FLUORIDE TOXICITY AND ITS EFFECT ON TWO VARIETIES OF *SOLANUM LYCOPERSICUM*

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ABSTRACT: Environmental pollutants are considered to be a serious threat not only to humans but also to flora and fauna. The fluoride ion (F) as present in water, in soil, and in the atmosphere may adversely affect plants and ultimately pose a threat to plant growth and development. This study aimed to observe the effect of F on the crop productivity of tomato plants. Two tomato plant varieties (Roma and Meiguodahong) were used to study, under controlled conditions, the effect of F, in concentrations of 0, 10, 25, 50, and 100 ppm, on the parameters of seed germination, leaf area, plant growth rate, net assimilation rate (NAR), and proteomic response. The results showed that the maximum adverse effect of F on seed germination, leaf area, plant growth rate, and NAR occurred with a F concentration of 50 ppm. Compared to the control values (F=0 ppm), the respective values for the Roma (R) and Meiguodahong (M) varieties at F=50 ppm were (i) growth rate: R: 5.5 mg/day (F=0 ppm), 1.1 mg/day (F=50 ppm); M: 7.1 mg/day (F=0 ppm), 3.1mg/day (F=50 ppm); (ii) average leaf area: R: 25 cm^2 (F=0 ppm), 8 cm^2 (F=50 ppm); M: 27 cm^2 (F=0 ppm), 11cm^2 (F=50 ppm); and (iii) NAR: R: 44 µg/day (F=0 ppm, 22 µg/day (F=50 ppm); M: 52 µg/day (F=0 ppm, 23 µg/day (F=50 ppm). At a F concentration of 25 ppm, both tomato varieties over expressed a unique band, a 55 kilodalton (kDa) protein. The Meiguodahong variety was more resistant to F toxicity than the Roma variety.

Key words: Physiological parameters; Roma; Meiguodahong; Protein; *Solanum lycopersicum*.

INTRODUCTION

Extensive urbanization around the globe has resulted in increased industrialization with the release of enormous quantities of toxic pollutants into the environment. Of these pollutants, the fluoride ion (F) is a highly toxic pollutant which negatively affects both the biotic and the abiotic factors of the environment. The main industrial contributors to F release into the environment are mining, coal combustion, using F as a fertilizer. and the manufacture of aluminum. In addition, the use of F containing pesticides in agriculture and the utilization of drinking water with high F levels also contribute to environmental F pollution. F is known to be highly phytotoxic, to diffuse easily in soil, and to be taken up more readily by plant roots when the soil pH is more acidic[1, 2].

F invades into plants primarily through two major pathways: (i) *aerial deposition*, which may occur via stomatal diffusion. F may enter the plant via the leaves, pass through the cell wall, and travel to the leaf tips and margins, which are vital areas for evaporation; and (ii) *through soil and water.* The plant's roots are the prime source of F absorption from the soil. The F then further proceeds further via xylematic flow to reach the plant leaves or stem. However, stems are considered to be the least preferred place for F to reside in plants.[3]

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F accumulation in plants, such as wheat or tomatoes, may also result from irrigating plants with F-containing water. High levels of F in plants may result in cell death, disturbance of the various processes of plant growth and development,[5-9] and ultimately reduced plant productivity.

The tomato belongs to the nightshade family, *Solanaceae*. Tomato is the second most important vegetable globally and is also a significant crop of Pakistan. It is a rich source of vitamins, minerals, sugar, amino acids, and dietary fiber. It also reduces the risk of cardiovascular disease and prostate cancer. The aim of the present study was to examine the effect of F on various quality parameters of two varieties of tomato plant, Roma and Meiguodahong.

MATERIALS AND METHODS

The experiment was conducted in a green house at the Institute for Biotechnology and Genetic Engineering, Peshawar, Pakistan. Two tomato plant varieties, Roma and Meiguodahong, were collected from the Horticulture Department, The University of Agriculture, Peshawar, Pakistan. In the experiment, three specimens of both varieties were subjected to four F treatments: 0 (control), 10, 25, 50, and 100 ppm. Treatment was continued for two weeks. The vital parameters of plant growth and development of net assimilation rate (NAR), plant germination rate, total plant leaf area, proteomic insight, and the relative growth rate were considered at regular intervals to assess the impact of F on the two varieties of tomato plant.

Germination rate: To find the germination rate, the media were supplied with sodium fluoride (NaF) in addition to distilled water and agar. Plant germination media were supplied with various amount of F and poured into beakers in a laminar flow hood (LFH). The tomato seeds were sterilized by the addition of a few drops of TWEEN® 20 into distilled water and the seeds were incubated for 10 min. The seeds were washed three times with distilled water before being placed on semi-solid media with flame-sterilized forceps. The media was supplemented with various doses of F: 0 (negative control), 10, 25, 50, and 100 ppm. The tomato varieties under consideration were grown on F media and the germination rate was calculated. The germination of the seeds were recorded per beaker and the weights of the plants were also determined.[9]

Leaf area: The leaf area of both tomato varieties was measured with the planimeter method as described by Joshi and Bhardwaj.[10]

Net assimilation rate: The net assimilation rate (NAR) was evaluated as described earlier by Watson.[11]

$$NAR = \frac{(\log_e L2 - \log_e L1) \times (W2 - W1)}{(L2 - L1) \times (t2 - t1)}$$

where:

L1 and L2 are the total leaf area
W1 and W2 are the dry weights
t1 and t2 are the times at the beginning and end of the observation period or the intervals

Relative growth rate: The relative growth rate (RGR) was evaluated following the formula as described earlier by Watson.[11]

$$RGR = \frac{(\log_e W2 - \log_e W1)}{t2 - t1}$$

where:

W1 and W2 are the dry weights
t1 and t2 re the times at the beginning and end of the observation period or the intervals
RGR = relative growth rate
The value of e in $\log_e$ = 2.306 as calculated by Rathore.[12]

Protein content: The protein content of the leaves of both tomato varieties was determined using the Laemmli method.

Statistical analysis: A randomized complete block design (ONE WAY ANOVA) and LSD test was used for analysis. The standard errors and means were calculated by using Microsoft Excel (2007).

RESULTS

The study was conducted in order to examine the effect of F accumulation in tomato plants. The tomato plant varieties, Roma and Meiguodahong, were used as a model to investigate the impact of different doses of F on various parameters related to growth and development. The experiments showed that F has a huge impact on plant growth and development that drastically affects the parameters of plant growth of leaf area, NAR, proteomic response, seed germination ratio, and growth rate.

Effect of F on germination rate: In the control condition with a F concentration of 0 ppm, the germination rates for the Roma and Meiguodahong varieties were 82% and 68%, respectively (Figure 1). F adversely effected seed germination at the F concentrations of 10, 25, 50, and 100 ppm. The maximal impairment of germination occurred with a F concentration of 50 ppm with the average germination rate for both Roma and Meiguodahong being 18%. At the F concentration of 10 ppm, the seed germination rate for Roma and Meiguodahong were 35% and 43%, respectively. Similar results were observed at 25 ppm, with the germination rates for Roma and Meiguodahong being 35% and 38%, respectively. Interestingly, the germination rates at the F concentration of 100 ppm for Roma and Meiguodahong, 26% and 23%, respectively, were higher than those with the F concentration of 50 ppm, 18% for both Roma and Meiguodahong.

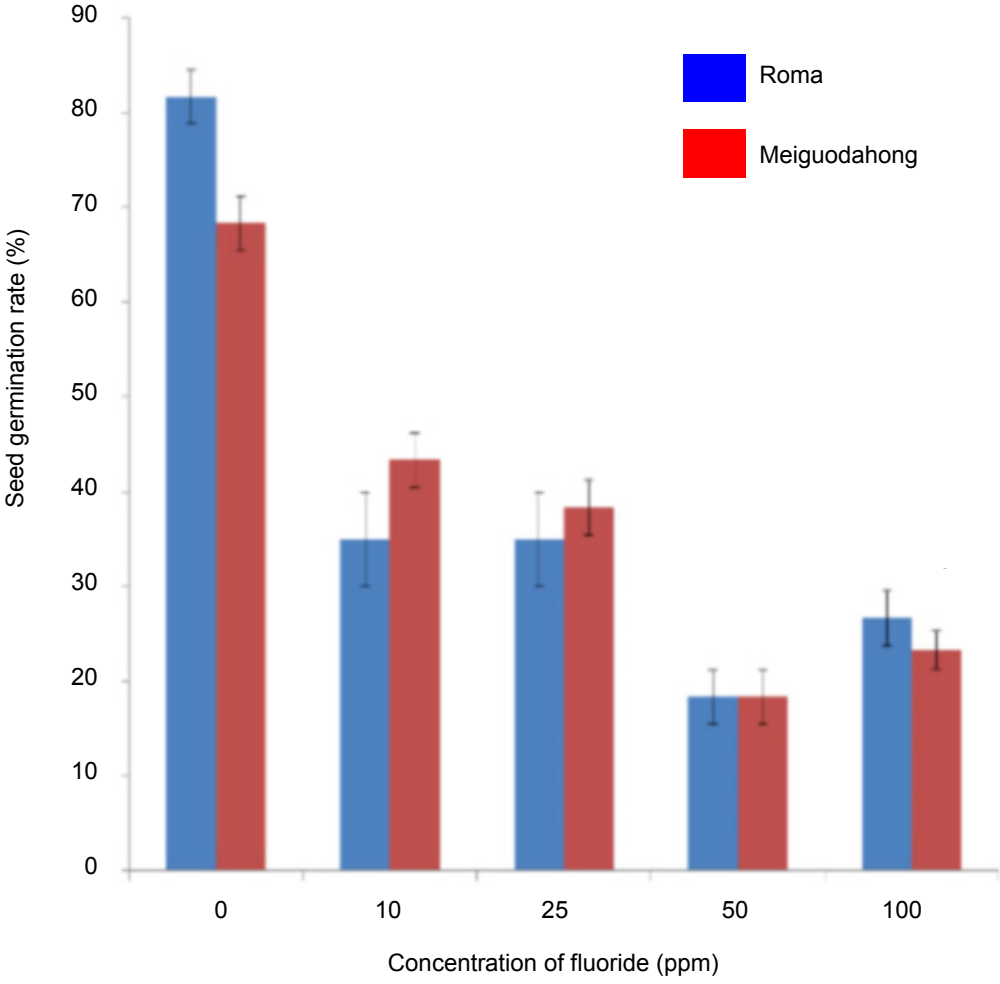

Figure 1. Germination data on the Roma and Meiguodahong tomato varieties under different concentrations of fluoride. Values are the means of three values with the experiment being done in triplicate.

Effect of F on leaf area: The control plants, with a F concentration of 0 ppm, of both varieties showed the maximum leaf area (Figure 2). At 10 ppm, the average leaf area of Roma was 20 cm^2 and for Meiguodahong it was 18 cm^2. At 25 ppm, the leaf areas of Roma and Meiguodahong were 14 cm^2 and 20 cm^2, respectively. The maximal reduction in leaf area was observed at 50 ppm, where the average leaf area of Roma was 8 cm^2 and that of Meiguodahong was 12 cm^2. At 100 ppm, the average leaf area for Roma was 14 cm^2 and for Meiguodahong it was 13 cm^2.

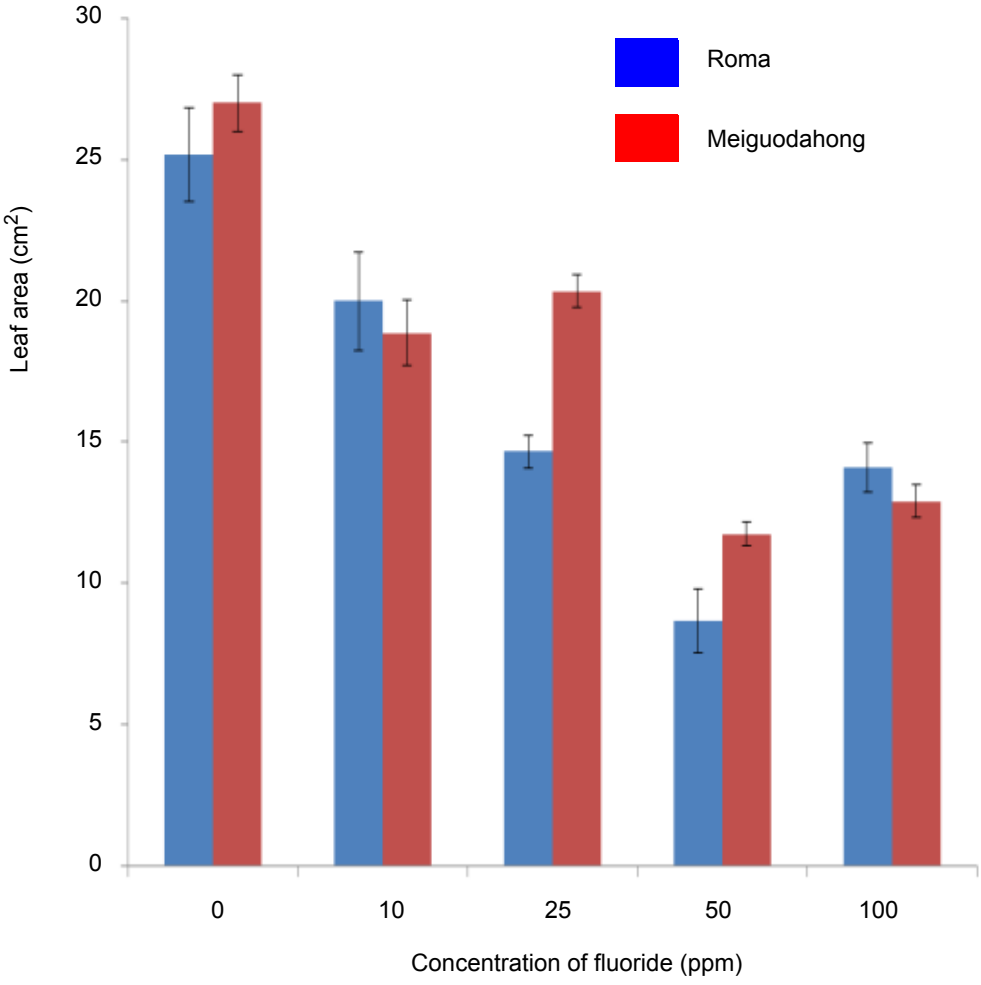

Figure 2. Leaf area data on the Roma and Meiguodahong tomato varieties under different concentrations of fluoride. Values are the means of three values with the experiment being done in triplicate.

Effect of F on the growth rate: The control plants (F= 0 ppm) for both varieties showed the maximum growth rate, which was 5.5 mg/day for Roma and 7.1 mg/day for Meiguodahong (Figure 3). F negatively affected the growth rate at 10 and 25 ppm but the effect was non-significant. At 10 ppm, the average growth rate recorded for Roma was 4.3 mg/day and for Meiguodahong it was 5.1 mg/day, while at 25 ppm it was 3.5 mg/day for Roma and 4.3 mg/day for Meiguodahong. The maximum effect of F on growth rate was noted at 50 ppm, at which level it was 1.5 mg/day for Roma and 3.1 mg/day for Meiguodahong. At the higher F level of 100 ppm, the growth rate was 2.5 mg/day and 4.33 mg/day for Roma and Meiguodahong, respectively.

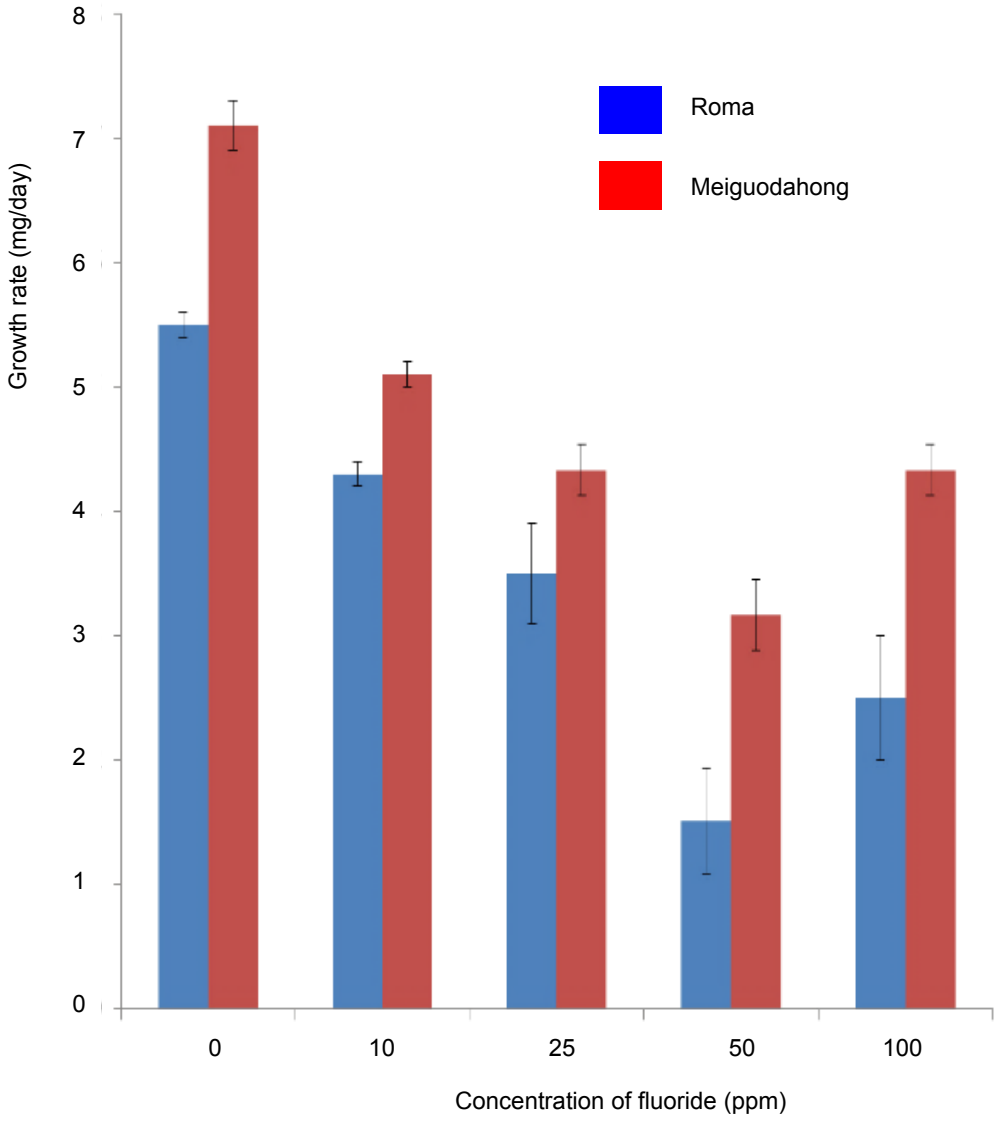

Figure 3. Growth rate data on the Roma and Meiguodahong tomato varieties under different concentrations of fluoride. Values are the means of three values with the experiment being done in triplicate.

Effect of F on the net assimilation rate (NAR): In the control condition (F=0 ppm) both varieties showed their maximum NAR which was 0.044 mg/day for Roma and 0.052 mg/day for Meiguodahong. At a F concentration of 10 ppm, the NAR was calculated as 0.03 mg/day for Roma and 0.04 mg/day for Meiguodahong. At 25 ppm, the NAR observed for Roma was 0.02 mg/day and for Meiguodahong it was 0.03 mg/day. The maximum reduction of the NAR was observed at a F concentration of 50 ppm with NAR values of 0.02 mg/day and 0.023 mg/day for Roma and Meiguodahong, respectively. At the F concentration of 100 ppm, the NAR activity

was 0.022 mg/day for Roma and 0.028 mg/day NAR for the Meiguodahong variety (Figure 4).

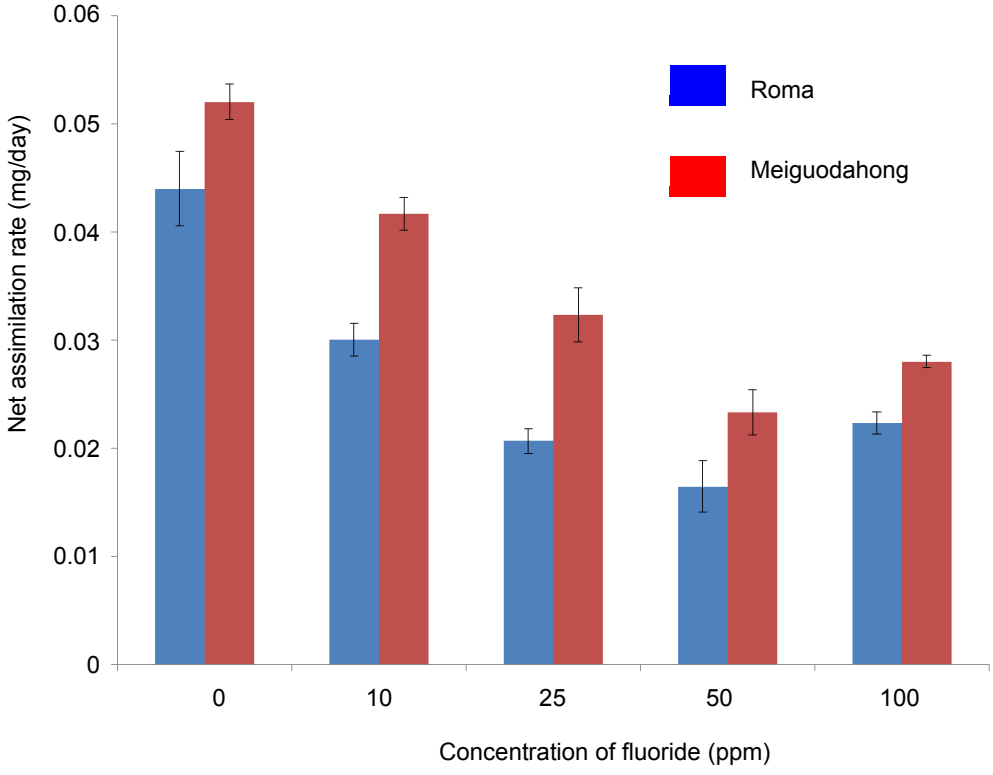

Figure 4. Net assimilation rate (NAR) data on the Roma and Meiguodahong tomato varieties under different concentrations of fluoride. Values are the means of three values with the experiment being done in triplicate.

Effect of F on the protein profile: The effect of F on the proteomics of the plants was examined and, after total protein isolation, the SDS-PAGE revealed an interesting profile for both tomato varieties. The gel analysis revealed that only seven detectable bands were observed on the gel. The range of the molecular mass on the banding was 15–170 kDa. Only four banding patterns, 25, 55, 70, and 170 kDa, were observed at the various levels of F. The four banding patterns were clearly observed in both the varieties of tomato. Interestingly, a unique banding pattern of 55 KDa was up regulated and observed for both the varieties at the F concentration of 25 ppm compared to the other F concentrations (Figure 5).

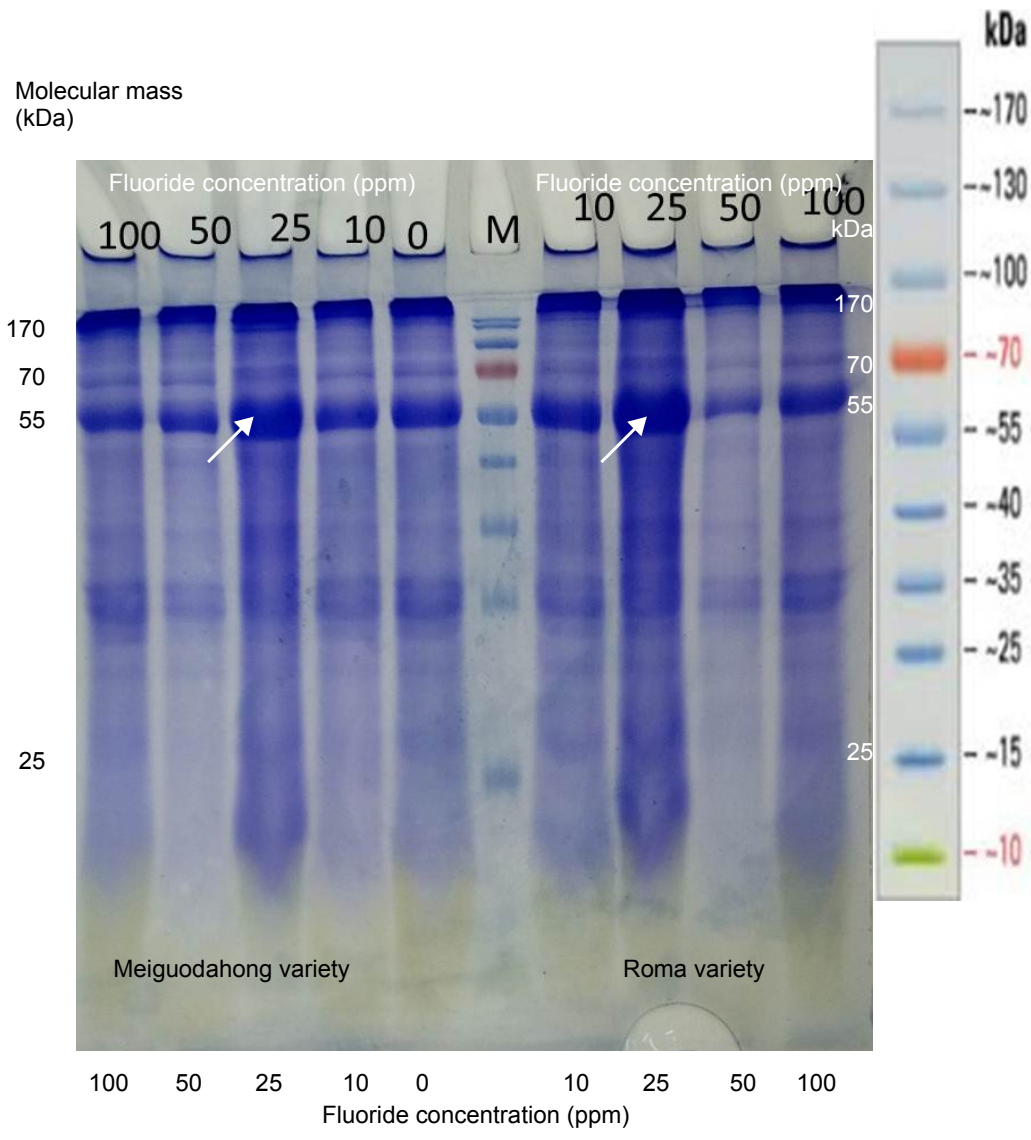

Figure 5. SDS-PAGE. Protein profiling of the Roma ad Meiguodahong tomato varieties at various levels of fluoride (0, 10, 25, 50, and 100 ppm) after 14 days. The white arrows indicate the unique banding pattern of a 55 kDa molecular mass with both the varieties at the F concentration of 25 ppm

DISCUSSION

F exposure can adversely the health of humans, animals and plants. Widespread air pollution and environmental damage may occur with F release into the environment by humans in various industries, including the industrial use of F as a flux, the processing of F-containing phosphate rock to produce fertilizer, and the burning of coal. The emission of inorganic F, over the decades, has damaged crop productivity, forests and vegetation. Plants are sensitive to F and F environmental contamination adversely affects plant growth and development. The present study revealed that F

had an adverse effect on the germination rate of both varieties of tomato at every concentration tested: 10, 25, 50, and 100 ppm. Our findings are in-line with those of Reddy and Kaur[13] and Gupta et al.[14] who found that F affected plant growth and development via the dephosphorylation of phytin compounds in plant seeds resulting in a lower germination rate and by retarding seedling growth. Similarly, Singh et al. found that F accumulation induced metabolic changes and a decreased germination rate.[15]

F accumulation in leaves negatively affects gaseous exchange and stomatal diffusion. F affects the process of photosynthesis by causing the degradation of chloroplasts and by reducing chlorophyll synthesis. The present study shows that F decreased the leaf area and the net assimilation rate at 10, 25, 50, and 100 ppm with the decreases being maximal at 50 ppm in both varieties of tomato. Yamauchi et al.[16] found similar results by observing that F treatment resulted in a decrease in the photosynthetic pigments, chlorophyll and carotenoids, and the content of anthocyanins, water-soluble vacuolar pigments. The reduction in the chlorophyll and carotenoid content may be attributed to increasing activity of chlorophyllase. Chlorophyllase is a enzyme that has a role in degrading chlorophyll and is also responsible for a reduction of Fe^{+2} ions which play an important role in the synthesis of chlorophyll pigments. Our findings also revealed that at a F concentration of 100 ppm, the leaf area and the net assimilation rate were higher than at 50 ppm. Our results are consistent with the findings of Elloumi et al.[4] who described F stress as being responsible for inhibiting the formation of reducing sugars in leaves such as fructose, mannose, and glucose. The researchers further reported that plants may adopt the special mechanism for coping with F toxicity of converting reducing sugars to non-reducing sugars.

F may reduce plant growth and development leading to reduced plant production. Our results shows that a high level of F may negatively affect the growth rate of two varieties of tomato, Roma and Meiguodahong. Negative effects on germination rate, leaf area, growth rate, and net assimilation rate (NAR) occurred with all the F concentrations studied, 10, 25, 50, and 100 ppm, with the maximum effects occurring at 50 ppm. In addition, F also leads to lipid peroxidation resulting in deprivation of lipids and destabilization of the cell membrane. As a natural defence, the plants showed some resistance to the increasing level of F with some tolerance and the start of recovery at the highest F level of 100 ppm. Plants posses antioxidant defence mechanisms including superoxide dismutase, etc. and non-enzymatic species. Li et al. reported that plants utilize enzymes such as superoxide dismutase as a protection mechanism to convert free radicals into non-radical molecular forms to protect cells from toxicity.[17] In our findings, the Meiguodahong variety showed more tolerance to F toxicity than did the Roma variety. The same mechanism of protection in response to F toxicity may be adopted by a variety of plants.

F is responsible for the alteration and modulation of the quantity as well as the quality of proteins. It has been reported that F may decrease the total protein content in plants. In plants, certain proteins perform a vital role in the structure and function of the cell and novel proteins may be expressed in response to F stress. We analysed the plants for the proteomic response to F toxicity and found the same banding pattern for both tomato varieties for all the F concentrations except for the up

regulation of a 55 kDa band with a F concentration at 25 ppm which was consistent with the work of Li et al.[18] Their research suggested that this over expression might be attributed to the expression of an exporter protein, FEX. The exporter protein FEX allows F to be expelled to outside of the cell and is a defence mechanism used by the plant to avoid F toxicity. In contrast to the finding of the 55 kDa band at 25 ppm of F, we found a different proteomic response at 10, 50, and 100 ppm of F which may not have had a significant effect on removing F from the cell. F may also adversely affect protein synthesis and prevent the exploitation of proteins for energy consumption. Excessive F accumulation in plants may also lead to the degradation of certain proteins which are considered as vital for plant growth and development. F may effect nucleotides and disrupt their sequencing or function leading to reduced RNA synthesis. F changes the free nucleotide ratio which ultimately reduces RNA synthesis. mRNA has a vital role in protein synthesis and there are reports of mRNA being disturbed or decreased at the sub-cellular level by F resulting in disrupted protein synthesis. The toxic effects of F in plants are also reduced by the uptake of thymine and uridine at both the DNA and RNA levels. Plants also over express proline in response to certain stresses, including F stress. We found that the Roma tomato variety exhibited a higher expression of transport proteins (ABC and FEX) compared to the Meiguodahong variety.

CONCLUSION

This study examined the effect of F stress, at concentrations of 10, 25, 50, and 100 ppm, on the Roma and Meiguodahong varieties of tomato plant and found that the maximal negative effect occurred with a concentration of 50 ppm on the parameters of germination rate, leaf area, growth rate, and net assimilation rate, in both varieties. We conclude that F, in the range of 10–100 ppm, has a negative impact on tomato plant growth and development and that the Meiguodahong variety is more resistant to F stress than the Roma variety.

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