is estimated to fall by 6 percent, mainly due to climate issues [15].

Germination is one of the most important criteria for crop seed quality assessment [16]. This process is the initiation of the first developmental phase in the life cycle of plants and is followed by the growth of the seedling [17]. Biochemical analysis is widely used for monitoring seeds during germination, but it is often focused on the final phase of germination (the start of seedling growth), and that is paid the most attention. However, the initial phase of germination, imbibition, also exhibits interesting behavior [18]. The imbibition phase is divided into three defined parts [19]. The first one (imbibition) lasts approximately one hour and consists mainly of the swelling of the embryo part of the seed. The second one, also known as main imbibition, consists of spatial expansion caused by water adsorption in the other parts of the seed. The third one is the growth phase, in which radicle protrusion commences, followed by continued root and seedling growth [18,20]. The results of imbibition include a rapid increase in mitochondrial activity and high glyoxysomal activity. Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) and a byproduct of the metabolic activities of both of those compartments [21]. ROS, which are released during the normal metabolism of oxygen, have significant signaling roles in the process of seed germination. Despite, this ROS must be maintained at a relatively low level for germination to proceed. However, if ROS levels are too low, seeds will never germinate. If they are too high, then seeds will incur excessive oxidative damage during seed imbibition and will be nonviable [22]. F^- is also one of the strongest oxidants, which leads to oxidative changes in plant cells. It may increase the content of ROS and inhibit the activity of antioxidative enzymes [23]. Catalase (CAT) and peroxidases (POX) are efficient, enzymatic free radical scavengers, particularly H_2O_2. Previous studies have shown the importance of CAT and POX activity in regulating H_2O_2 levels in embryos during seed germination [24]. However, there is not enough information about the activity of those enzymes during the imbibition phase and radicle growing of wheat seeds under fluoride-induced stress.

The aim of this study was to assess the effect of different NaF concentrations on the morphological parameters and some antioxidant enzyme activity during the germination and root growth phases of three winter wheat cultivars.

2. Materials and Methods

2.1. Experimental Design

The experiment was carried out in laboratory conditions with three cultivars of winter wheat seeds (*Triticum aestivum* L.): Tobak, Dalewar, and Arkadia. The seeds were selected and obtained from the Polish Seed Station. Delawar has moderate freezing resistance, high drought resistance, and exceptional resistance to all leaf diseases, and it is characterized by a high yield of around 11,000 kg/ha. Tobak has high freezing and drought resistances and a high yield potential of 11,300 kg/ha. Arkadia has extremely high freezing and cereal disease resistances, low soil requirements, and a good yield of 7600 kg/ha. Our previous research showed that wheat is one of the most sensitive species to fluoride in the medium [25].

Therefore, it was decided to compare the sensitivity of different cultivars of winter wheat to this element in the presented studies. Seeds were placed on plastic Petri dishes lined with two layers of filter paper (Whatman 1), moistened with 20 cm^3 of sterile water (control) or an NaF solution at concentrations: 2.5 mmol·dm^{-3} (47 mg F·dm^{-3}), 5.0 mmol·dm^{-3} (94 mg F·dm^{-3}) 8.0 mmol·dm^{-3} (150.4 mg F·dm^{-3}), and 10.0 mmol·dm^{-3} (188 mg F·dm^{-3}). 100 seeds were used per treatment, with 20 seeds in each Petri dish. All the plates with the seeds were stored in the dark at a constant temperature of 23 ± 1 °C. During the imbibition phase (after 24 h), the seeds were removed from the solutions and the embryo parts were carefully removed with a scalpel by pushing them out. The activities of the catalase (CAT) and peroxidase (POX) were determined in the embryo parts. Moreover, after 72 h, the number of germinating seeds, germination index, root length, catalase (CAT) activity, and peroxidase (POX) activity were also determined. All measurements were performed in three repetitions.

2.2. Determination of Biometric Parameters and Germination Index

Root length was measured with a ruler after 72 h. On the basis of the number of germinated seeds and the length of the roots, the germination index (GI%) was calculated using the Barbero et al. [26] formula:

$$GI\% = (Gs \cdot Ls)/(Gc \cdot Lc) \cdot 100$$

where Gs is the number of germinated seeds exposed to NaF, Ls is the root length in the plant exposed to NaF, Gc is the number of germinated seeds in the control group, and Lc is the root length in the control plant.

2.3. Determination of Antioxidant Enzymes

CAT activity was determined according to the Lück [27] method. 0.1 g of either the embryo or the root was homogenized in frozen mortars with the phosphate buffer at a pH level of 7.0. The extracts were centrifuged at 14,800× *g* at 4 °C. The obtained supernatants were used to determine CAT activity. This method consists of measuring the decline in absorbance in the samples caused by H_2O_2 decomposition at λ = 240 nm. CAT activity was calculated by using a molar absorbance index for H_2O_2 of 43.6 and was expressed as μmol H_2O_2 g^{-1}·FW·min^{-1}.

POX activity was determined using the method described by Chance and Maehly [28], which is based on pyrogallol oxidation into purpurogallin in the presence of H_2O_2. 0.1 g of either the embryo or the root was homogenized in frozen mortars with the acetate buffer set to a pH level of 6.8. The extracts were centrifuged at 14,800× *g* at 4 °C. The obtained supernatants were used to determine POX activity. The absorbance was measured at λ = 415 nm. POX activity was expressed as μmol purpurogallin g^{-1}·FW·min^{-1}.

A spectrophotometer UV–1800 (Shimadzu, Kyoto, Japan) was used to determine the activity of antioxidant enzymes.

2.4. Data Analysis

The results were processed statistically with Statistica 13.3 software (StatSoft, Kraków, Poland). The significance of the observed differences was verified using a two-way analysis of variance, followed by the post hoc Tukey's HSD (Honest Significant Difference) test ($p = 0.05$). The contribution of independent variables to dependent variables for antioxidant enzymes was determined by calculating the coefficient η^2 with ANOVA.

3. Results

3.1. Antioxidant Enzyme Activity in Wheat Embryos During Imbibition

CAT activity in the embryos of winter wheat seeds placed in sterile water differed significantly between cultivars. The highest enzyme activity was found for the Tobak cultivar and the lowest one

was for the Delawar cultivar. In all concentrations, sodium fluoride showed significant inhibition of CAT activity. Moreover, the observed effect strengthened with the increase in NaF concentration. Compared with the control, the highest decrease in CAT activity was found for the NaF concentration of 10.0 mmol·dm^{-3}, and it was 64%, 87%, and 63% for the Delawar, Tobak, and Arkadia cultivars, respectively (Table 1).

Table 1. Catalase activity (μmol H_2O_2 g^{-1}·FW·min^{-1}) in wheat embryos during imbibition in NaF solutions.

NaF Concentration (mmol·dm^{-3})	Winter Wheat Cultivar		
	Delawar	Tobak	Arkadia
0 (control)	145.2 ± 1.5 c	249.7 ± 3.3 a	182.7 ± 6.1 b
2.5	118.4 ± 1.5 d	144.4 ± 10.8 c	143.9 ± 2.4 c
5.0	116.1 ± 1.2 d	124.8 ± 1.2 d	138.7 ± 2.9 c
8.0	58.4 ± 0.9 fg	89.8 ± 1.8 e	71.1 ± 4.2 f
10.0	52.5 ± 0.4 g	32.2 ± 6.8 h	67.0 ± 3.2 f

Data were given as means ± D (n = 3). The values marked with the same letters do not differ statistically at $p = 0.05$ (post hoc Tukey HSD test).

Similar to CAT activity, POX activity in the control embryos differed significantly between cultivars. The highest enzyme activity was found in embryos of the Tobak cultivar, and the lowest activity was found in the Delawar cultivar. However, the results of the NaF effect on POX activity were not the same as for CAT activity. A concentration of NaF at a level of 2.5 mmol·dm^{-3} resulted in a significant increase in POX activity, but only in embryos of the Tobak cultivar (43% compared with the control), whereas for a concentration of 5.0 mmol·dm^{-3}, a significant stimulation of POX activity was reported in the embryos of all cultivars. The observed increases in activity for the Delawar, Tobak, and Arkadia cultivars were 28%, 58%, and 23%, respectively. The further increase of the NaF concentration resulted in stimulations of activity only for the Arkadia cultivar, which for concentrations of 8.0 mmol·dm^{-3} and 10.0 mmol·dm^{-3} were 30% and 14%, respectively. Meanwhile, a decrease in POX activity occurred in embryos of the Delawar and Tobak cultivars at the highest NaF concentrations. The greatest inhibition of enzyme activity was found in the embryos of Delawar seeds placed in the NaF concentration of 10.0 mmol·dm^{-3}; it was 57%, compared with the control (Table 2).

Table 2. Peroxidase activity (μmol purpurogallin·g^{-1} FW·min^{-1}) in wheat embryos during imbibition in NaF solutions.

NaF Concentration (mmol·dm^{-3})	Winter Wheat Cultivar		
	Delawar	Tobak	Arkadia
0 (control)	33.9 ± 0.4 j	52.0 ± 0.72 e	46.9 ± 0.6 f
2.5	35.2 ± 0.7 ij	74.2 ± 0.38 b	47.6 ± 1.2 f
5.0	43.5 ± 0.5 g	82.3 ± 0.66 a	57.6 ± 0.7 d
8.0	31.0 ± 0.2 k	40.7 ± 1.19 h	60.8 ± 0.5 c
10.0	19.3 ± 0.8 l	36.7 ± 1.71 i	53.4 ± 1.4 e

Data were given as means ± SD (n = 3). Values marked with the same letters do not differ statistically at $p = 0.05$ (post hoc Tukey HSD test).

3.2. Antioxidant Enzyme Activity in Wheat Roots

The comparison of CAT activity in the roots of the control seedlings showed that the Delawar cultivar had the highest enzyme activity, whereas the Tobak cultivar had the lowest activity. Similarly, as in wheat seed embryos in the imbibition phase, CAT activity in the roots of all cultivars decreased significantly with the increase in NaF concentration. Compared to the control, the highest inhibition of enzyme activity was observed for the NaF concentration of 10.0 mmol·dm^{-3}, and it was 90%, 94%, and 61% for the Delawar, Tobak, and Arkadia cultivars, respectively (Table 3).

Table 3. Catalase activity (μmol H_2O_2 g^{-1}·FW·min^{-1}) in wheat roots after 72 h of incubation in NaF solutions.

NaF Concentration (mmol·dm^{-3})	Winter Wheat Cultivar		
	Delawar	Tobak	Arkadia
0 (control)	268.1 ± 14.6 a	114.3 ± 11.7 ef	163.5 ± 1.6 c
2.5	182.7 ± 5.5 b	154.2 ± 1.5 cd	124.8 ± 3.6 e
5.0	143.6 ± 2.9 d	144.0 ± 2.6 d	102.6 ± 1.0 fg
8.0	58.4 ± 0.9 h	38.9 ± 6.0 i	95.9 ± 6.8 g
10.0	27.3 ± 3.7 i	7.2 ± 3.3 j	63.3 ± 1.2 h

Data were given as means ± SD (n = 3). Values marked with the same letters do not differ statistically at $p = 0.05$ (post hoc Tukey HSD test).

Contrary to the wheat seed embryos in the imbibition phase, no significant differences in root POX activity were found between cultivars. A significant stimulation of POX activity was observed in the roots of the Arkadia cultivar for all NaF concentrations. The highest increase of activity was shown for the NaF concentration, and it was 82% compared with the control (Table 4). However, a significant stimulation of POX activity occurred in the roots of the Dalewar cultivar for concentrations of 8.0 mmol·dm^{-3} and 10.0 mmol·dm^{-3}, and they were 37% and 62% compared with the control, respectively, whereas a significant increase in activity was reported in the roots of the Tobak cultivar only for a concentration of 10.0 mmol·dm^{-3} (136%, compared with the control).

Table 4. Peroxidase activity (μmol purpurogallin g^{-1} FW min^{-1}) in wheat roots after 72 h of incubation in NaF solutions.

NaF Concentration (mmol·dm^{-3})	Winter Wheat Cultivar		
	Delawar	Tobak	Arkadia
0 (control)	41.9 ± 4.7 fg	36.7 ± 1.4 gh	38.8 ± 1.9 fgh
2.5	44.2 ± 1.2 fg	30.4 ± 0.8 h	54.9 ± 1.6 de
5.0	47.5 ± 2.2 ef	41.2 ± 2.9 fg	58.7 ± 2.1 cd
8.0	57.1 ± 2.7 cd	45.1 ± 0.7 fg	65.2 ± 3.1 bc
10.0	67.8 ± 5.3 b	86.5 ± 2.8 a	70.5 ± 5.3 b

Data were given as means ± SD (n = 3). Values marked with the same letters do not differ statistically at $p = 0.05$ (post hoc Tukey HSD test).

3.3. Share of Factors in Formation of Antioxidant Enzyme Activities

The coefficient η^2 in ANOVA showed that CAT activity in the embryos and roots, as well as the activity of POX in roots, were affected to the greatest extent by concentrations of NaF, while the activities of POX in embryos were most affected by the wheat cultivar (Figure 1). It is also interesting that the contribution of the interaction between the NaF concentration and the winter wheat cultivar to the formation of CAT and POX activity was high.

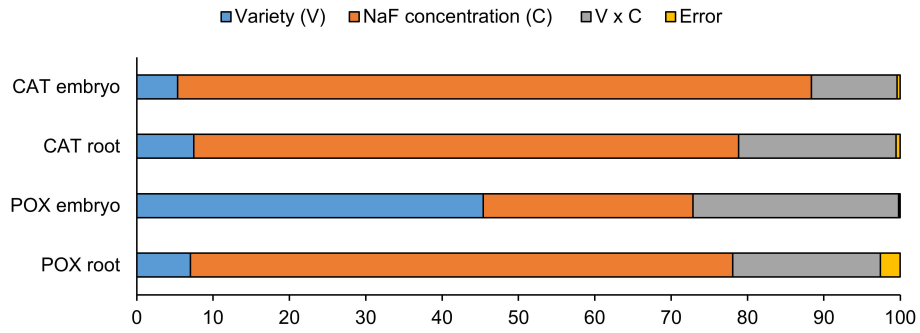

Figure 1. The share of independent variables in the evolution of the activity of catalase (CAT) and peroxidase (POX) in winter wheat embryos and roots.

3.4. Germination and Root Length

The application of sodium fluoride in all of the concentrations led to a significant decrease ($p = 0.05$) of root length in all cultivars, compared with the control plants. The highest total reduction of root length was for the Delawar (−73%) and Arkadia (−84%) cultivars (Figure 2). On the other hand, the reduction was at a level of 43% for the Tobak cultivar, and the root length in 10 mmol·dm^{-3} of NaF was still up to 1 cm.

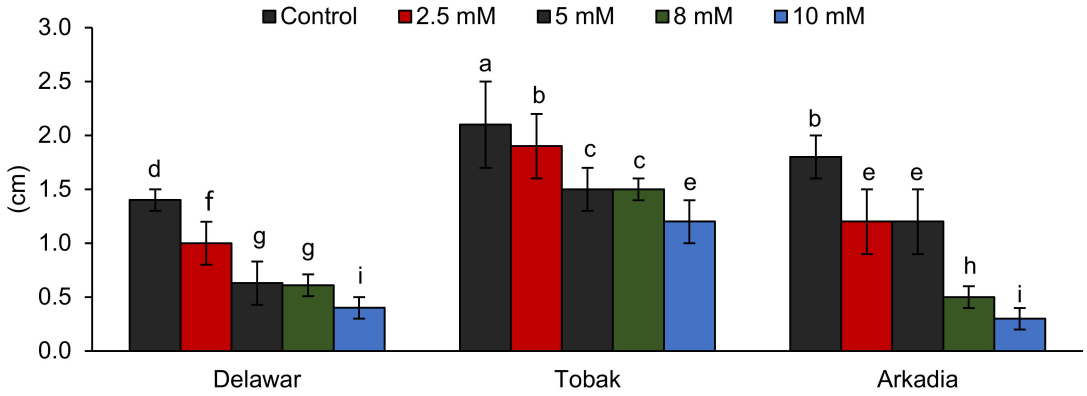

Figure 2. Root length in wheat after 72 h of incubation in NaF solutions. Data are given as means ± SD (n = 100). Values marked with the same letters do not differ statistically at $p = 0.05$ (post hoc Tukey HSD test).

The seed germination index decreased with the increase in concentration of NaF (Figure 3). Among the treatments, the application of 10 mmol·dm^{-3} of NaF reduced the highest number of germinated seeds compared with the control (100%) to 17%, 50%, and 14%, respectively. However, in a concentration of 5 mmol·dm^{-3} of NaF and 8 mmol of NaF in the Tobak cultivar, and in a concentration of 2.5 mmol·dm^{-3} of NaF and 5 mmol·dm^{-3} of NaF in the Arkadia cultivar, the GI% was at the same levels of 71% and 67%, respectively. The most rapid decrease in the GI% was observed for the Delawar cultivar from 2.5 mmol·dm^{-3} of NaF to 5 mmol·dm^{-3} of NaF; the reduction equaled 34% compared with the Tobak and Arkadia cultivars.

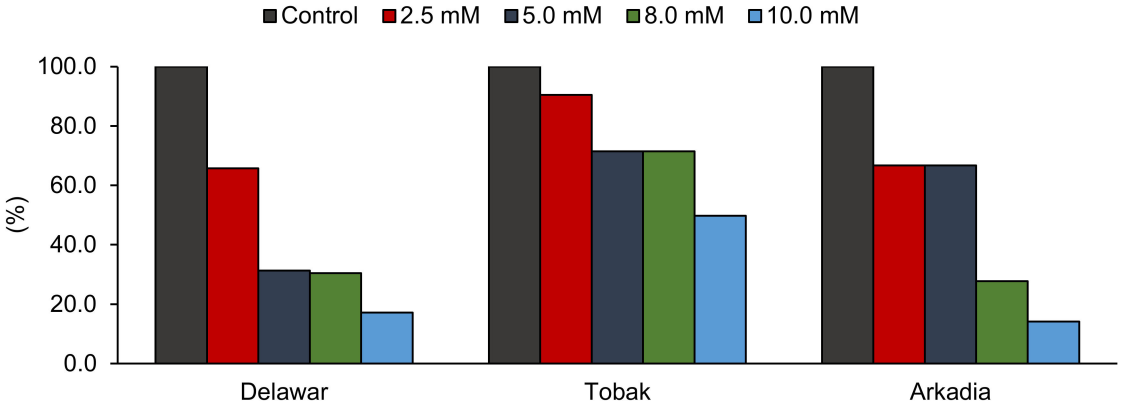

Figure 3. Germination index in wheat seeds after incubation in NaF solutions.

4. Discussion

The importance of the process of breaking seed dormancy, which leads to the initiation of germination, is widely known, and this study has been used by many authors as a model for assessing crop resistance to biotic and abiotic stresses [29–31]. From physiological and biochemical points of

view, germination occurs after restored metabolic activity. Then, a large number of specific metabolic events, such as antioxidants enzymes activity, take place. When the seeds initiate growth, they need appropriate internal and environmental conditions. However, changes in these conditions caused by toxic substances such as NaF result in changes in the seed's metabolism [32–34]. The high levels of fluoride lead to the production and accumulation of ROS, as well as changes in the enzymatic antioxidant system. This system comprises of antioxidant enzymes, such as CAT and POX. Many authors suggest that oxidative damage is a major effect of F toxicity to plants. Due to the effects on these antioxidant enzymatic systems, many researchers have also studied the adverse effect of F on the metabolic processes of plants [1,35,36].

In the current study, the analysis of CAT and POX activities has been used for the first time in wheat embryos under fluoride-induced stress. In addition, the activities of those enzymes in wheat roots have been presented, which are not examined very often. The results have shown the inhibiting effects of sodium fluoride on CAT activity in both embryos and roots. The activity decreased with the increase in the sodium fluoride concentration in all of the cultivars, and the highest inhibition of CAT activity in embryos and roots has been observed for the Tobak cultivar at 87% and 97%, respectively. The reduced activity of enzyme antioxidants such as CAT can lead to lipid peroxidation and membrane damage [37]. CAT activity during the seed imbibition phase is usually low and initially increases during the seedling stage [21]. However, in our study, because of the NaF solutions, CAT activity in the embryos and roots was lower than in the control samples, while the level of inhibition was dependent on the cultivar. Differences in CAT activity among the cultivars under fluoride-induced stress were also observed by Tak and Asthir [38]. In their study, CAT activity decreased drastically in the roots of the F-tolerant wheat cultivar HD 3086, compared with the less tolerant WH1105. Fluoride combines easily with many heme enzymes such as CAT, which has iron in its structure. Moreover, fluoride anion coordinated with the heme iron is proven to be hydrogen bonded to the distal histidine (His64) and to a water molecule in this enzyme. Hence, iron which allows CAT to react with the hydrogen peroxide is blocked [39]. However, wheat under fluoride-induced stress may also cause both an increase and a decrease in CAT activity. Such changes are noted by Śnioszek et al. [40] in leaves of the winter wheat cultivar Skagen. After the application of NaF and KF into the soil, CAT activity boosts with the increases of fluoride concentrations in the soil. The differences in CAT activity are observed among different plant cultivars as well. Many similar changes are recognized in different crops. Mondal [41] discovered that CAT activity steadily escalates with the increase of NaF in four rice cultivars. In the studies by Rao et al. [42], CAT also showed its increased activity in tea leaves under fluoride-induced stress.

As has been mentioned earlier, POX, similar to CAT, also reacts with H_2O_2. The distal histidine contributes to the stability of the fluoride complex in POX in a similar way to CAT, and it also leads to the decrease of this enzyme's activity [39]. However, the increase in the POX activity under fluoride-induced stress is observed more often. In our study, POX activity has been different in the cases of embryos and roots and between cultivars. In embryos, POX activity in the Delawar and Tobak cultivars increased with 5.0 mmol·dm^{-3} of NaF, and for 8.0 mmol·dm^{-3} of NaF it started to decrease the most in the Tobak cultivar (58%). Only the Arkadia cultivar saw increases in POX activity in all of the NaF concentrations. POX activity may increase during germination phase due to plant response to wounding stress induced by radicle penetration through the micropylar endosperm which is accompanied by the rupture, or wounding of this tissue. [43]. However, it is largely unknown how F is deposited in seeds and the levels at which it is toxic to embryos [44]. Contrary to the embryos, the POX activity in roots escalated with the increase in the NaF concentration, while the decrease was only observed in the Tobak cultivar in 2.5 mmol·dm^{-3} of NaF. We have also remarked that the activities for roots differed among cultivars. Roots have usually shown a greater tendency to accumulate fluoride than other parts of plants [35]. In the study by Tak and Asthir [38], greater POX root activity was characteristic for HD 3086 wheat cultivar resistance to F in normal conditions and under F-induced stress, and it demonstrated superior tolerance mechanisms in terms of H_2O_2 production and utilization.

Hence, those differences may indicate the existence of more tolerant cultivars which have a better ability to scavenge H_2O_2 [44].

However, we have reported that the NaF concentration has a higher impact on CAT and POX activities than the cultivar of wheat. In our previous study on winter wheat seedlings, we also observed that the fluoride concentration in the soil had the greatest influence on the activity of antioxidant enzymes [40].

The evaluation of the germination of seeds is important for the provision of successful, high-yield crop production. Many authors also report that increased levels of F negatively affect wheat germination [16,31,45–47]. In our study, sodium fluoride also reduced a number of germinated seeds (GI%) in all of the wheat cultivars, but at different levels. The lowest GI% was observed in 10 mmol·dm^{-3} of NaF, and it was 14% for the Arkadia cultivar and 17% for the Delawar cultivar. However, in the same concentration, the GI% for the Tobak cultivar was 50%. Alim et al. [45] suggested that this might be caused by the inhibition of phytase enzymes by fluoride. This enzyme is very important during germination and contributes to the degradation of phytate (also known as phytic acid). Phytate is widely recognized as a main source of stored energy for germinating seeds and a compound of phosphorus (up to 80% of total P in seeds). Baunthiyal et al. [48] also suggested that fluoride inhibits the carbohydrate metabolism during germination; hence, seeds are unable to grow. Yadu et al. [49] considered that it may be due to a reduction of amylase activity.

Plant root systems play a vital role in water and nutrient uptakes, and thus in the growth, physiology, and metabolism of all plants [50]. Root length measurement is considered a rapid phytotoxicity test method for chemicals such as fluoride [51]. Study of this indicator is also used to identify plant cultivars tolerant to many abiotic stresses [13,52,53]. The reduction of root length due to F toxicity has been reported in many crops such as wheat, rice, barley, rye, and various plant species (e.g., peas, cucumbers, radishes, tomatoes, and sunflowers) [25,31,38,40,41,46,54]. In the study by Sachan and Lal [55] on the root and shoot growth of chickpeas, an inhibitory effect of even 1.0 mmol·dm^{-3} of a NaF concentration was revealed. In our study, we also reported a decrease in root length, which was dependent on the cultivar. These results are analogous to the GI%; the highest reduction of root length in 10 mmol·dm^{-3} of NaF was observed for the Delawar (73%) and Arkadia (84%) cultivars and the lowest for the Tobak (43%) cultivar. We also reported some anomaly among the cultivars, for Tobak and Arkadia there was no GI% reduction observed, respectively at 5.0 - 8.0 mM and at 2.5 – 5.0 mM NaF. Crops cultivars of the same species differ in response to stress effects due to the morphological features of plants [55]. However, most often, fluoride reduces the root length in plants by up to 70%, compared with the control plants [38]. This root reduction may be related to the decrease in turgor pressure, which causes the inhibition of cell growth in roots. Some authors also support the view that fluoride induces alterations in seed metabolism, which results in the inhibition of root growth [38,49,56]. Moreover, the morphological and physiological characteristics of roots play a major role in shoot growth determination, as well as entire plant production [57].

5. Conclusions

The decrease in germination, inhibition of root growth, and inhibition of catalase activity in embryos and roots have been reported for all tested winter wheat cultivars. These effects have been strengthened with the increase in fluoride concentration. Changes in the peroxidase activity induced by NaF have been dependent on the winter wheat cultivar. The results of the η^2 analysis have shown that the NaF concentration has a greater effect on the activity of the determined antioxidant enzymes than the winter wheat cultivar.

Author Contributions: Conceptualization, J.P.; methodology, J.P. and M.Ś.; formal analysis, J.P. and M.Ś.; investigation, J.P., M.Ś., and A.T., writing—Original draft preparation, J.P., M.Ś., J.W., and A.T; writing—Review and editing, J.P., M.Ś., and A.T.; supervision, A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research and APC was funded by the Subsidy of Polish Ministry of Science and Higher Education for West Pomeranian University of Technology in Szczecin number 503-07-083-08/04.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cai, H.; Dong, Y.; Peng, C.; Li, Y.; Xu, W.; Li, D.; Wan, X. Fluoride-induced responses in the chlorophyll content and the antioxidant system in tea leaves (Camellia sinensis). *Fluoride* **2017**, *50*, 59–78.
2. Vithanage, M.; Bhattacharya, P. Fluoride in the environment: Sources, distribution and defluoridation. *Environ. Chem. Lett.* **2015**, *13*, 131–147. [CrossRef]
3. Cronin, S.J.; Manoharan, V.; Hedley, M.J.; Loganathan, P. Fluoride: A review of its fate, bioavailability, and risks of fluorosis in grazed-pasture systems in New Zealand. *N. Z. J. Agric. Res.* **2000**, *43*, 295–321. [CrossRef]
4. Gadi, B.R.; Pooja, V.; Ram, A. Influence of NaF on seed germination, membrane stability and some biochemical content in Vigna seedlings. *J. Chem. Biol. Phys. Sci.* **2012**, *2*, 1371–1378.
5. Petersen, P.E.; Lennon, M.A. Effective use of fluorides for the prevention of dental caries in the 21st century: The WHO approach. *Community Dent. Oral Epidemiol.* **2004**, *32*, 319–321. [CrossRef]
6. World Health Organization (WHO). *Fluoride in Drinking-Water. Background Document for Development of WHO Guidelines for Drinking-Water Quality*; WHO: Geneva, Switzerland, 2004; pp. 1–5.
7. Dutkiewicz, T. Forms of human exposure to environmental factors. In *Environment and Health*; Karski, J.B., Pawlak, J., Eds.; Center for Health Organization and Economics: Warsaw, Poland, 1995; pp. 107–113. (In Polish)
8. Gautam, R.; Bhardawaj, N. Bioaccumulation of fluoride in different plants parts of Hordeum vulgare (Barley) var. RD-2683 form irrigation water. *Fluoride* **2010**, *43*, 57–60.
9. Fujiwara, T.; O'Hagan, D. Successful fluorine-containing herbicide agrochemicals. *J. Fluor. Chem.* **2014**, *167*, 16–29. [CrossRef]
10. Lushchak, V.I.; Matviishyn, T.M.; Husak, V.V.; Storey, J.M.; Storey, K.B. Pesticide toxicity: A mechanistic approach. *EXCLI J.* **2018**, *17*, 1101–1136. [PubMed]
11. Kumar, T.S.; Dhakaand, K.P.; Singh, A. Effect of fluoride toxicity on the growth and yield of wheat (*Triticum aestivum* L.). *Int. J. Forest. Crop Impr.* **2013**, *4*, 59–62.
12. Choudhary, S.; Rani, M.; Devika, O.S.; Patra, A.; Singh, R.K.; Prasad, S.K.; Devika. Impact of fluoride on agriculture: A review on it's sources. toxicity in plants and mitigation strategies. *Int. J. Chem. Stud.* **2019**, *7*, 1675–1680.
13. Lethin, J.; Shakil, S.S.M.; Hassan, S.; Sirijovski, N.; Töpel, M.; Olsson, O.; Aronsson, H. Development and characterization of an EMS-mutagenized wheat population and identification of salt-tolerant wheat lines. *BMC Plant. Biol.* **2020**, *20*, 15–18. [CrossRef] [PubMed]
14. Kumar, P.; Yadava, R.K.; . Gollen, B.; Kumar, S.; Verma, R.K.; Yadav, S. Nutritional contents and medicinal properties of wheat: A review. *Life Sci. Med. Res.* **2011**, *22*, 1–10.
15. Giraldo, P.; Barzana, M.E.B.; Manzano-Agugliaro, F.; Giménez, E. Worldwide Research Trends on Wheat and Barley: A Bibliometric Comparative Analysis. *Agronomy* **2019**, *9*, 352. [CrossRef]
16. Filho, J.M. Seed vigor testing: An overview of the past, present and future perspective. *Sci. Agric.* **2015**, *72*, 363–374. [CrossRef]
17. Wolny, E.; Betekhtin, A.; Rojek-Jelonek, M.; Braszewska-Zalewska, A.; Lusinska, J.; Hasterok, R. Germination and the Early Stages of Seedling Development in Brachypodium distachyon. *Int. J. Mol. Sci.* **2018**, *19*, 2916. [CrossRef] [PubMed]
18. Lev, J.; Blahovec, J. Imbibition of wheat seeds: Application of image analysis. *Int. Agrophysics* **2017**, *31*, 475–481. [CrossRef]
19. Rathjen, J.R.; Strounina, E.V.; Mares, D. Water movement into dormant and non-dormant wheat (*Triticum aestivum* L.) grains. *J. Exp. Bot.* **2009**, *60*, 1619–1631. [CrossRef]
20. Ruttanaruangboworn, A.; Chanprasert, W.; Tobunluepop, P.; Onwimol, D. Effect of seed priming with different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice (*Oryza sativa* L.). *J. Integr. Agric.* **2017**, *16*, 605–613. [CrossRef]
21. Hite, D.; Auh, C.; Scandalios, J. Catalase activity and hydrogen peroxide levels are inversely correlated in maize scutella during seed germination. *Redox Rep.* **1999**, *4*, 29–34. [CrossRef]

22. El-Maarouf-Bouteau, H.; Bailly, C. Oxidative signaling in seed germination and dormancy. *Plant. Signal. Behav.* **2008**, *3*, 175–182. [CrossRef]
23. Śnioszek, M.; Telesiński, A.; Smolik, B.; Zakrzewska, H. Effect of Fluoride and Bentonite on Biochemical Aspects of Oxidative Stress in *Pisum sativum* L. *J. Ecol. Eng.* **2018**, *19*, 164–171. [CrossRef]
24. Cakmak, I.; Strbac, D.; Marschner, H. Activities of Hydrogen Peroxide-Scavenging Enzymes in Germinating Wheat Seeds. *J. Exp. Bot.* **1993**, *44*, 127–132. [CrossRef]
25. Pelc, J.; Smolik, B.; Krupa-Małkiewicz, M. Effect of Sodium Fluoride On Some Morphological And Physiological Parameters Of 10-Day-Old Seedlings Of Various Plant Species. *Folia Pomeranae Univ. Technol. Stetin. Agric. Aliment. Piscaria Zootech.* **2017**, *338*, 151–158. [CrossRef]
26. Barbero, P.; Beltrami, M.; Baudo, R.; Rossi, D. Assessment of Lake Orta sediments phytotoxicity after limiting treatment. *J. Limnol.* **2001**, *60*, 269–276. [CrossRef]
27. Lück, H. *Catalase*; Elsevier: Amsterdam, The Netherlands, 1965; pp. 885–894.
28. Chance, B.; Maehly, A. [136] Assay of catalases and peroxidases. *Methods Enzym.* **1955**, *2*, 764–775. [CrossRef]
29. Saleh, A.; Abdel-Kader, D.Z. Metabolic responses of two Helianthus annuus cultivars to different fluoride concentration during germination and seedling growth stager. *Egypt J. Biol.* **2003**, *5*, 43–54.
30. Elloumi, N.; Abdallah, F.B.; Mezghani, I.; Rhouma, A.; Boukhris, M. Effect of fluoride on almond seedlings in culture solution. *Fluoride* **2005**, *38*, 193–198.
31. Gupta, S.; Banerjee, S.; Mondal, S. Phytotoxicity of fluoride in the germination of paddy (*Oryza sativa* L.) and its effect on the physiology and biochemistry of germinated seedlings. *Fluoride* **2009**, *42*, 142–146.
32. Montagnolli, R.N.; Lopes, P.R.M.; Cruz, J.M.; Claro, E.M.T.; Quiterio, G.M.; Bidoia, E.D. The effects of fluoride based fire-fighting foams on soil microbiota activity and plant growth during natural attenuation of perfluorinated compounds. *Environ. Toxicol. Pharmacol.* **2017**, *50*, 119–127. [CrossRef]
33. Baunthiyal, M.; Ranghar, S. Physiological and biochemical responses of plants under fluoride stress: An overview. *Fluoride* **2014**, *47*, 287–293.
34. Finch-Savage, W.E.; Leubner-Metzger, G. Seed dormancy and the control of germination. *New Phytol.* **2006**, *171*, 501–523. [CrossRef] [PubMed]
35. Sharma, R.; Kaur, R. Insights into fluoride-induced oxidative stress and antioxidant defences in plants. *Acta Physiol. Plant.* **2018**, *40*, 181. [CrossRef]
36. Ghassemi-Golezani, K.; Farhangi-Abriz, S. Biochar alleviates fluoride toxicity and oxidative stress in safflower (*Carthamus tinctorius* L.) seedlings. *Chemosphere* **2019**, *223*, 406–415. [CrossRef] [PubMed]
37. Scandalios, J. Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res.* **2005**, *38*, 995–1014. [CrossRef]
38. Tak, Y.; Asthir, B. Fluoride-induced changes in the antioxidant defense system in two contrasting cultivars of *Triticum aestivum* L. *Fluoride* **2017**, *50*, 324–333.
39. Neri, F.; Kok, D.; Miller, M.A.; Smulevich, G. Fluoride Binding in Hemoproteins: The Importance of the Distal Cavity Structure†. *Biochemistry* **1997**, *36*, 8947–8953. [CrossRef]
40. Śnioszek, M.; Telesiński, A.; Biczak, R.; Płatkowski, M.; Pawłowska, B.; Wróbel, J. Comparison of the effects of soil treatment with NaF and KF on antioxidant enzymes in winter wheat (*Triticum aestivum* L.) seedlings. *Fluoride* **2019**, *52*, 199–208.
41. Mondal, N.K. Effect of fluoride on photosynthesis, growth and accumulation of four widely cultivated rice (*Oryza sativa* L.) varieties in India. *Ecotoxicol. Environ. Saf.* **2017**, *144*, 36–44. [CrossRef]
42. Rao, A.; Ahmad, S.D.; Sabir, S.M.; Awan, S.I.; Shah, A.H.; Abbas, S.R.; Shafique, S.; Khan, F.; Chaudhary, A. Potential Antioxidant Activities Improve Salt Tolerance in Ten Varieties of Wheat (*Triticum aestivum* L.). *Am. J. Plant. Sci.* **2013**, *4*, 69–76. [CrossRef]
43. Morohashi, Y. Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion. *J. Exp. Bot.* **2002**, *53*(374), 1643–1650. [CrossRef]
44. Saini, P.; Khan, S.; Baunthiyal, M.; Sharma, V. Effects of fluoride on germination, early growth and antioxidant enzyme activities of legume plant species Prosopis juliflora. *J. Environ. Biol.* **2013**, *34*, 205–209. [PubMed]
45. Alim, H.; Ahmad, M.A.; Munir, I.; Khan, I.; Mustafa, G.; Ullah, I.; Ahmad, M.N.; Khan, H.; Yasinzai, M.; Zia, A.; et al. The effect of different concentrations of the fluoride ion on the growth and nutritional value of two elite genotypes of *Triticum aestivum*. *Fluoride* **2017**, *50*, 143–150.
46. Bhargava, D.; Bhardwaj, N. Effect of sodium fluoride on seed germination and seedling growth of Triticum aestivum var. RAJ. 4083. *J. Phytol.* **2010**, *2*, 41–43.

47. Osuna, D.; Prieto, P.; Aguilar, M. Control of Seed Germination and Plant Development by Carbon and Nitrogen Availability. *Front. Plant. Sci.* **2015**, *6*, 6. [CrossRef]
48. Baunthiyal, M.; Bhatt, A.; Ranghar, S. Fluorides and its effects on plant metabolism. *Int. J. Agric. Technol.* **2014**, *10*, 1–27.
49. Yadu, B.; Chandrakar, V.; Kreshavkant, S. Responses of plants to fluoride: An overview of oxidative stress and defense mechanisms. *Fluoride* **2016**, *49*, 293–302.
50. Shen, Y.; Li, S.; Shao, M. Effects of Spatial Coupling Of Water And Fertilizer Applications On Root Growth Characteristics And Water Use Of Winter Wheat. *J. Plant. Nutr.* **2013**, *36*, 515–528. [CrossRef]
51. Zhao, B.Q.; Zhang, F.S.; Li, Z.J.; Li, F.C.; Shi, C.Y.; Zhang, J.; Zhang, X.C.; Shen, J.Q.; Pan, H.J.; Zhao, J.M.; et al. The vertical distribution and its change of root quantity and activity of the inter-planted winter wheat. *Plant Nutr. Fertil. Sci.* **2003**, *9*, 214–219.
52. Rout, G.R.; Samantaray, S.; Das, P. Differential chromium tolerance among eight mung bean cultivars grown in nutrient culture. *J. Plant Nutr.* **1997**, *20*, 473–483. [CrossRef]
53. Kim, Y.; Chung, Y.S.; Lee, E.; Tripathi, P.; Heo, S.; Kim, K.-H. Root Response to Drought Stress in Rice (*Oryza sativa* L.). *Int. J. Mol. Sci.* **2020**, *21*, 1513. [CrossRef]
54. Smolik, B.; Pelc, J. Efficacy of the use of biologically active substances to relieve the stress induced by sodium fluoride on the basis of morphological, biochemical and physiological parameters in spring wheat (*Triticum aestivum* L.) var. *Bryza. Agron. Sci.* **2017**, *62*, 27–35.
55. Sachan, P.; Lal, N. Effect of sodium fluoride on germination, seedling growth and photosynthetic pigments in *Cicer arietinum* L. and *Hordeum vulgare* L. *MOJ Ecol. Environ. Sci.* **2018**, *3*, 1. [CrossRef]
56. Singh, J.; Singh, D.; Chauhan, S.V.S. Effect of sodium fluoride on growth and yield in wheat (*Triticum aestivum*). *Ind. J. Agric. Sci.* **2001**, *71*, 41–43.
57. Ghosh, D.; Xu, J. Abiotic stress responses in plant roots: A proteomics perspective. *Front. Plant. Sci.* **2014**, *5*, 5. [CrossRef] [PubMed]

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