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FLUORIDE RESISTANT BACTERIA ALLEVIATE FLUORIDE STRESS IN *TRITICUM AESTIVUM* L. THROUGH MODULATING GAS EXCHANGE CHARACTERISTICS AND ENHANCED PLANT GROWTH

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ABSTRACT: The aim of the work was to determine the role of bacteria to mitigate the effect of fluoride on the growth, gas exchange characteristics, and productivity of the two wheat varieties (Galaxy-13, Chakwal-50). Experiment was conducted in the Botanical Garden (University of the Punjab, Lahore Pakistan) according to the Design Expert software 11. Four concentrations of NaF (0 ppm, 150 ppm, 250 ppm, and 350 ppm) and three bacterial strains (ss5a, ss5b, and ss10a) were used. Growth parameters, gas exchange characteristics, fresh and dry weight of root and shoot, and productivity were measured. The results observed were less harmful in case of plants treated in combination with NaF+bacteria as compared to plants treated with NaF alone. Fluoride affected various parameters, chlorosis, leaf tip burn, leaf necrosis, growth, and productivity of the wheat plants. Galaxy-13 was least influenced by F compared to the Chakwal-50 variety. It was concluded that growth was increased with the application of beneficial fluoride resistant bacteria and proved helpful in minimizing the harmful effects of fluoride salt on wheat varieties.

Keywords: Fluoride resistant bacteria; Gas exchange characteristics; Leaf necrosis; Productivity.

INTRODUCTION

Now-a-days, the whole world is facing fluoride contamination problem.[1] It is estimated that 350 ppm of fluorine roughly present in soil, in some soil 1000 ppm while in disrupted soil 3500 ppm.[2] Phosphate from fertilizers and contaminated smoke from brick kilns and industries of ceramic enhances the F concentration in agricultural water.[3] Fluoride absorbance in soil is affected by pH and it ranges from 5.5 – 6.5. In acidic soil, F can diffuse easily and taken up by plant roots and if highly absorbed by the plants it was phytotoxic.[4] It caused necrosis at the plant leaf margins and tips, leaf notching, leaf falling, and chlorosis which leads to the reduction in the plant growth and yield which ultimately reduces the economic value of plant.[5] Photosynthesis, transpiration rate, and other processes were strongly inhibited by fluoride.[6] Fluoride response depends upon dose, duration of exposure, age, and genotypes of plants.[7] Fluoride absorbed by plants from air, soil, and water with various means of uptake. Its uptake in gaseous forms by leaves is relatively high due to its high solubility and high accumulation in leaves than other plant parts. In roots, it absorbed by passive diffusion from soil solution and then into the xylem by the apoplastic and symplastic pathways through the shoots in unidirectional flow.[8] In wheat, fluoride acts as an accumulative poison in foliage.[9] It absorbed through the roots or stomata, moves in the transpiration stream and accumulated gradually in the leaf margins.[10] Seed germination and early seedlings growth are the important phases for the successful growth and survival of plants and these physiological parameters are affected by F stress.[11] Fluoride stress caused deficiency of nutrient uptake,

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reduction in plant biomass, and enzymatic activities,[12] and increased the catalase, peroxidase, and super oxidase among the wheat varieties.[13]

Bacteria have evolved mechanisms for metal resistance and salt toxicity.[14] Fluoride resistance bacteria, *Bacillus flexus,* are available as bio-absorption of F. Plant growth-promoting bacteria prevent the toxicity of fluoride to plants and promote growth and development by nitrogen fixation, siderophores, production of phytohormones, and mineral solubilization such as phosphorus.[15] Fluoride retained in soil for long duration exerts negative effect on soil microorganisms.[16]

MATERIALS AND METHODS

Design of the experiment: Design expert software 11 was used to design the experiment. Seeds of two wheat varieties (Galaxy-13, Chakwal-50) were collected from the seed center of the University of the Punjab, Lahore, Pakistan. Experiment was conducted in November 2018 in the Botanical Garden of the University of the Punjab, Lahore, Pakistan. Four different concentrations of fluoride, viz., 0 (control), 150, 250, and 350 ppm F were evaluated on seed germination, growth, productivity, and gas exchange characteristics of two wheat varieties (Galaxy-13, Chakwal-50) in the presence of fluoride resistant bacteria given as a soil drench. Bacterial treatment was given to wheat varieties to prepare the plants to bear the stress of sodium fluoride. Three bacterial strains were used such as, SS5a, SS5b, and SS10a which can bear 500 ppm of fluoride stress Fluoride was applied as a solution of NaF. All the experimental wheat seeds of the respective varieties were surface sterilized. Seeds were sown in thoroughly cleaned plastic pots filled with 1 kg soil. Stock solution was prepared by dissolving required amount of NaF in distilled water to get 1000 mL fluoride solution. Appropriate dilutions were made to prepare 150, 250 and 350 ppm NaF concentrations. Control experiments were also set up by using only tap water. Each treatment was replicated thrice following complete block. Same field conditions were given during the entire experimental period. All the growth parameters gas exchange characteristics, biomass assessment, and productivity were recorded during the experimental period.

Plate 1. Experimental site and growth stages of wheat varieties

Determination of growth parameters: Plant roots were detached carefully from each treatment and washed gently in tap water. Then plants were placed carefully in labelled polythene bags and carried to the laboratory for measuring the vegetative growth parameters,i.e., Root Length (cm), Shoot Length (cm), Leaf length (cm), Leaf area (cm^2), and Leaf numbers.

Biomass assessment: Biomass assessment was carried out after 70 days. Fresh weight of shoot and root was taken in grams on electronic weighing balance. Samples were put in electric oven for measuring dry weight parameters, (Wiseven, Model WOF-105, Korea) at 70ºC for 72 hours. Then after 72 hours their dry weight of shoot and root were measured.

Determination of gas exchange characteristics: Gas exchange characteristics of leaf, i.e., photosynthetic rate (*p*n), stomatal conductance (*g*s), internal concentration of CO_2(*C*i), projected leaf area, CO_2 flowing from leaf chamber (C' an), 'reference' CO_2 into leaf chamber (Cref), and rate of transpiration (E) were measured by using a portable IRGA (infra-red gas analyzer) machine of model "LCA 4 ACD." Measurements took place between 10:00–11:30 am, while leaf temperature within the chamber was adjusted to 36±2ºC, and photosynthetic photon flux density was 1200 µmol/m^2/sec at the ambient CO_2 concentration. All gas-exchange measurements started 4 hours after the onset of the photoperiod and were replicated in 4 plants per treatment and on two fully expanded, healthy, sun-exposed leaves per plant.

Yield parameters: Data for different yield parameters (Plant length, Number of spikelets, Size of spikelet, Grains per spikelet, and Grains weight were noted at the time of harvest.

Statistical analysis: For statistical analysis a software IBMSPSS was used.

RESULTS AND DISCUSSION

Germination study: Germination percentage was checked after 7 days of seed sowing. Germination percentage of replicates of both wheat varieties (Galaxy-13 and Chakwal-50) was random. Bacteria categorized in *Synergistetes phylum* (fluoride resistant bacteria) was reported which reduced F effect on crop.[17] Study results indicate that both varieties showed decrease of germination percentage with increase in the concentration of F solution. It was studied that fluoride slowed down the enzymes activity which affected the germination process.[12] Percent reduction of germination was recorded for concentration (150 ppm, 250 ppm, and 350ppm) with respect to control for both varieties. Percentage of replicates of both wheat varieties (Galaxy-13 and Chakwal-50) was random. In Galaxy-13 variety germination percentage was greater as compared to the Chakwal-50. However, at higher concentrations, i.e., 250 ppm and 350 ppm, percent reduction ranged from 26% to 59%. Almost similar observation was reported by Datta et al. in 2012 and Kamaluddin and Zwiazek in 2003.[18,19] Seed germination is an energy (ATP) dependent process and during germination many enzymes are critically involved in transforming complex seed storage into simpler forms and translocating them to the growing region of seedlings.[20] Therefore, higher level of F may interfere in enzymatic activity and liberation of energy which eventually accounted for the death of seed tissues and inhibition of seed germination. Almost comparable results were

reported in case of different crops and vegetables such as wheat (*Triticum aestivum* L.), Bengal gram (*Cicer arietinum* L.), mustard (*Brassica juncea* L.) and tomato (*Lycopersicon esculentum* L.), where there was steady decrease in the growth of roots and shoots with increased concentration of NaF.[21,22]

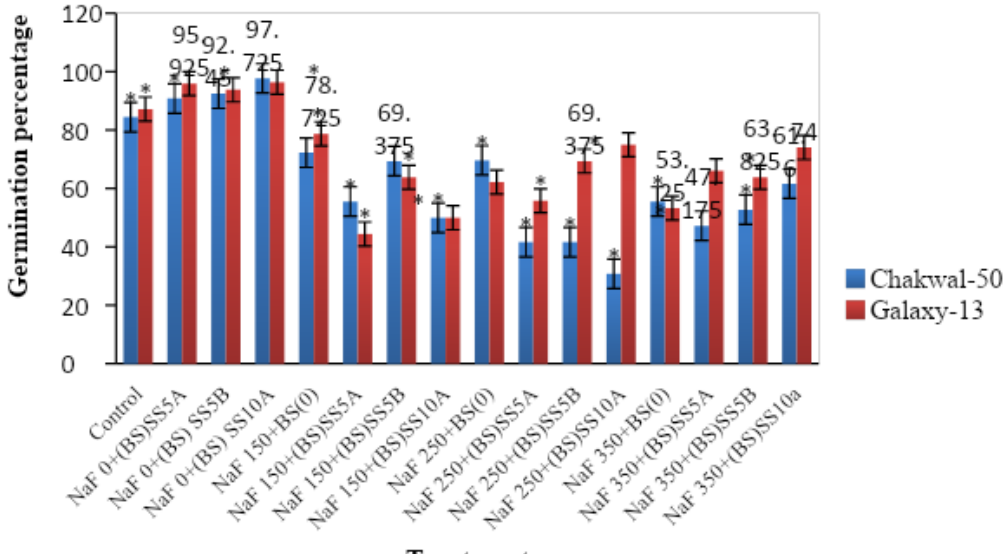

Figure 1. Germination percentage of both wheat varieties after 7 days of seed sowing. Data presented here are the means of 16 values per treatment. Alphabets are representing Duncan's multiple range test value at p=0.05.

Study of leaf morphology: Fluoride phytotoxicity was manifested through leaf tip burning and leaf-margin necrosis after exposure of the seedlings to the 350ppm F solution for four weeks. The peripheral leaf margins of both varieties exhibited F induced necrosis although not very significantly. Chlorosis induced by F may also resemble symptoms of iron, magnesium, and boron deficiency along with loss of apical dominance.[23] The phyto-hormone auxin that plays a crucial role in sustaining apical dominance seemed to be considerably perturbed, which might have resulted in scrub-vegetation becoming a serious demerit for agro-forestry.[23]

Plate 2. Leaf tip burning and necrosis of wheat varieties due to fluorosis.

Determination of growth parameters: Plant growth related parameters were affected by saline conditions of fluoride (Tables 1 and 2). Shoot and root length, leaf number, leaf length and leaf area of *Triticum acetivum* L. showed a declining pattern in an increased NaF conditions. Highest reduction found in NaF 350ppm with no bacterial strain. However, the application of NaF along with different bacterial strains showed less reduction in growth. Plants with bacterial strain (ss5a, ss5b, ss10a) in the absence of F stress gave good results. Galaxy-13 variety gave good response to stress environment as compared to var. Significant reduction in the growth of wheat cultivars may be due to disturbance of physiological and biochemical processes.[24] Fluoride decreased root and shoot length due to unequal supplement taken-up by seedlings in the vicinity of fluoride.[11] The number of leaves decreased in the following fashion in both varieties, NaF-150> NaF-250> NaF-350> and NaF-150+BS> NaF-250+BS> NaF-350+BS. Plant leaf showed leaf tip burning and the necrosis of leaf margins with the increased concentration of NaF.[25] Structural and ultra-structural damage occurred in the cells and tissues of the leaves which changed the leaf length.[26] Leaf area was minimum in both wheat varieties with NaF 150ppm+BS (ss5b). There was significant difference among the leaf area of both varieties. Stomata of leaves and epidermis get damaged when they came in contact with fluoride (F) and disturbed the mechanism of stomatal aperture.[27]

Biomass assessment: Fluoride solution showed inhibitory effect on subsequent fresh and dry biomasses of wheat plants (Figures 2–5). Plant biomass (Fresh and dry weight of shoot and root) was decreased significantly with increased NaF concentration in var. Chakwal-50 whereas var. Galaxy-13 showed resistance against stress condition. Fresh weight of shoot and root also increased when treated only with bacterial strains. Reduction was found because of decreased metabolic movement in the presence of fluoride.[28] Fluoride stored in roots for long time which decreased nutrition absorption. Fluoride easily extracted by roots through mild washing procedures leading to reduced plant biomass.[29]

Gas Exchange Characteristics: Spearmint leaf photosynthetic rate, stomatal conductance, internal CO concentration, and transpiration rate were affected (P = 0.05) by high salinity (Figures 6–9). Gas exchange characteristics showed significant difference between both varieties except some characters such as leaf temperature and leaf area which remain constant. Temperature of leaf varied from 35.7ºC–35.9ºC. Gas exchange characteristics showed good result with bacterial strains in the absence of NaF and in combination with NaF. Maximum CO_2 found into the leaf chamber of var. Chakwal-50 with treatment NaF250ppm+ BS (ss5b, ss5a). Overall Galaxy-13 variety showed high CO_2 concentration Higher CO_2 concentration from leaf chamber found in Galaxy-13 with treatment NaF 150ppm+BS (ss5a) lowest concentration also found for same treatment in Chakwal-50 cultivar. Treatment of NaF and NaF+BS significantly affected the rate of photosynthesis in both varieties of *Triticum aestivum* L. Highest photosynthetic rate found for both varieties in the presence of BS (ss5a). While highest transpiration rate found in both cultivars with treatment NaF 250ppm+BS (0) and 350ppm+ BS (ss5b). Transpiration rate increased with the increased NaF concentration in the following order 350ppm>250ppm>150ppm.

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Table 1. Growth parameters of variety Galaxy-13 of wheat after 70 days treated with different concentrations NaF and Bacterial Strain (BS)

Treatment	Shoot length (cm)	Root length (cm)	Leaf number	Leaf length (cm)	Leaf area (cm^2)
Control	74.5ab ± 1.84	11.58ab ± 0 .61	32.0a ± 3.08	17.87a ± 2.87	40.34a ± 4.82
NaF0+(BS)SS5A	80.12ab ± 2.78	11.12a ± 1.5	24.25a ± 6.89	23.75abcd ± 0.75	45.12abc ± 5.43
NaF 0+(BS) SS5B	77.12ab ± 2.43	14.97abc ± 1.89	21.75a ± 6.56	27.25cd ± 1.03	41.4ab ± 10.1
NaF 0+(BS) SS10A	78.87ab ± 2.18	14.86abcd ± 1.71	22.5a ± 3.94	22.0abcd ± 0.97	53.09abcd ± 1.93
NaF150+BS(0)	77.5ab ± 2.36	13.81abc ± 0 .71	23.5a ± 4.87	20.37ab ± 0.74	40.74ab ± 9.85
NaF150+(BS)SS5A	74.5ab ± 5.85	18.64d ± 0.47	24.0a ± 1.41	19.42ab ± 2.56	69.68abcd ± 3.98
NaF150+(BS)SS5B	78.0ab ± 1.08	14.97abcd ± 0.71	28.5a ± 2.39	21.62abc ± 1.02	39.95a ± 0 .72
NaF150+(BS)SS10A	82.75b ± 1.49	16.86cd ± 1.49	22.5a ± 1.19	22.42abcd ± 1.03	78.96a ± 10.7
NaF250+BS(0)	82.45b ± 2.33	14.79abcd ± 0.97	19.5a ± 1.89	22.55abcd ± 1.19	52.9abcd ± 2.58
NaF250+(BS)SS5A	74.25ab ± 1.43	15.95bcd ± 0.68	21.0a ± 3.13	24.75bcd ± 1.92	62.12cde ± 2.12
NaF250+(BS)SS5B	75.5ab ± 1.32	12.4abc ± 1.08	20.25a ± 3.63	22.5abcd ± 0.6	68.51de ± 2.44
NaF250+(BS)SS10A	78.0ab ± 0.57	14.91abcd ± 2.29	22.75a ± 3.03	28.5d ± 2.75	56.6abcde ± 5.71
NaF350+BS(0)	76.0ab ± 2.82	12.27abcd ± 1.21	27.25a ± 3.77	23.25abcd ± 3.49	61.42bcde ± 5.99
NaF350+(BS)SS5A	76.5ab ± 0.95	14.51abc ± 1.35	27.5a ± 6.76	20.0ab ± 2.45	70.17de ± 8.75
NaF350+(BS)SS5B	72.12a ± 3.65	14.53abcd ± 1.18	27.25a ± 4.02	23.0abcd ± 2.44	76.36e ± 8.07
NaF350+(BS)SS10A	74.62ab ± 1.28	14.45abcd ± 1.65	27.25a ± 4.02	22.25abcd ± 1.79	76.36abcd ± 8.07

Presented data are the means of 16 values per treatment. Alphabets are representing Duncan's multiple range test value at p=0.05.

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Table 2. Growth parameters of variety Chakwal-50 of wheat after 70 days treated with different concentrations NaF and Bacterial Strain (BS)

Treatment	Shoot length (cm)	Root length (cm)	Leaf number	Leaf length (cm)	Leaf area (cm^2)
Control	79.65a ± 4.41	16.28ab ± 1.12	31.75a ± 2.39	18.27a ± 1.4	55.59a ± 8.87
NaF0+(BS)SS5A	76.12abc ± 1.12	14.99a ± 1.14	24.25a ± 3.11	21.65abcd ± 2.86	52.87abc ± 2.4
NaF 0+(BS) SS5B	75.15abc ± 0.43	17.97abc ± 0.78	23.5a ± 2.21	17.87cd ± 2.04	70.92ab ± 16.73
NaF 0+(BS) SS10A	82.25c ± 2.75	17.11abcd ± 0.96	18.5a ± 2.59	20.25abcd ± 1.58	76.07abcd ± 21.2
NaF150+BS(0)	79.07bc ± 3.66	17.06abc ± 1.31	16.75a ± 1.18	20.0ab ± 1.17	60.74ab ± 6.62
NaF150+(BS)SS5A	80.75bc ± 2.65	17.64d ± 1.01	24.0a ± 7.32	22.0ab ± 2.85	87.19abcd ± 14.9
NaF150+(BS)SS5B	77.0abc ± 1.08	16.12abcd ± 0 .64	18.0a ± 4.16	20.17abc ± 1.65	39.95a ± 0.72
NaF150+(BS)SS10A	79.65bc ± 2.88	16.86cd ± 1.49	17.0a ± 0.7	19.72abcd ± 1.27	83.70a ± 20.75
NaF250+BS(0)	73.62ab ± 2.77	15.29abcd ± 1.06	15.75a ± 0.62	19.75abcd ± 2.04	61.23abcd ± 6.03
NaF250+(BS)SS5A	77.25abc ± 0.47	15.45bcd ± 0.25	18.5a ± 7.44	21.12bcd ± 5.66	62.12cde ± 2.12
NaF250+(BS)SS5B	76.65abc ± 2.18	13.25abc ± 0.84	19.0a ± 5.04	16.87abcd ± 0.65	68.51de ± 2.44
NaF250+(BS)SS10A	79.25bc ± 2.39	17.16abcd ± 1.37	16.75a ± 0.94	18.0d ± 0.57	56.6abcde ± 5.71
NaF350+BS(0)	79.0bc ± 2.51	13.02abcd ± 0.75	10.25a ± 1.8	13.1abcd ± 0.53	62.17bcde ± 5.39
NaF350+(BS)SS5A	78.92bc ± 0.36	15.76abc ± 1.44	12.5a ± 1.65	15.37ab ± 0.23	92.25de ± 16.56
NaF350+(BS)SS5B	76.82abc ± 1.49	15.03abcd ± 0.74	15.5a ± 1.65	17.12abcd ± 1.47	76.36e ± 8.07
NaF350+(BS)SS10A	80.0bc ± 0.7	16.7abcd ± 0.94	16.5a ± 1.5	18.05abcd ± 1.37	57.56abcd ± 3.43

Presented data are the means of 16 values per treatment. Alphabets are representing Duncan's multiple range test value at p=0.05.

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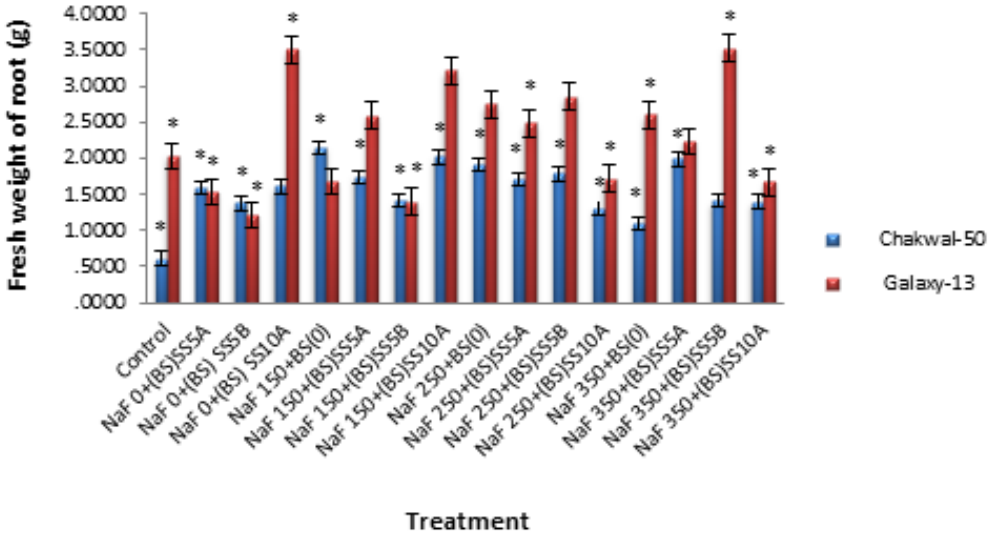

Figure 2. Comparative fresh weight of root vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both cultivars. Data used are the means of 16 values per treatment and the significant difference between them at p=0.05 according to Duncan's multiple range tests.

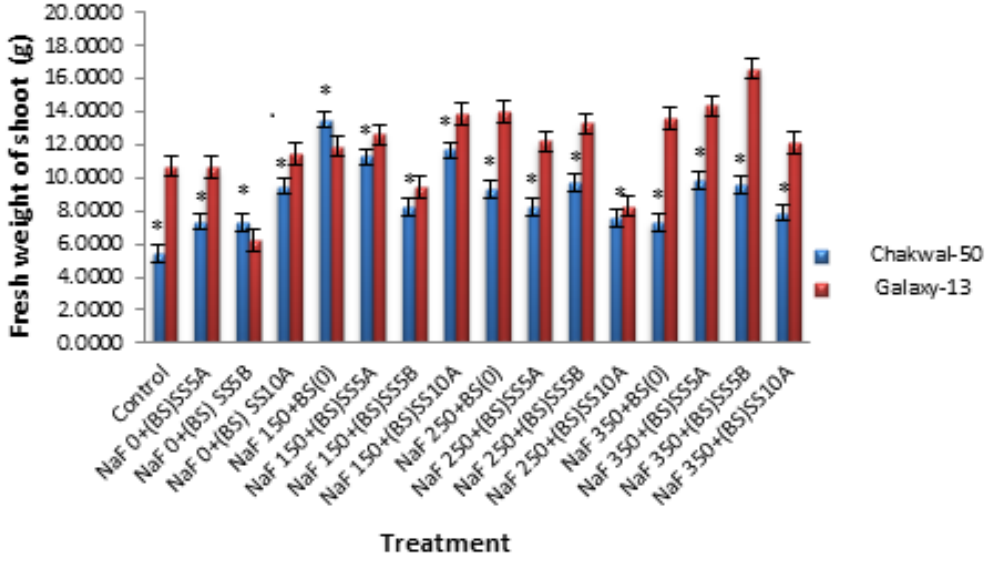

Figure 3. Comparative fresh weight of shoot vs wheat varieties (Galaxy-13 & Chakwal-50)treated with different concentrations of NaF and bacterial strain involving control of both cultivars. Data used are the means of 16 values per treatment and the significant difference between them at p=0.05 according to Duncan's multiple range tests.

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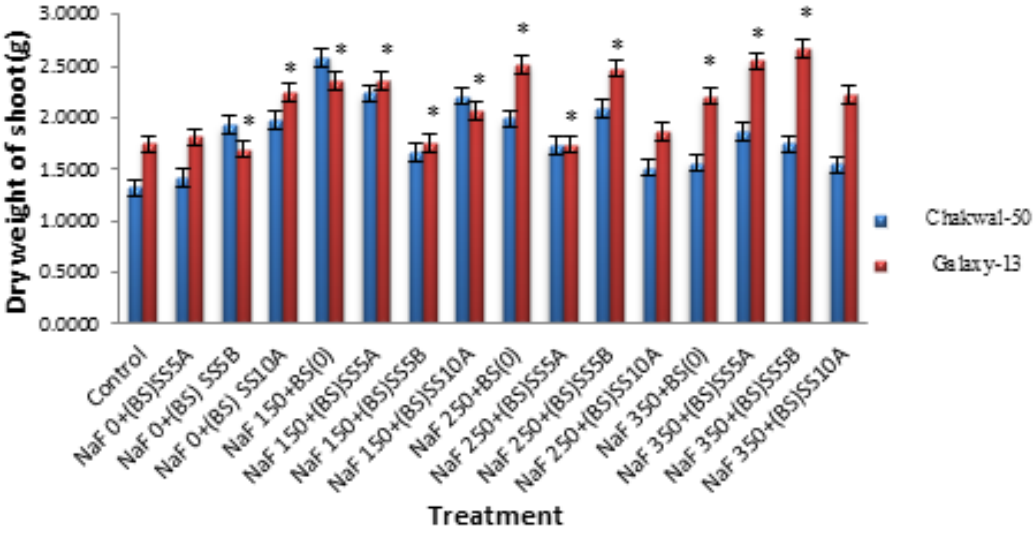

Figure 4. Comparative dry weight of shoot vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both cultivars. Data used are the means of 16 values per treatment and the significant difference between them at p=0.05 according to Duncan's multiple range tests.

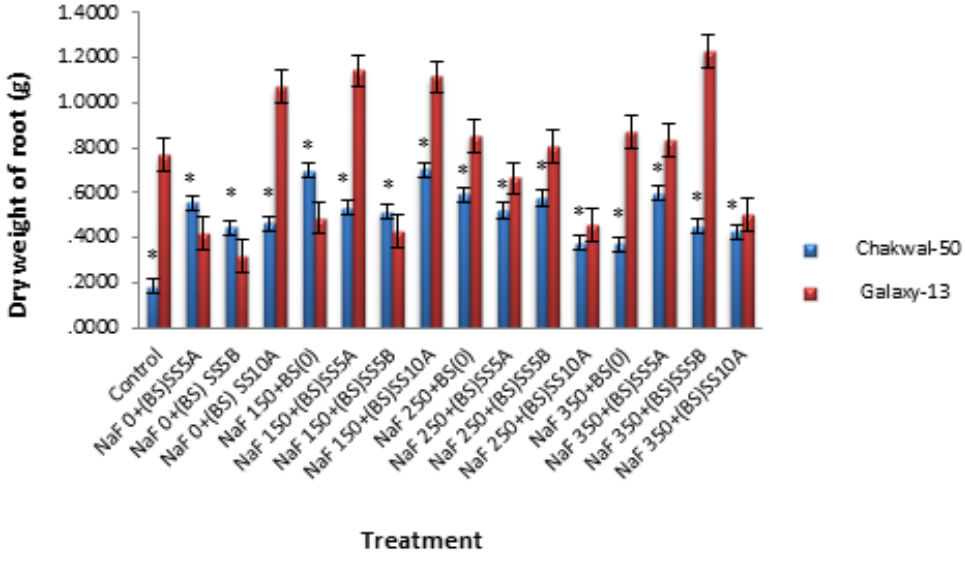

Figure 5. Comparative dry weight of root vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both cultivars. Data used are the means of 16 values per treatment and the significant difference between them at p=0.05 according to Duncan's multiple range tests.

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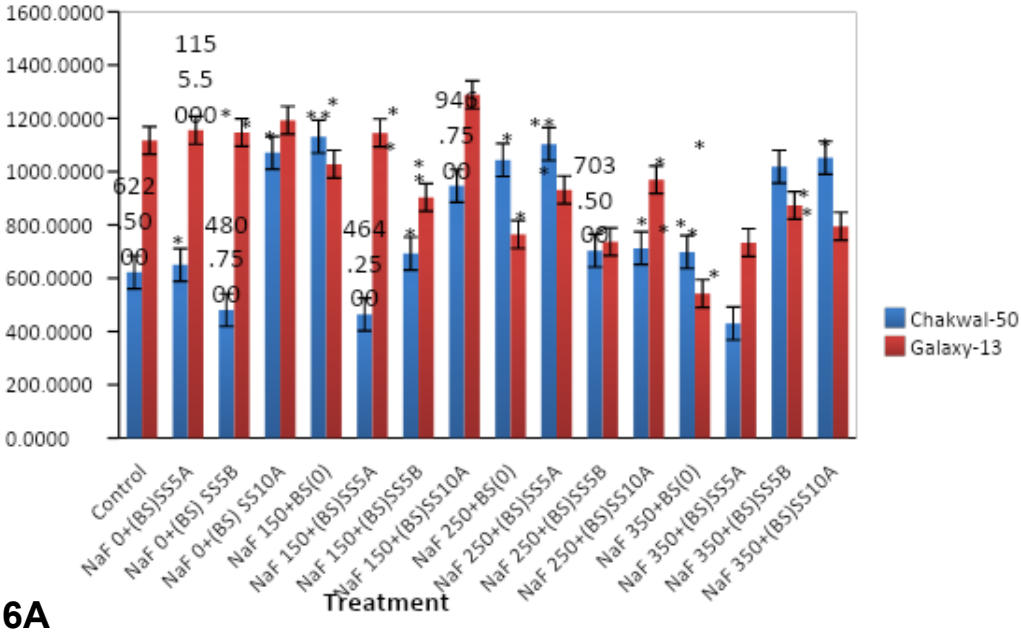
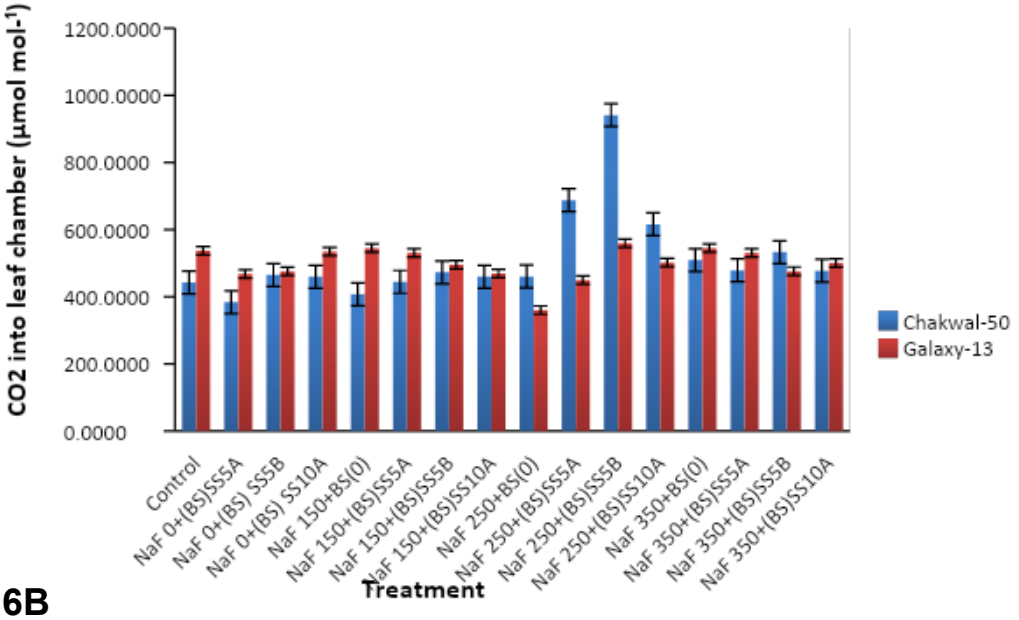


6A



6B

Figures 6A and 6B. Comparative photon flux density and CO_2 into leaf chamber vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both varieties. Data used are the means of 16 values per treatment the significant difference between them at p=0.05 according to Duncan's multiple range tests.

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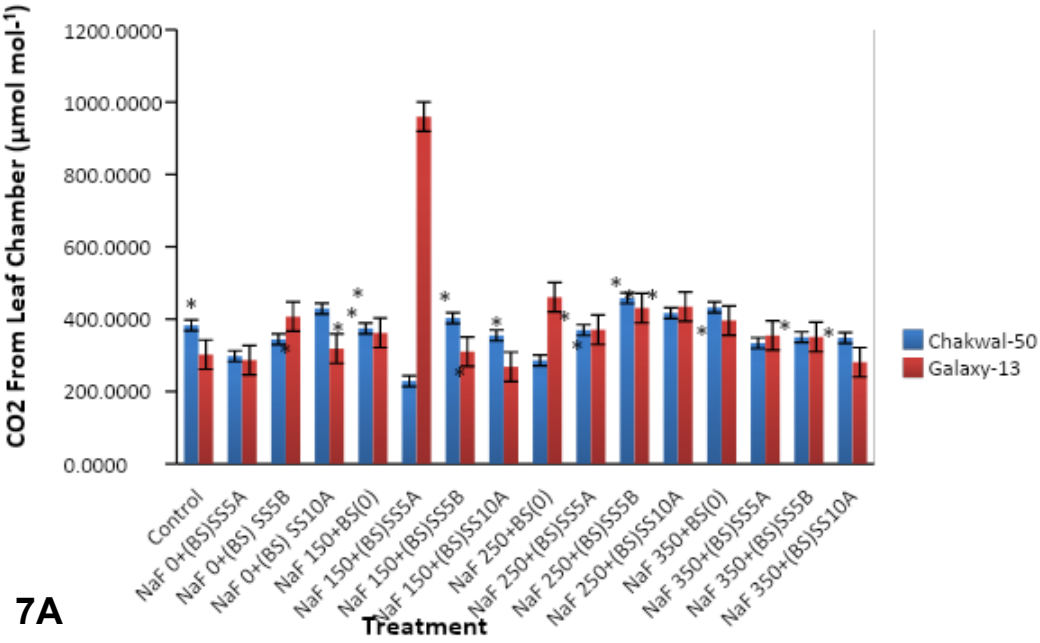

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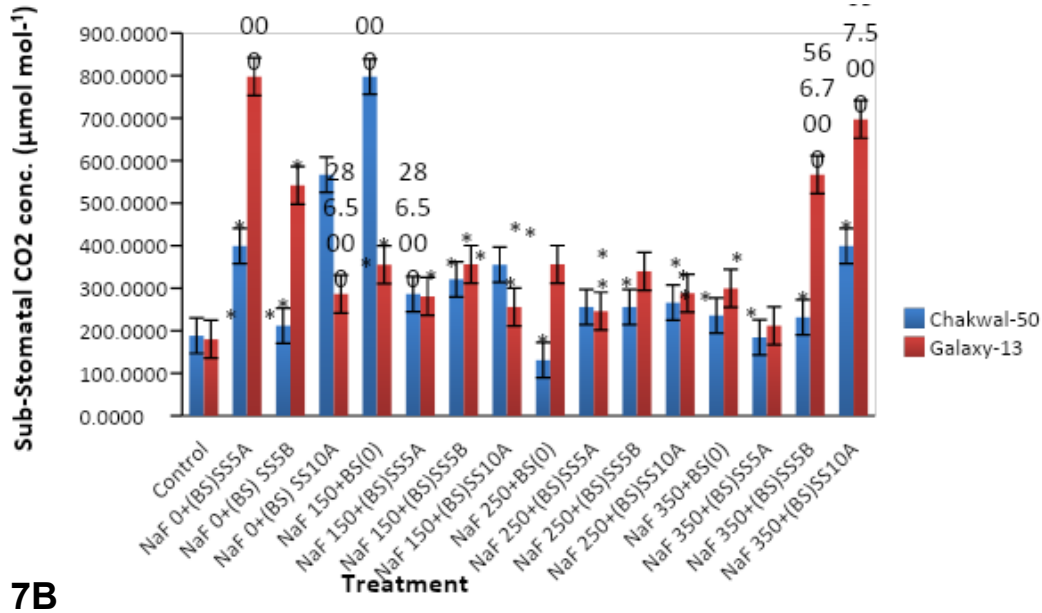

7B

Figures 7A and 7B. Comparative CO_2 from leaf chamber and Sub-stomatal CO_2 conc. vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both varieties. Data used are the means of 16 values per treatment the significant difference between them at p=0.05 according to Duncan's multiple range tests.

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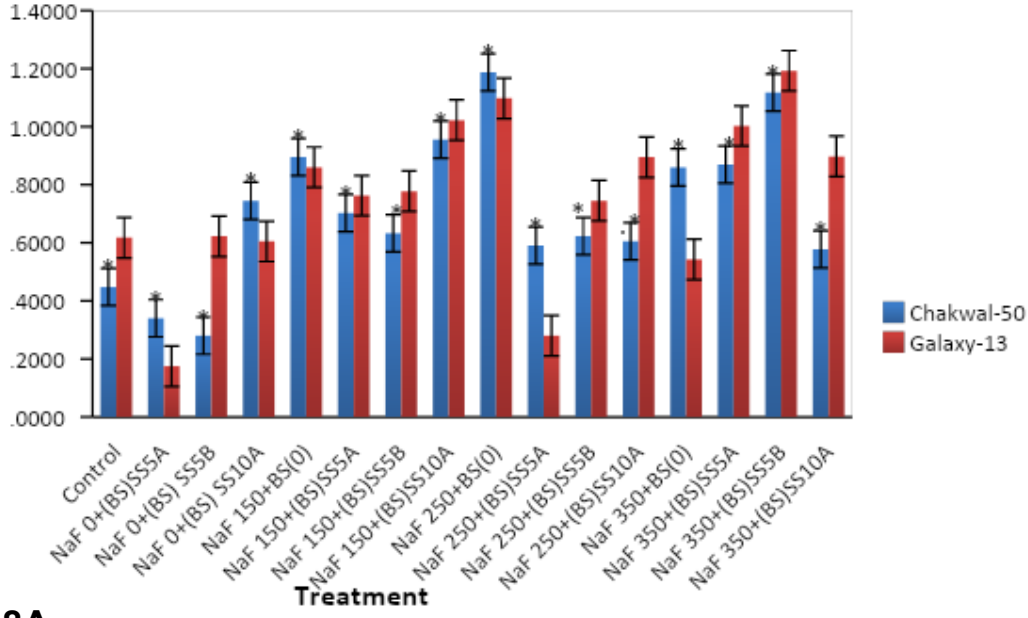
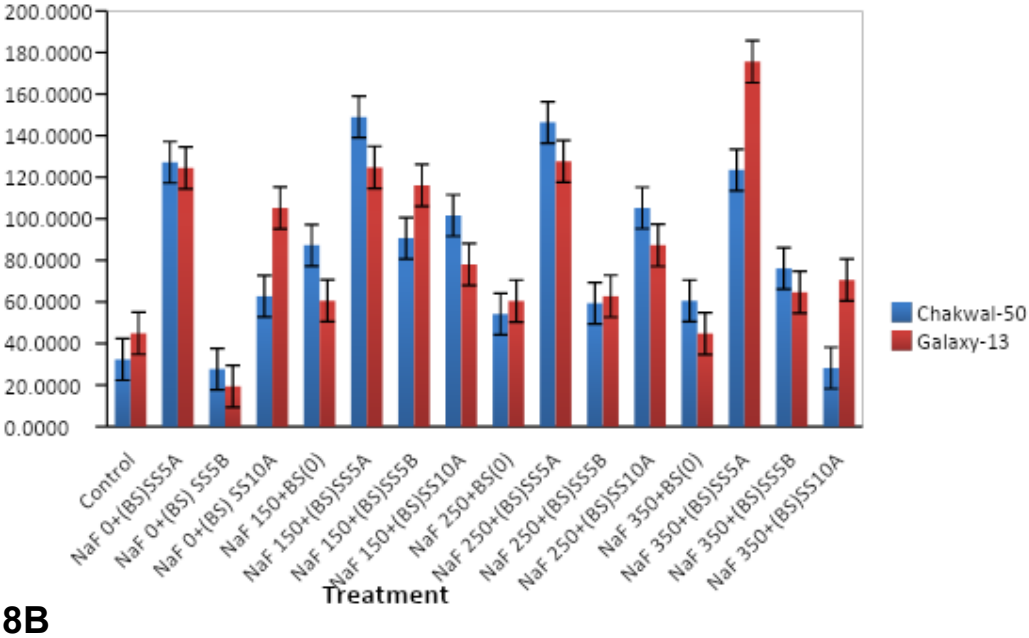


8A



8B

Figures 8A and 8B. Comparative Transpiration and Photosynthetic rate vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both varieties. Data used are the means of 16 values per treatment the significant difference between them at p=0.05 according to Duncan's multiple range tests.

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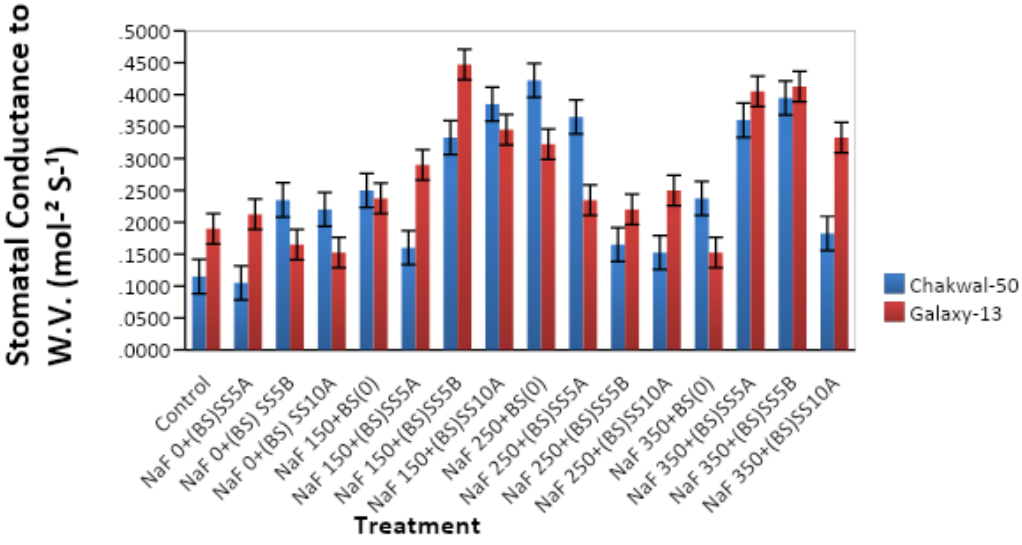

Figure 9. Comparative Stomatal Conductance vs wheat varieties (Galaxy-13 & Chakwal-50) treated with different concentrations of NaF and bacterial strain involving control of both varieties. Data used are the means of 16 values per treatment the significant difference between them at p=0.05 according to Duncan's multiple range tests.

Comparatively gas exchange characteristics were higher in Chakwal-50 variety as compared to Galaxy-13. Maximum reduction was noted in stress condition. [30] Both varieties showed significant difference in transpiration and photosynthetic rate. Sub-stomatal CO_2 and photon flux density was also significantly different in both varieties. Salt stress induced Stomatal closure and decreased the photosynthetic rate because stomatal closure reduced CO_2 availability in the mesophyll cells.[31] Fluoride accumulation altered or inhibited the rate of respiration because of increased use of pentose phosphate pathway.[32]

Projected leaf area: Projected leaf area was constant physiological parameter. It is same (6.25 cm^2) for both varieties in all types of treatment.

CONCLUSIONS

This study was carried out to evaluate phytoremediation potential of two varieties of *Triticum aestivum* L. for fluoride. It was observed that increasing the level of sodium fluoride results in an increased reduction in growth of wheat plants. But var. Galaxy-13 has maximum decontamination potential than Chakwal-50 with reference to the highest fluoride accumulation in the shoot and root length. On the other hand, greater rate of physiological activities related with the induction of fluoride and it was positively correlated with other parameters. Bioremediation of fluoride by using bacterial strains proved quit helpful. Three bacterial strains were used in the experiment, out of which BS (ss10a) was proved more resistant to fluoride stress. Bacterial strains decreased the harmful effects of NaF on wheat cultivars.

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