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MECHANISTIC INSIGHTS INTO THE INTERACTION OF FLUORIDE RESISTANT BACTERIA WITH WHEAT ROOTS TOWARD ENHANCING PLANT PRODUCTIVITY BY ALLEVIATING FLUORIDE STRESS

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ABSTRACT: Wheat productivity is threatened by fluoride contamination in the environment. Fluoride's toxicological effects on plants have been hotly discussed due to lower growth indices, metabolic inhibition, and reduced photosynthetic activity. Fluoride toxicity was reduced in this study by using fluoride-resistant bacterial strains (SS-10a and SS-5a) in the soil. Plants of wheat were treated to varying sodium fluoride (NaF) concentrations of 0 (control), 150, 250, and 350 ppm in a randomized full block design. At 7DAT (Day After Transplantation), fluoride-resistant bacteria were inoculated into the soil of two wheat types, Ujala-15 and Faisalabad 2008. Fluoride stress reduced all growth, biomass, yield, and biochemical parameters significantly, although fluoride-resistant bacteria assisted the plants in overcoming this stress and mitigating the total plant productivity loss. In cellular enzymatic extract from bacterial strain infected plants, total chlorophyll content, carotenoid content, and total soluble proteins all increased. SS-10a and SS-5a inoculated plants demonstrated an increase in ascorbate peroxidase (APX) and catalase (CAT) activity at a low level of NaF, 150 ppm. CAT and APX activity were elevated in plants treated with SS-10a and SS-5a to protect plant cells against oxidative stress at high fluoride levels (250 and 350 ppm). Plant malondialdehyde (MDA) content was also much higher in plants inoculated with bacterial strains than in treated plants, indicating that MDA plays an important role in neutralizing ROS. In both kinds, the efficacy of SS-5a in decreasing fluoride stress was much lower than that of SS-10a. Inoculation with fluoride-resistant bacterial strains can thus be a viable and practicable strategy to decrease fluoride stress under current pollution from an economic and agricultural standpoint.

Key words*:* Antioxidant enzyme, Fluoride toxicity, Oxidative stress, Wheat productivity

INTRODUCTION

The top priority of commercial producers, farmers, and plant breeders is to maximize the production of economically significant crops such as wheat. Wheat is the most significant cereal, accounting for around 20% of total calories and more than 25% of total protein. However, the increase in wheat productivity over the last decade has not been adequate to fulfil future demand, and extreme abiotic stress events would reduce wheat production by 20–30% [1]. Fluoride is naturally generated when rocks and volcanic ash weather [2]. Fluoride exposure over a long length of time, above the allowed limit of 1.5 mg/L, has severe implications for both plants and animals. The most major inorganic fluorides that have had a negative impact on the

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environment are sodium fluoride, hydrogen fluoride, sulphur hexafluoride, calcium fluoride, and silicofluoride [3].

Fluorosis, one of the world's main public health concerns, can be caused by an increase in fluoride levels [4]. Fluoride pollution in the environment, both natural and man-made, has two basic characteristics. The first is crop damage caused by producing distinctive necrotic lesions and tip burning of leaves in plants sensitive to it [5], [6]. and the second is fluoride accumulation within the plant body larger than 50 ppm, which affects yield and productivity [7]. Fluoride dosages of 100–200 ppm were found to be detrimental to biochemical and physiological markers such as chlorophyll, carotenoids, total soluble proteins, catalase, and ascorbate peroxidase [8], [9]. Fluoride is a potent oxidant that causes oxidative changes in plant cells. It has the ability to increase the amount of reactive oxygen species (ROS) in the body while blocking antioxidant enzyme function. Catalase (CAT) and peroxidases (POX) are efficient free radical scavengers. The activity of CAT and POX has been discovered to be significant in controlling H_2O_2 levels in embryos during seed germination [10].

Several conventional strategies, such as ion exchange, excavation, and landfilling, have been developed to mitigate fluoride toxicity [11]. Conventional remediation procedures are costly and harmful to biological pathways. Bioremediation via plant growth promoting rhizospheric bacteria has proven to be the most desirable and convincing way for soil defluorination among all documented techniques [12]. The rhizosphere is a diverse ecosystem that contains a variety of Plant Growth-Promoting Microorganisms (PGPM). Plants' release of root exudates is critical for microbial colonization in the rhizosphere [13]. The co-inoculation of Rhizobia and Azospirilla significantly reduced the negative effects of abiotic stressors on root development and nodulation, resulting in increased productivity of wheat, mung bean, and maize. The physiological mechanism underlying stress reduction remains unknown [14]. The use of the plant growth encouraging bacterium strain *Pseudomonas fluorescence* increased the pea plant's ability to withstand fluoride stress [15]. A similar investigation was carried out on tomato plants using *Archromobacteri piechaudii*, which enhanced plant biomass by 40% when compared to 172 mM NaCl stress [16].

The goal of this study was to see how varying NaF concentrations altered antioxidant enzyme activity as well as morpho-physiological activities in two wheat cultivars during germination and development. It will also look at the effect of fluoride-resistant microorganisms on wheat physiology and productivity by reducing fluoride toxicity.

MATERIALS AND METHODS

Experimental Site: The experiment was carried out at the Botanical Garden's wire house at the University of the Punjab in Lahore. Certified seeds of two wheat types, Faisalabad-2008 and Ujala-15, were obtained from the University of the Punjab's Seed Center. They were arranged in accordance with size and health equality. Any diseased seeds (grains) were carefully discarded.

Bacterial Strains: Fluoride resistant bacterial strains were isolated by repeatedly exposing them to increasing fluoride dosages. Fluoride-resistant bacterial strains (SS-10a and SS-5a) were revived in LB-agar media with increasing concentrations of Fluoride. In LB-broth culture media, an inoculum was created [17].

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Stock solutions of Sodium Fluoride: Stock solution of sodium fluoride (NaF) was prepared by dissolving 0.45g of NaF in 1000ml of dist. water in a round bottom flask. The solutions of NaF (150,250,350 ppm) were prepared according to dilution law, in which calculated volume was taken from the stock and diluted in distilled water to get the desired concentration. Sodium fluoride was applied as soil drench once in a week during experiment. First treatment of sodium fluoride was given at 15(DAT) on 1st Jan- 2021. Pots were irrigated with measured amount of NaF (150,250,350) ppm. Tap water was supplied at regular intervals to minimize dehydration.

Determination of Plant Pigments: The quantification of plant pigments was done according to standard protocol of Wellburn & Lichtenthaler [18]. Dimethylsulphoxide (DMSO) buffer was used as an extraction buffer. A mass of 0.5 g of leaf was used for extraction. The extract was centrifuged at 14000 rpm for 20 min. By spectrophotometer the absorbance was taken at 480, 645 and 663 nm respectively. The chlorophyll *a*, *b*, total chlorophyll content and carotenoids were measured.

Assessments of Total Soluble Proteins: Bradford method was used for the estimation of total protein content. The plant enzymatic extract was prepared by using liquid nitrogen. Further the extract was centrifuged at 12000 rpm. The comassive blue was added in supernatant obtained after centrifugation. Later on the absorbance was measured at 595 nm. The value of Absorbance was substituted in total soluble protein standard curve [19].

Evaluation of Malondidaldehyde: For the determination of Malondidaldehyde (MDA), 1.0 ml of enzyme solution was dissolved in 2.0 ml of reaction solution. The reaction solution was prepared by dissolving an appropriate amount of thioburbituric acid and thiochloroacetic acid in a deionized water. The test tubes were heated in a water bath at 95 ^{0}C for 30 min, after this the tubes were quickly collected at ice bath. The extract was centrifuged at 12,000 rpm for 10 min. the supernatant was shifted to another tube. Absorbance of the sample was measured at 532 nm and 600 nm [20].

Assessment of Catalase Activity (CAT): Catalase activity was evaluated on the basis of H_2O_2 decomposition rate at 240nm once 10-20 sec in 60-80 sec. 25 µl enzyme extract was added in 3 ml of reaction mixture having 50 mM PBS (pH 7.4) and 15 mM H_2O_2 [21].

Assessment of Ascorbate Peroxidase (APX): For the measurement of APX activity 0.55 mg of fresh leaf was dipped in 3 ml of reaction solution for the enzyme extraction. The reaction solution was composed of Hac-NaAc, EDTA, H_2O_2 and ASA. The absorbance was noted at A290 once each 10 seconds for 60 to 80 seconds. UV cuvettes were used [22].

Statistical Analysis: Experiment was designed by Design- Expert Software-11. Data collected from experiment was analyzed by using one-way ANOVA followed by Bonferroni post-hoc multiple comparison test by using Graph pad prism.

RESULTS AND DISCUSSION

High level of fluoride stress, especially 350 ppm, significantly decreased the growth parameters in both wheat varieties. The present experimental findings

were in conformity with the studies of Singh et al. [23], Panda and Cai et al. [6], Zafar-Ul-Hye et al. [24], conducted on *Raphanus sativus* L., *Abelmoschus esculentus* L. *Camellia sinensis* and spinach leaves. According to a study, fluoride enters in cell in the form of fluoride ion, which directly interferes with photosynthesis and respiration by inhibiting important ATP generating enzymes. This ultimately reduces energy currency and sugar transport, causes reduction in growth of plant [25], [26].

Maximum values of growth parameters were recorded in SS-10a inoculated plants under 350 ppm of fluoride (NaF) stress in both wheat varieties. In var. Ujala-15, the maximum plant height 70.1 cm, leaf area 65 cm^2 and number of tillers 18 were recorded. In var. Faisalabad 2008 maximum plant height 62 cm, leaf area 61 cm^2 and number of tillers 16 were recorded. Efficiency of SS-5a was significantly less as compared to SS-10a. Results were in consensus with study that assessed the defluorination ability of fluoride resistant *Actinobactor* sp. RH5 in soil [27]. Reduction of fluoride stress in wheat may attributed to sequestration of fluoride inside bacterial cells. This prevent the accumulation of fluoride inside the plant body and also decreases the bioavailability [28], [29]. Ujala-15 variety was genetically tolerant to salt stress so that is the reason it showed best performance under NaF stress [30].

SS-10a inoculated plants showed a significant increase in yield parameters and biomass under 350 ppm of fluoride stress as compared to control (350 ppm). In var. Ujala-15 maximum spike length 26 cm, grains per spike 56, 1000 grain weight 44 g, shoot fresh weight 30.21 g, root fresh weight 10.01 g, shoot dry weight 6.03 g and root dry weight 5.21 g were recorded. In var. Faisalabad 2008, maximum spike length 22 cm, grains per spike 48 and 1000 grain wt. 42 g, shoot fresh weight 29.34 g, root fresh weight 9.87 g, shoot dry weight 5.93 g, root dry weight 5.11 g were recorded. Role of resistant bacteria in enhancement of yield was scantly reported in previous literature. Results Fig. 1 (a &b) and Fig. 2 (a & b) were supported by the findings of Singh et al. [31].

The maximum values of total chlorophyll, carotenoids and total soluble protein content were recorded in SS-10a inoculated plants under 350ppm in both varieties and minimum values were recorded in control under 350 ppm. Results showed consensus with the previous studies that had been conducted on several plants like *Triticum aestivum* [31] and *Withiana somnifera* [32] by inoculating zinc resistant and heavy metal resistant bacteria. The reduction in chlorophyll content in control under fluoride stress is attributed to degradation resulting from fluoride, as fluoride has great potential for replacement of heavy metals. Carotenoid reduction was also due to disruption in their structure [33]. High fluoride stress disrupts the proteinaceous structures and soluble proteins, hence decreases the total soluble protein content. Results regarding present study (Fig. 1(a&b), Fig.3 (a&b), Fig4 (a &b) and Fig 5(a & b) were supported in an experimental study conducted on wheat with resistant rhizospheric bacteria [34] and [35].

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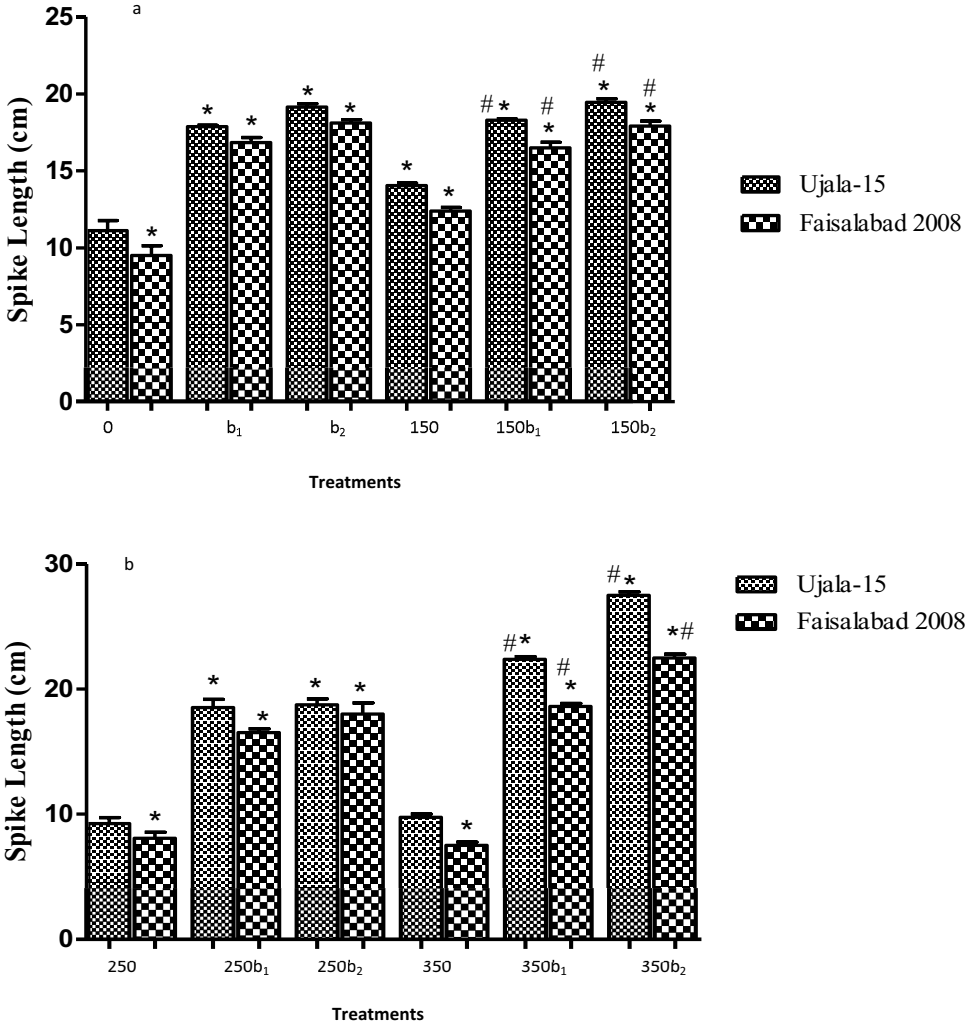

Fig. 1 (a & b): Effect of different treatment levels on spike length. Values are mean±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control (0) and (250), # indicates $p < 0.05$ with respect to control (150 and 350), (b_1=SS5a and b_2=SS10a).

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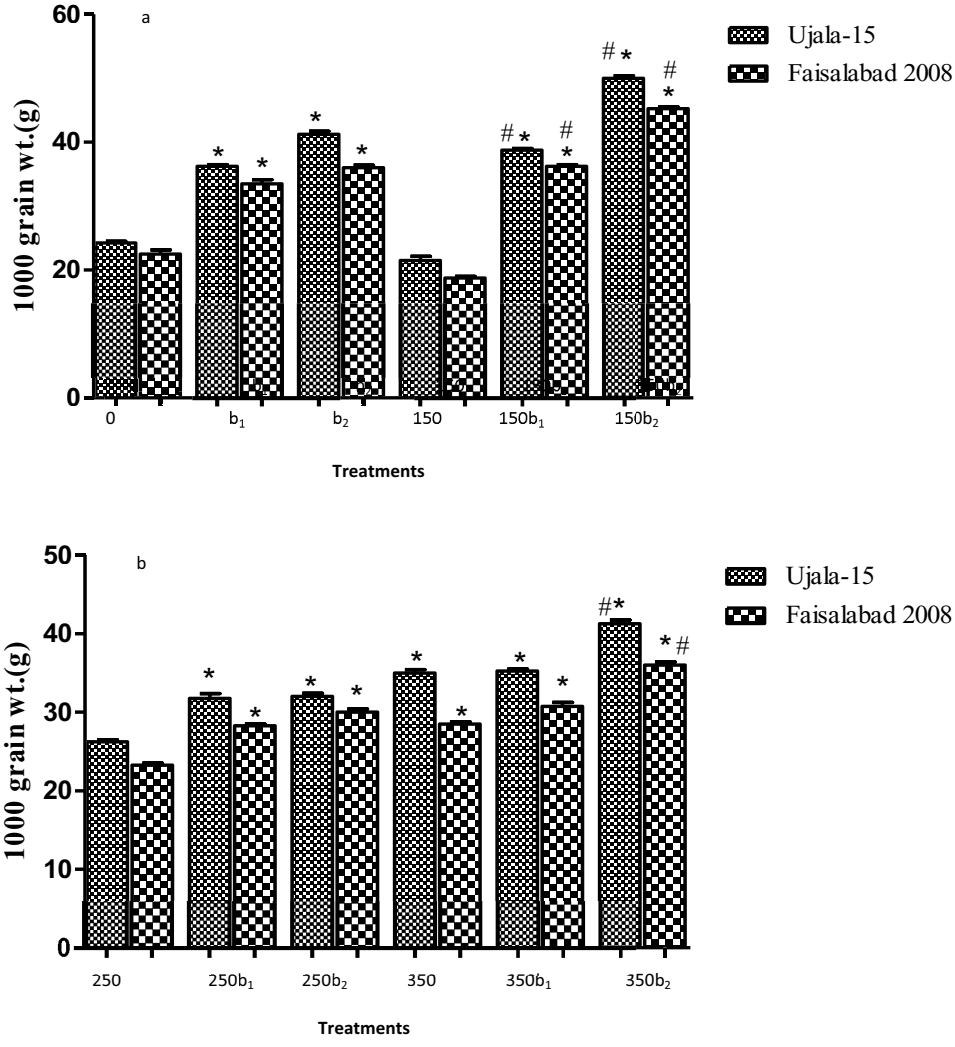

Fig. 2 (a & b): Effect of different treatment levels on 1000 grain weight. Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control (0) and (250), # indicates $p < 0.05$ with respect to control (150 and 350), (b_1=SS5a and b_2=SS10a).

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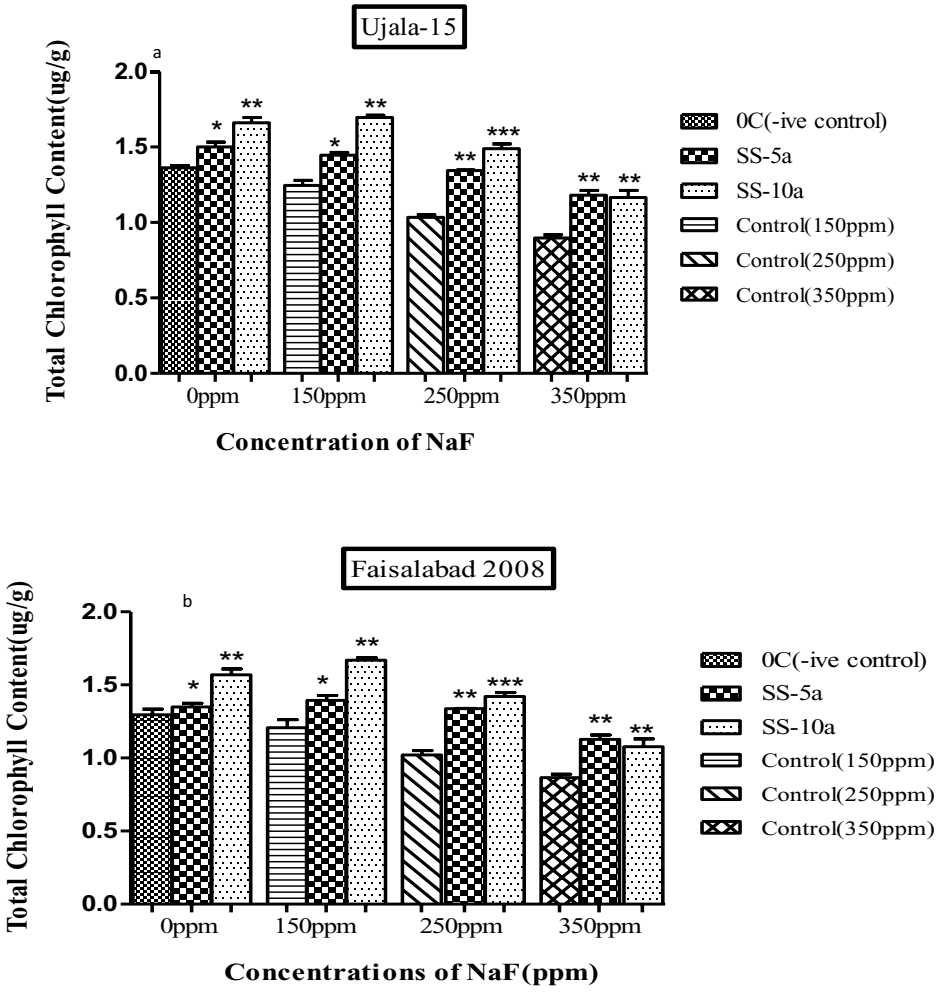

Fig. 3 (a & b): Effect of different treatment levels on the total chlorophyll content. Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control indicates $p < 0.05$.

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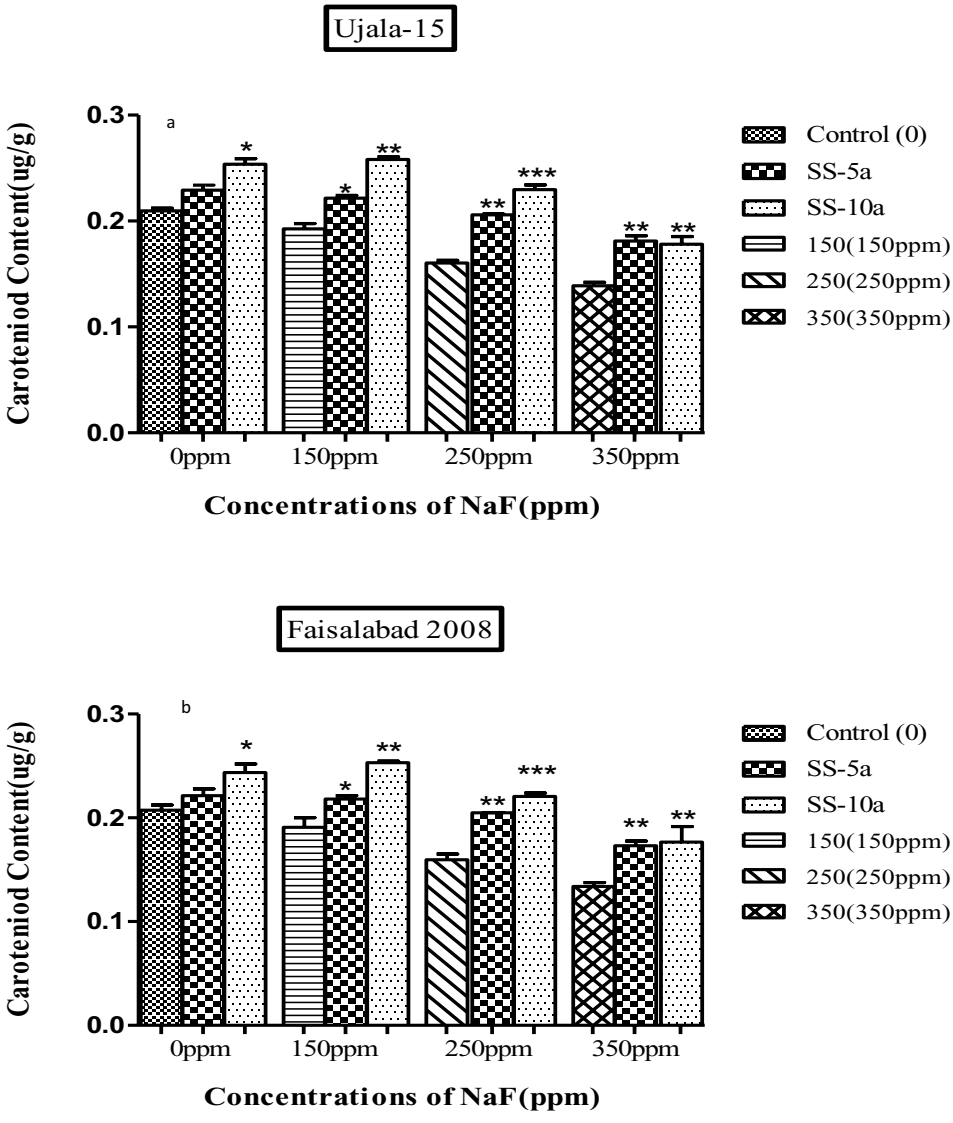

Fig. 4 (a & b): Effect of different treatment levels on the carotenoid content. Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control indicates $p < 0.05$.

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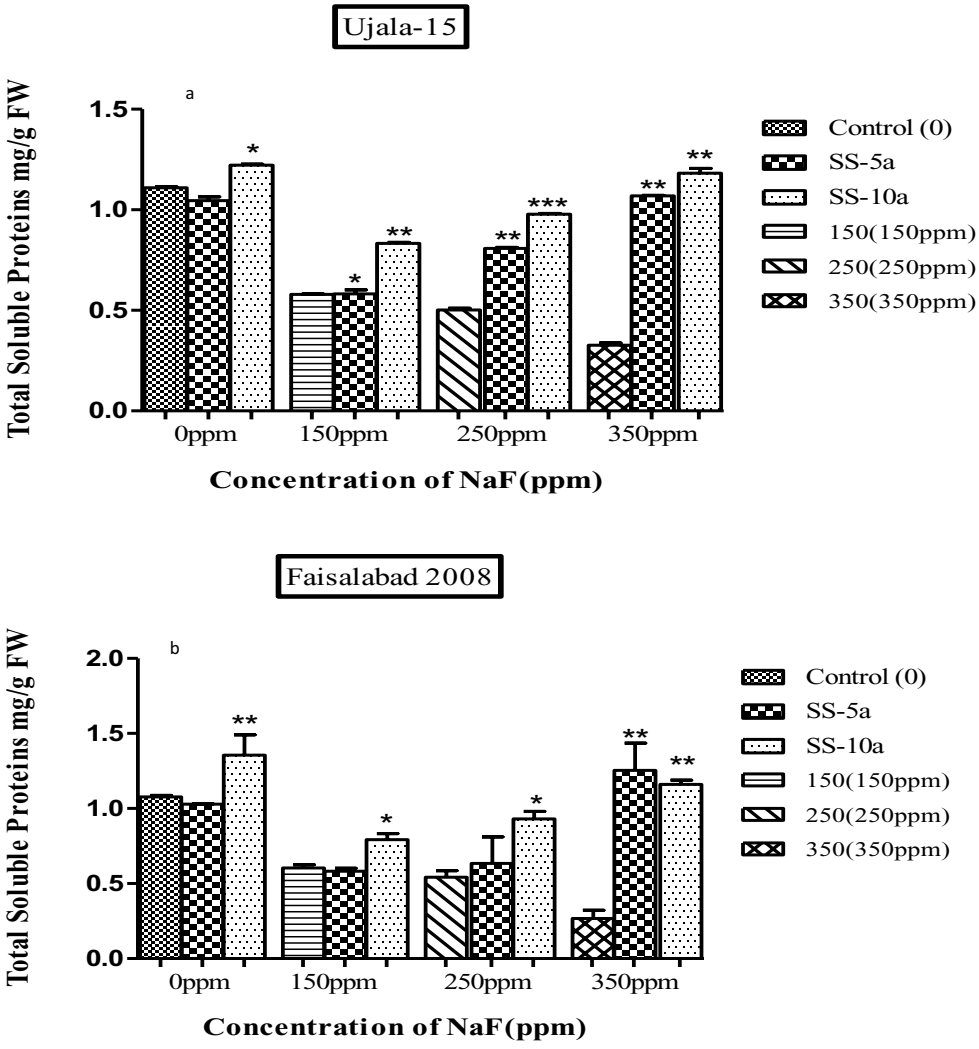

Fig. 5 (a & b): Effect of different treatment levels on the total soluble proteins. Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control indicates $p < 0.05$.

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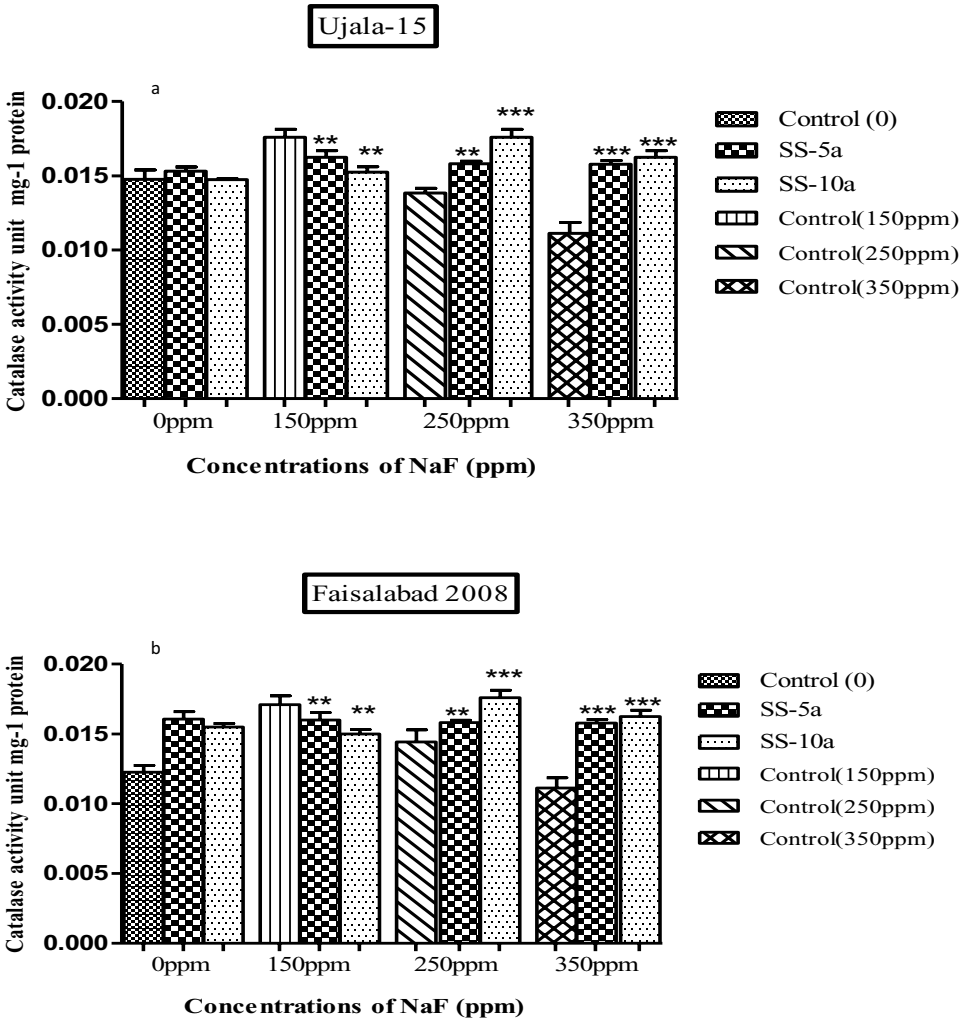

Fig. 6 (a & b): Effect of different treatment levels at CAT (catalase activity). Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control.

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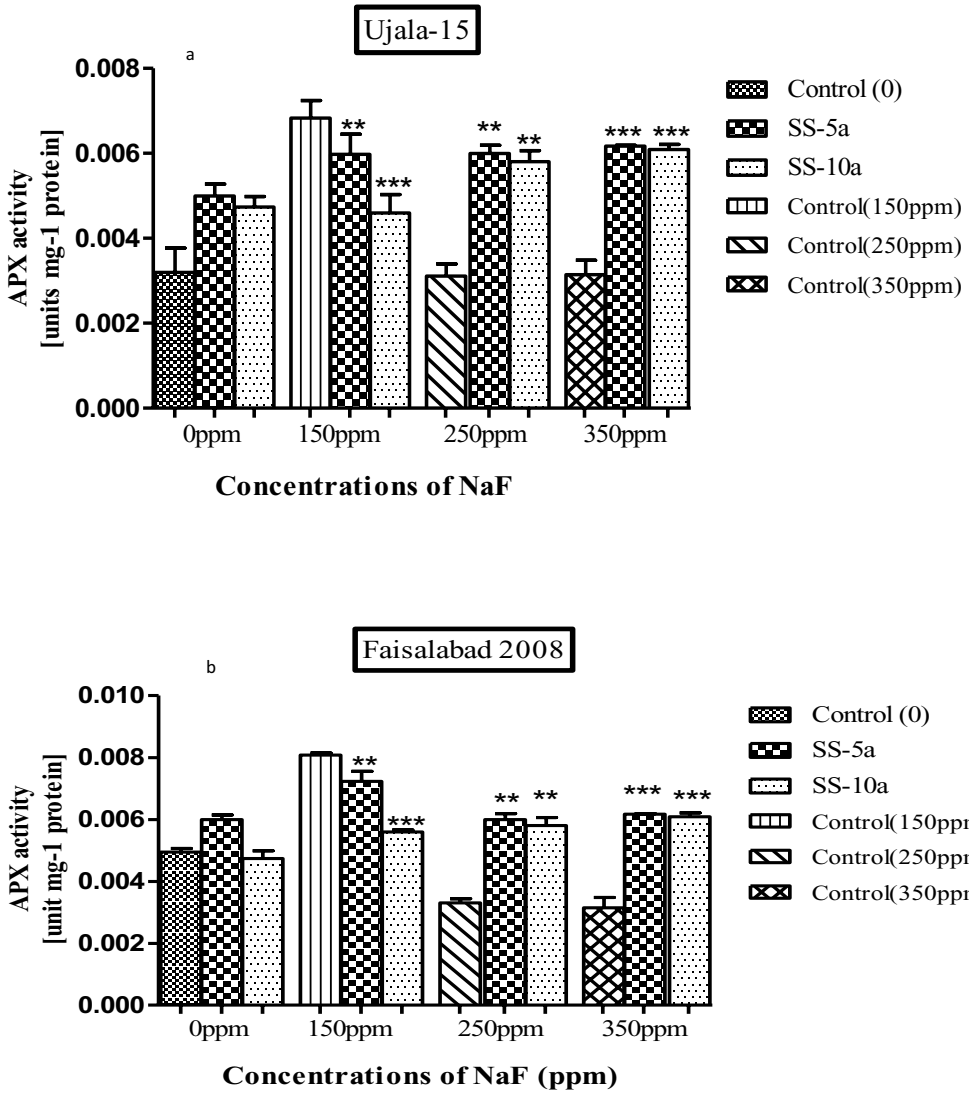

Fig. 7 (a & b): Effect of different treatment levels at APX. Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control.

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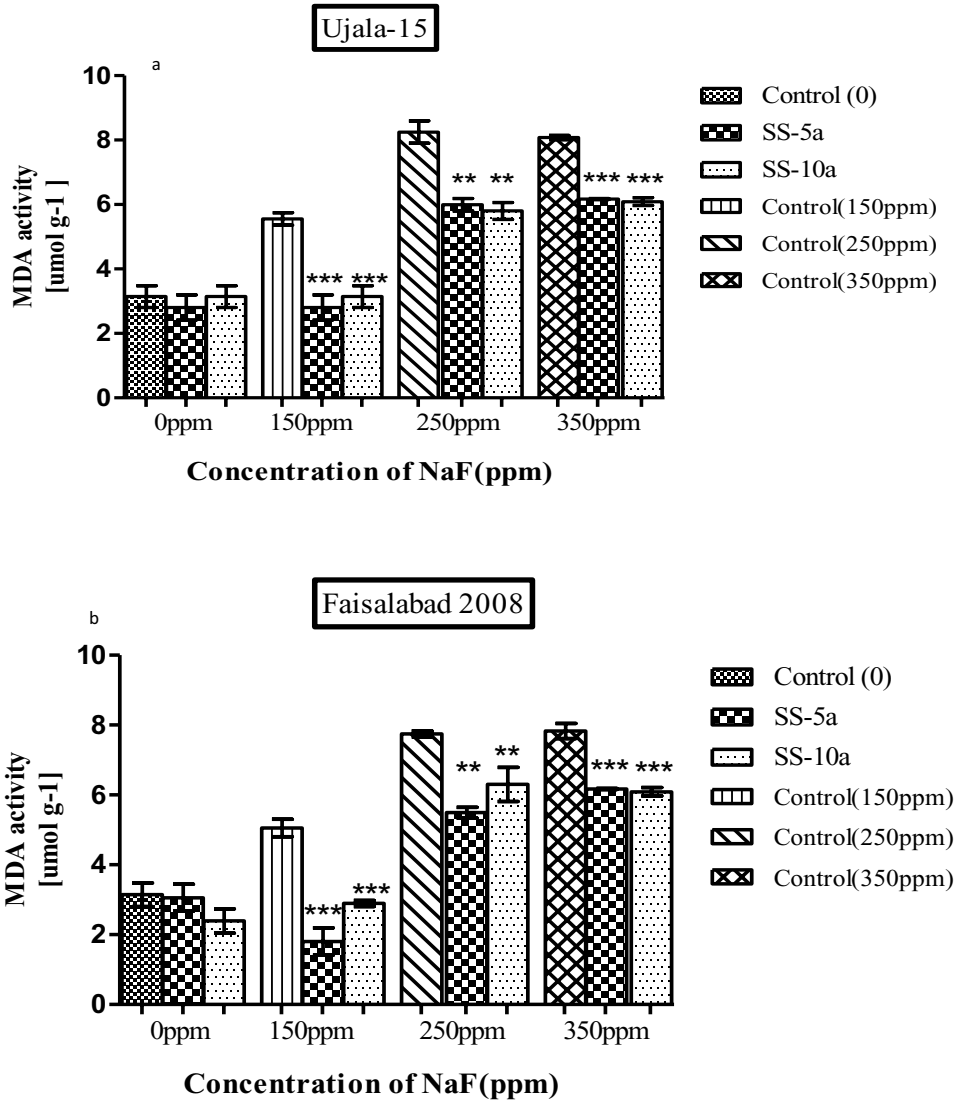

Fig. 8 (a & b): Effect of different treatment levels at MDA. Values are mean ±SEM (n=4). The results are considered significant (*) if $p < 0.05$. (*) indicates $p < 0.05$ with respect to control.

A significant reduction in CAT and APX activity was noticed under 350 ppm of fluoride. Present experimental results were supported by Singh *et al*., [31]. High level of fluoride disrupt the globular structure of enzymatic proteins like CAT and APX. This will lead to reduction in antioxidant enzymes. Ultimately ROS surpass the normal limit, and injury symptoms appear in the form of tip burning [35], [36]. SS-10a inoculated plants under low fluoride doses 150 and 250 ppm showed a gradual increase in CAT and APX activity while at 350 ppm a significant reduction was observed in both varieties. Maximum efficiency of SS-10a was recorded at 350 ppm. As high fluoride activates the fluoride resistant genes that increase the fluoride sequestration inside bacterial cell and prevent accumulation inside plant body results in significant reduction of oxidative stress (Fig. 6 a &b and Fig 7 a & b).

It was observed that increase in fluoride stress gradually increased the MDA content. Maximum value of MDA was recorded in enzymatic extract of 350 ppm stressed plants in both varieties. Previous studies reported the similar findings in leaves of Tea (*Camellia sinensis*) [37] and in *Oryza sativa* [38]. The increase in MDA content mainly due to degradation of lipids by reactive oxygen species. Under stress, plant produce ROS that cause lipid peroxidation and releases MDA as a by-product. MDA content is the quantification of damage by stress [38]. Fluoride resistant bacterial strains SS-5a and SS-10a significantly reduced the MDA content. No significant difference was observed in MDA content of (Fig.8 a&b) SS-5a and SS-10a inoculated plants.

CONCLUSIONS

All of the wheat cultivars studied in this study had lower germination and plant growth inhibition. Bioremediation using plant-growth-promoting rhizospheric bacteria has found to be the most effective of all the methods studied. The goal of this research was to lessen fluoride toxicity in the soil by introducing fluoride-resistant bacterial strains (SS-10a and SS-5a). Plant growth promoting bacteria (PGPB) that are fluoride-tolerant assist the plant in surviving stress. Plants inoculated with bacterial strains had considerably greater antioxidant levels (CAT, APX, and MDA) than fluoride-treated plants, which play a key role in neutralising ROS. As a result, inoculating fluoride-resistant bacterial strains can be a viable and realistic strategy for relieving fluoride stress under current pollution from an economic and agricultural standpoint.

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