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ORIGINAL ARTICLE

Antioxidant defense activity under elevated fluoride levels during early seedling growth in wheat (*Triticum aestivum*L.).

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ABSTRACT
Activities of antioxidant enzymes and cell membrane integrity 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. Under elevated F activity of antioxidative enzyme superoxide dismutase increased but that of catalase decreased. Increased proline content in F treated seedlings indicated that such treatment caused stressful environment, however, increased malondialdehyde (MDH) content indicated that F toxicity had adverse effects on cell membrane integrity. It is inferred that decreased membrane integrity on account of derangement in antioxidant defense mechanism are the major factors for poor seed germination and seedling growth under F toxicity in wheat.
Keywords:*Antioxidants, fluoride, malondialdehyde, proline, wheat*

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INTRODUCTION

Fluorine is an anion and a member of the halogen family (reduced form). Rocks, water, and soil all contain the naturally occurring element fluoride (F). Many meal samples, including water, flora, and the atmosphere, all contain F^- (16). Fluoride impacts a number of physiological and biochemical processes in plants, affecting their growth and development and potentially having a significant detrimental influence on their economic productivity.The cereal crop wheat (*Triticum aestivum*) is vulnerable to high fluoride levels (23). Plant defence systems against oxidative stress, such as fluoride toxicity, are essential for this adaptation. As a result, it is critical to comprehend the antioxidant defence activity in wheat at high fluoride levels if you want to increase plant sturdiness and production. Including (26).

According to reports, the excessive fluoride accumulation in agricultural soils has a detrimental effect on wheat yield and growth. Fluoride is a toxic compound that may cause oxidative stress in plants by releasing reactive oxygen species (ROS). Increased ROS production has the potential to harm cells and interfere with metabolic processes, which would limit growth and lower crop production (2).Elevated fluoride levels triggered oxidative stress and damage to plant cells, according to increased lipid peroxidation and hydrogen peroxide concentration. Yet, in order to scavenge the excess reactive oxygen species, wheat seedlings activated their antioxidant defence system by elevating the activities of superoxide dismutase, peroxidase, and catalase (26).

Fluoride may cause oxidative stress and restrict plant growth by producing ROS such as superoxide anion ($O2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (^-OH) (26). Fluoride induced oxidative stress in wheat has been reported to lower the activity of antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), all of which are important for scavenging reactive oxygen species (ROS) (2).Several physiological responses have been linked to fluoride stress, including an increase in the production of reactive oxygen species (ROS), which destabilize plant metabolism and increase MDA, protein denaturation, and DNA damage in plants (24). Superoxide dismutase (SOD) and

catalase (CAT), two antioxidative enzymes, are part of an antioxidant system that regulates the level of ROS in live cells. It has been shown that proline, an amino acid and ROS scavenger, increases in response to diverse abiotic stresses (19).

Increased fluoride levels preserved greater proline content in two sunflower varieties (22). SOD was discovered to rise in response to rising NaF concentrations (14). Under fluoride stress, seedling germination, growth, and the membrane stability index were observed to decline. Osmolytes such proline were significantly enriched after sodium fluoride treatment (12). Wheat seedlings exposed to sodium fluoride accumulated higher phenolics, researchers revealed (18).

MATERIAL AND METHODS

The present research was carried out using the wheat variety HUW-234 (Malviya Wheat 234), during the rabi (winter season) of 2019-20 and 2020-21. HUW-234 is extensively cultivated in India's North Eastern Plain Zone. The Department of Genetics and Plant Breeding, Banaras Hindu University's Institute of Agricultural Sciences, in Varanasi, was where the seeds were purchased. The Department of Plant Physiology's Tissue Analysis Laboratory and Central Instrumentation Facility carried out biochemical investigations. The data collected during the course of the experiment's two years are displayed here. Standard statistical techniques were used to evaluate the data for the factorial complete randomized design.

Germination Paper Roll Test:

A piece of butter paper the same size as the germination paper (44 x 30 cm) was laid on top, leaving the bottom 4 cm of the germination paper accessible. Germination paper sheets were extensively saturated with fluoride solutions made by dissolving NaF at concentrations of 0 (T_1), 100 (T_2), 200 (T_3), or 300 (T_4) ppm. Control was a sheet that had been wet with distilled water. On germination paper sheets, seeds were uniformly distributed and positioned 15 cm above the bottom line. It was covered with a piece of saturated germination paper that was the same size. Over the germination paper, the butter paper was wrapped and folded within. Rolls were inserted into a 500 mL beaker filled with a fluoride solution at the same concentration such that the bottom portion of the roll that was not covered with butter paper remained submerged in the solution. Seeds were allowed to sprout at room temperature, and observations were made 3, 6, and 9 days following germination by unwrapping the sheets.

Biochemical Parameters:

(a) Superoxide dismutase (SOD)

The activity of superoxide dismutase enzyme was measured in seedling at 9 days after sowing according to the protocol given by (10).

(b) Catalase (CAT)

The activity of enzyme catalase was measured in seedling at 9 days after sowing. Enzyme was assayed according to the protocol given by (1).

(c) Malondialdehyde (MDA) content

Malondialdehyde (MDA) content was determined in seedling at 9 days after sowing. The level of peroxidation was determined as MDA content, according to the method of (15).

(e) Proline content

Proline content was determined in seedling at 9 days after sowing by the method described by (4).

RESULTS

Enzymatic antioxidants and biochemical

Superoxide dismutase activity (µmol min^{-1} mg^{-1} protein)

Superoxide dismutase activity increased as seedling growth progressed under all the treatments. On the 9th days after observation, superoxide dismutase activity increased in steadily as fluoride levels increased in the solution of germination paper (Fig. 1).

Catalase activity (µmol min^{-1} mg^{-1}protein)

At 9th day after of germination, the maximum catalase activity was found in seedling under T_1 (control). Catalase activity in seedling increased as seedling growth progressed under all treatments. At all stages catalase activity declined as fluoride content increased(Fig.1).

Malondialdehyde (MDA) content (µmol g^{-1} fresh weight)

The MDA content increased inall treatments as seedling growth progressed. The MDA content increased proportionally as fluoride levels increased. Seedlings treated with T_4 had the maximum MDA content (Fig.1).

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

The maximum proline concentration was recorded in leaves grown under T_4 treatment at 9th day after growth. Proline content increased as seedling growth advanced in all treatments. Proline content increased steadily as fluoride concentration increased (Fig.1).

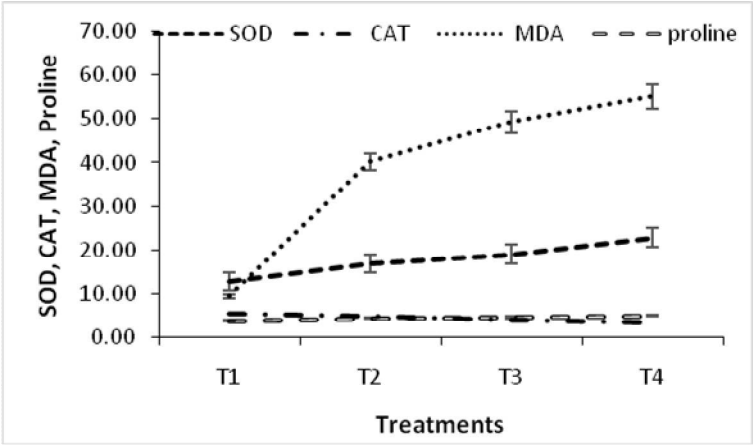

Fig. 1: Effect of different concentrations of fluoride on SOD (µmol min^{-1} mg^{-1} protein), CAT (µmol min^{-1} mg^{-1} protein), MDA (µmol g^{-1} fresh weight) and proline (µmol g^{-1} fresh weight) in wheat genotype HUW-234 at 9 days after sowing

DISCUSSION

Antioxidants are essential determinants that protect cells from the oxidative damage caused by free radicals. During seed germination and the early phases of seedling growth, several biotic and abiotic factors induce oxidative stress. Fluoride is one of the environmental pollutants that may induce oxidative stress in plants (5). Fluoride toxicity has an effect on plant growth and development by inhibiting growth and limiting seedling emergence. These frequent and more severe as the fluoride concentration is increased. Fluoride's deleterious influence on seed germination and initial seedling growth is due to fluoride accumulation in plant tissues, which causes oxidative stress and cellular damage (8).

Superoxide dismutase is an enzyme that degrades O_2 while maintaining the integrity of the cell membrane. Fluoride enhanced seedling SOD activity (Fig.1). Our findings were consistent with previous research that found a significant increase in SOD activity in rice fields when exogenous F (10-30 mg/L) was applied (6). This increased activity might be a positive feedback mechanism or an adaptive response to oxidative stress changes (7).

Cells were protected from the damaging effects of hydrogen peroxide by catalysing the dismutation of H_2O_2, which produced O_2 and H_2O. (13). Catalase is required for the elimination of H2O2 produced in peroxisomes by oxidases engaged in fatty acid oxidation, photorespiration, and purine catabolism. The presence of F reduced CAT activity. Since CAT scavenged H_2O_2 or encouraged the formation of water and oxygen, they are harmless (9). CAT activity was dramatically lowered when exposed to 300 ppm NaF. Our findings show that F suppresses CAT activity in wheat seedlings (Fig.1). Our findings validated the statements of numerous previous research that F inhibits CAT activity (24; 17,6, 7, 25).

Fluoride-stressed plants produced more reactive oxygen species, which resulted in membrane lipid peroxidation and consequent membrane damage. Fluoride toxicity stimulates the production of reactive oxygen species, which increases MDA levels (20). Mulberry genotypes with low MDA content have been shown to survive greater fluoride concentrations when subjected to fluoride stress (17). In the present study, higher fluoride levels resulted in higher MDA levels in wheat seedlings (Fig.1). Although though the levels of systems that scavenge reactive oxygen species were not tested in this study, it was anticipated that increased fluoride produced an increase in the quantity of reactive oxygen species, which in turn caused more damage to bio membranes and an increase in the content of MDA.

There have been studies of proline concentration changes while under F stress. Our results are consistent with studies by (6, 12, 3, 25), which demonstrated that greater fluoride concentrations in the root zone enhanced the amount of proline in wheat and other crops. Moreover, a stressful situation brought on by a rise in fluoride concentration in the root zone induces the plant to accumulate osmotic such soluble sugars and proline in order to maintain cellular water status (Fig.1).

CONCLUSION

Increased fluoride concentrations significantly affect wheat seed germination and early seedling development. Fluoride accumulates in plant tissues, causing oxidative stress and cellular damage, which is what causes the harmful consequences of fluoride toxicity. Plants need the antioxidant defence system to prevent oxidative damage brought on by fluoride toxicity. Although catalase activity decreased as fluoride levels in the solution increased, superoxide dismutase activity increased. Wheat seedlings exposed to high fluoride levels showed an increase in proline and malondialdehyde (MDA) content along with an increase in fluoride toxicity. The molecular mechanisms behind the antioxidant defence system in plants exposed to fluoride toxicity need more research.

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