Endogenous calcium mediates seedling growth and fluoride stress tolerance in four bean genotypes

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Highlights

- *F stress negatively affected bean germination and seedling growth.*
- *F mainly accumulated in the shoots of bean varieties.*
- *Ca concentration in the roots played a crucial role in mitigating F accumulation.*

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Abstract

Fluoride (F) pollution is a global environmental problem representing a severe risk for food and vegetables grown in contaminated soils. *Phaseolus vulgaris* L. is widely cultivated in arid and semi-arid regions and F-contaminated areas of the world. For that reason, F tolerance during germination and seedling growth was evaluated for four bean genotypes: Borlotto nano (commercial variety) and three African genotypes (Lyamungu 85, Lyamungu 90, and Jesca). Seeds were grown in sand enriched with NaF or KF at three different levels (0, 80, and 200 mg kg^{-1}). NaCl was used as a benchmark to determine the potential effect of different Na levels in the plant. Total F content and mineral accumulation (Na, K, and Ca) in roots and shoots were measured. The translocation factor, growth ratio, and F tolerance index were evaluated to estimate plant-salt response. Germination rate decreased with increased F level. Borlotto was more F sensitive (0% germination with 200 mg $kg^{-1}$of KF and NaF) than the African genotypes. Under the highest F concentration (200 mg kg^{-1}), F preferentially accumulated in shoots (Jesca 75.7 mg kg^{-1}, Lyamungu 85 100.1 mg kg^{-1}, and Lyamungu 90 115.4 mg kg^{-1}). Ca content in roots was negatively correlated to F absorption, suggesting its antagonistic role to F mobility. Based on these parameters, Jesca and Lyamungu 85 were the most tolerant species, recording a low F uptake and a high Ca content in the root. This study highlighted the central role of Ca as a key secondary messenger in regulating plant growth and development under F stress.

Introduction

Fluorine soil and groundwater contamination represent a severe risk to human health in several countries. The maximum permissible limit of fluoride (F) in drinking water is 1.5 mg L^{-1} (International Programme on Chemical Safety *et al.*, 1984). However, there is no stringent threshold limit of F content in soil and plant tissues, above which ingestion is considered detrimental to human health. In America, Asia, the Middle East, and Africa, people consume water with fluoride concentrations greater than

1.5 mg L^{-1} (Frencken, 1992). Contaminated soils pose a threat to human, animal, and plant health. Although F is considered an essential element in animal diet to improve bone and teeth development, excessive F in the diet can cause harmful alterations to teeth, bone, and other body systems (Loganathan *et al.,* 2003). Excess F in water, air, and soils is caused by weathering of volcanic ashes (Cronin *et al.,* 2003), the application of phosphate fertilisers, and the (illegal) release of industrial wastes (Choubisa and Choubisa, 2016).

In nature, fluorine forms F water-soluble compounds such as sodium fluoride (NaF) or other alkali-metal fluorides such as potassium fluoride (KF). Moreover, it can also be part of some non-dissociated salts such as calcium fluoride (CaF_2), also known as fluorite or fluorspar. The toxicity of F and its cumulative effects depend on the species susceptibility and the duration of the exposure to F over the solubility of the F salt.

High levels of F inhibit seed germination and early growth of many plants (Elloumi *et al.,* 2005; Gupta *et al.,* 2009), inducing several morphological symptoms like chlorosis, tip and marginal necrosis (Dey *et al.,* 2012; Fornasiero, 2003). Indeed, F absorbed from the soil is translocated to the shoots, causing physiological, biochemical, and structural damage, depending on its concentration in the substrate and translocation from roots to shoots (Dey *et al.,* 2012). Furthermore, a high concentration of F adversely affects the growth and survival of plants as a consequence of its inhibitory effects on respiration, photosynthetic pigments function and synthesis (Kamaluddin and Zwiazek, 2003), mineral and water uptake, and enzyme activity (Fornasiero, 2003).

Sensitivity to F is highly species-dependent (Beast and Haeck, 1983) indeed some plants can accumulate F at high concentrations (up to 4000 µg F g^{-1}), displaying no sign of toxicity (Jha *et al.,* 2008), while others show signs of toxicity at much lower concentrations, with some species being enormously sensitive to a level <20 µg g^{-1} (Jha *et al.,* 2008).

Common bean (*Phaseolus vulgaris* L.) is an important herbaceous annual grain legume grown as a cheap source of protein, carbohydrates, and iron among most Sub-Saharan African countries. Tanzania ranks fifth worldwide in bean production and is the leading producer of beans in Africa (FAOSTAT, 2014). The common bean varieties grown are Tikyakuponza, Soya, Lyamungu-85, Lyamungu-90, Canadian Wonder, Selian-94, Masai Red, Jesca, and Calima (Katungi *et al.,* 2009). However, a high proportion of agricultural land in Tanzania is contaminated by F of volcanic origin, as is common across many districts of the Rift Valley in East Africa.

While many studies explored plant response to NaCl salinity, fragmented information on plant tolerance to F is available.

Nevertheless, the processes and mechanisms behind plant adaptative methods to NaCl could be useful in paving the way for an investigation into the adaptation strategies of plants to F. Many studies revealed that the ability of plant genotypes to maintain higher levels of K and Ca and low levels of Na and Cl within the tissues is one of the critical mechanisms contributing to high salt tolerance. In screening plant genotypes for salt tolerance, the shoot K:Na and Ca:Na ratios and tissue Na concentration have been proposed as a useful screening tool to assess the salt tolerance of different crop species (Aktas *et al.,* 2006; Shabala and Cuin, 2008).

Three common bean African genotypes and one commercial variety cultivated widely in Europe were selected to compare the effect of different sources of F (NaF and KF) in selected morpho-physiological traits and principal mineral contents. We hypothesised that the effects of excess F content and other ions (Ca^{2+}, K^+, Na^+) in the substrate are different in commercial and African bean varieties currently grown in F-rich soils and that these effects can be detected in the early growth stages of beans, which are crucial for subsequent plant growth, development, and yield. The objective of the experiment was to study: i) the effects of increasing F levels in the substrate on germination and seedling growth traits of African bean genotypes; ii) the possible different responses of African bean genotypes to F at the seedling level; iii) the contribution of ion accumulation to the osmotic adjustment and the role of Na, K and Ca homeostasis in the determination of salt tolerance; iv) the possible differences of ion profiles under F between the shoots and the roots. To the best of our knowledge, this is the first study of the early development stages of different bean genotypes as influenced by increasing exposure to F-based salts. The aim was to contribute to the rapid identification of promising genotypes which can be used for selecting F-tolerant bean varieties, capable of growth in F-rich soils and to minimise the F absorption and translocation in edible plant organs.

Materials and methods

Plant material and experimental conditions

Mature seeds of four bean (*Phaseolus vulgaris* L.) genotypes were used for the germination experiments. In addition, three African genotypes, Lyamungu 85 (LYA85); Lyamungu 90 (LYA90); Jesca (JES) (provided by the Nelson Mandela University of Science and Technology in Tanzania), and the commercial cultivar Borlotto nano (BOR) were used. The experiment was carried out in a growth chamber (24±2°C, 18 h photoperiod, with an average irradiance of 1500 lux) for 14 days.

Three salt sources were added to the germination substrate (silica sand): sodium fluoride (NaF); potassium fluoride (KF), and sodium chloride (NaCl), and for each salt, three levels were evaluated: 0 (Control), 80 mg kg^{-1}, and 200 mg kg^{-1}; treatment was reported as salt name and level (*i.e.*, KF 80 mg kg^{-1}, is reported as KF80). The choice of salt concentration levels was based on the range of soil F contents observed in F-rich sites of the East African Rift Valley in Tanzania, as reported by Rizzu *et al*. (2020). Control (F0) contained only silica sand. Plastic containers (58×72 mm) containing two seeds each were used for each salt* level* variety combination. Fifteen replicates (containers) were used for each treatment; the experiment was repeated twice.

During the experiment, irrigation was performed by adding 5 mL of water to each box every 24 h. The F and Na^+ contents in the enriched sand were monitored using the potentiometric method with an ion-selective electrode (ORION 4 star) and inductively coupled plasma (ICP) atomic emission spectrometry (PerkinElmer), respectively.

Germination and growth measurements

Germination was recorded daily. Seedlings with a hypocotyl-radicle axis >3 cm were considered germinated. The germination proportion was calculated using Eq. 1. After 14 days, the seedlings were gently washed with water to remove the sand, and the plant was scanned using a Mustek flatbed scanner (model A3 USB 1200S). The length of the aerial part (L-AP) was measured using Image J software (Image processing and analysing in Java). Root length (R-L) was estimated with the GIA ROOTS software (Galkovskyi *et al.,* 2012).

Seedlings were wrapped in labelled blotting paper and oven-dried at 65°C until constant weight. Aerial part (AP) and root (R)

dry weight (DW) were measured. Germination percentage (%G) (Eq. 1), growth ratio (GR) (Eq. 2), and F tolerance index (TI) (Eq. 3) were calculated using the following equations (Baker, 1983):

$$\%G = \frac{No.seeds\ germinated}{Total\ no.seeds} * 100 \quad (1)$$

$$GR = \frac{Plant\ biomass\ with\ salt}{Plant\ biomass\ without\ salt} * 100 \quad (2)$$

$$TI = \frac{Root\ length\ with\ salt}{Root\ length\ without\ salt} \quad (3)$$

Determination of F content in root and shoot

The total F content was estimated using an acid digestion method and subsequently quantified by an F ion-selective electrode (Rizzu *et al.,* 2020).

The lyophilised samples (150 mg) were weighed in a Teflon reactor and were then subjected to a microwave-assisted wet digestion process (Milestone Ethos Easy), using 65% HNO_3 (2 mL), 3 mL of hydrogen peroxide, and 5 mL of dehydrogenised water. The power applied was 800 W, and the program used was: Step 1: ramp from room temperature to 200°C for 15 min; Step 2: hold at 200°C for 15 min; Step 3: cool to room temperature. When the Teflon reactor had cooled, the digestate was kept in a refrigerated bath (–30°C) for 30 min to avoid loss of F in the form of HF, then adjusted to a pH close to 7 using sodium hydroxide solution (NaOH, 8 mol L^{-1}). Then the extraction solution was mixed with 10% (v/v) of total ionic strength adjustment buffer 'TISAB II' solution. The mixture was analysed by an ion-selective electrode (ORION 4 star). Finally, the digestion was applied at least in duplicate to each of the samples analysed.

Translocation factor (TF) was calculated for F as:

$$TF = \frac{F\ concentration\ in\ shoot}{F\ concentration\ in\ root} \quad (4)$$

Macro and micro-nutrient content

Minerals (Na, K, and Ca) were extracted with perchloric acid digestion (Maggio *et al.,* 2000). Concentrations of minerals were analysed by inductively coupled plasma (ICP) atomic emission spectrometry (PerkinElmer).

Statistical analysis

The experiment was laid out as a completely randomised design with fifteen replicates. The normality of data was assessed using the Shapiro-Wilk test. The not-normally distributed data Na-AP, Na-R, K-R, K/Na (AP), Ca/Na (AP) were transformed using bounded distribution Sb. F-AP was transformed using Log-normal distribution SL. While we calculated the arcsine of the square root of the percentage of germination (%G), then it was transformed into normalised data using unbounded distribution (George and Ramachandran 2011). A two-way ANOVA was performed (Minitab 17 Statistical Software 2010). When significant effects were observed ($P<0.01$), multiple comparisons were investigated with the Tukey post-hoc test ($P<0.01$). The histograms were presented as averages and standard error.

Results

Germination study

A significant decrease in germination percentage (G%) was observed for all genotypes as a consequence of the F treatments used ($P<0.0001$) (Table 1). Under both KF and NaF treatments with 80 mg kg^{-1} of F, a significant reduction of germination was observed. Among genotypes under comparison, LYA 85 and JES showed the highest G% under NaF80 and KF 80, respectively (100% and 93%). Under both KF and NaF treatments with 200 mg kg^{-1} of F, germination was significantly affected for all genotypes. Using KF, the African bean genotypes survived with a low G% (7-28%), while no germination occurred using NaF. Nevertheless, with NaCl 200 mg L^{-1}, G% for LYA 85 and LYA90 did not statistically differ from control (100%), while for BOR and JES, it was significantly lower (93%).

Effect of F on plant growth

The effects of F treatments on the AP and R length and biomass in *P. vulgaris* seedlings were explored using two parameters: tolerance index (TI) and growth ratio (GR) (Table 2). All bean genotypes were more tolerant to KF than NaF, and a significant effect on the treatment level was observed ($P<0.0001$). Besides treatment, the genotype also differentially responded to the F treatments ($P<0.01$). BOR was the most susceptible variety, and the TI value was detectable until 80 mg kg^{-1} (0.72 and 0.76, respectively, for KF and NaF). Indeed, BOR seeds did not germinate under

Table 1. Effect of different salts and concentrations on bean germination.

Treatment (mg kg^{-1})	Genotype			
	BOR	JES	LYA85	LYA90
F0	100^{a}	100^{a}	100^{a}	100^{a}
KF80	79^{d}	93^{b}	69^{b}	83^{c}
KF200	0^{e}	10^{d}	7^{c}	28^{d}
NaF 80	86^{c}	83^{c}	100^{a}	90^{b}
NaF 200	0^{e}	0^{e}	0^{d}	0^{e}
NaCl80	93^{b}	93^{b}	100^{a}	100^{a}
NaCl200	93^{b}	93^{b}	100^{a}	100^{a}

Germination (%) of bean genotypes: Borlotto (BOR), Jesca (JES), Lyamunguru 85 (LYA85) and Lyamunguru 90 (LYA90). The results for the control condition (F0) and treatments with different salts (KF, NaF, and NaCl) at two different concentrations (80 and 200 mg kg^{-1}) are reported. $^{a-e}$Different letters indicate significant differences at $P<0.01$ according to the Tukey *post-hoc* test based on each genotype.

NaF200 and KF200. LYA90 showed the highest TI value (1.04 and 0.94) under KF80 and NaF80, respectively (Table 2). Under KF200, the African genotypes showed a significant decrease in TI values (JES: 0.52, LYA85: 0.49, and LYA90: 0.62). A slight decrease of TI was observed for both BOR and JES under NaCl200, while it remained unchanged for LYA85 and increased for LYA90 (Table 2).

As observed for TI, GR% was significantly affected by treatment and genotype (P=0.0091; P<0.0001, respectively). Indeed, GR% for BOR followed the same trend observed in TI. In addition, under KF80, both JES and LYA90 (76%) showed the lowest GR%, while under NaF80, GR% was much lower for JES (48%) and LYA85 (59%). Under KF200, LYA 90 showed the lowest GR (62%). In contrast, JES showed the lowest GR % under NaCl200 (74%) (Table 2).

The DW showed a different trend for R and AP, with highly significant differences (P<0.0001) (Table 3). Indeed, DW-R decreased drastically with F salt concentration (Table 3). The effect of F treatments (80 mg kg^{-1}) was much more negative on roots than on the aerial parts. Under NaF 80, the highest DW-R reductions were registered (BOR 50%, JES 79%, LYA85 69%, and LYA90 47%), while moderate reductions were observed under KF80 (BOR 40%, JES 39%, LYA85 16% and LYA90 40%). Under KF200, DW-R decreased respectively by 50% (JES), 76% (LYA85), and 73% (LYA90), while the parameter was not computed for BOR since there was no germination. Under NaCl200, the highest decline observed was for JES (44%) and LYA90 (31%). In the present study, the R/AP dry weight ratio decreased in all treatments except for NaCl 80 in BOR and JES compared to the control. This indicated that the R/AP of all genotypes responded similarly to the treatments, except for BOR and JES at NaCl 80. LYA 85 and LYA90 showed the highest ratio under NaCl 200 (Table 3). The highest R/AP ratio was observed in JES under KF200 and in LYA90 under NaF 80 treatment, while the lowest was observed in LYA85 under KF200 and in JES and BOR under NaF 80.

Fluoride uptake

Overall, the type of salt (treatment) and salt concentrations (treatment levels) significantly impacted F tissue content, while no genotype effect was observed. Under F0, LYA90 showed the highest F content in AP (8.15 mg kg^{-1}) while BOR in R (6.75 mg kg^{-1}) (Table 4). Under KF 80, no significant differences were recorded regarding F accumulation among all the genotypes for both AP and

Table 2. Effect of different salts (KF, NaF, and NaCl) at two different concentrations (80 and 200 mg kg^{-1}) on growth ratio % (GR%) and tolerance index (TI) in 4 bean varieties: Borlotto, Jesca, Lyamunguru 85, Lyamunguru 90 (BOR, JES, LYA85, and LYA90, respectively).

Genotype	Treatment (mg kg^{-1})	TI	GR%
BOR	F0	1.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
	KF80	0.72 ± 0.01^{De}	91.08 ± 0.11^{Bc}
	KF200	NA	NA
	NaF80	0.76 ± 0.00^{Bd}	84.01 ± 0.37^{Ad}
	NaF200	NA	NA
	NaCl80	0.96 ± 0.01^{Bb}	99.32 ± 0.29^{Aa}
	NaCl200	0.88 ± 0.01^{Cc}	95.05 ± 0.73^{Ab}
JES	F0	1.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
	KF80	0.82 ± 0.01^{Cc}	75.66 ± 1.34^{Ca}
	KF200	0.52 ± 0.04^{Ae}	80.39 ± 20.74^{Aa}
	NaF80	0.62 ± 0.00^{Cd}	47.91 ± 0.95^{Db}
	NaF200	NA	NA
	NaCl80	1.00 ± 0.01^{Aa}	78.73 ± 0.39^{Ca}
	NaCl200	0.90 ± 0.01^{BCb}	73.87 ± 0.20^{Cab}
LYA85	F0	1.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
	KF80	0.92 ± 0.00^{Ba}	95.42 ± 0.74^{Aa}
	KF200	0.49 ± 0.11^{Ac}	77.64 ± 19.25^{Ab}
	NaF80	0.77 ± 0.00^{Bb}	58.95 ± 0.06^{Cc}
	NaF200	NA	NA
	NaCl80	0.92 ± 0.01^{Ba}	102.54 ± 1.85^{Aa}
	NaCl200	0.92 ± 0.01^{Ba}	88.71 ± 1.14^{Bab}
LYA90	F0	1.00 ± 0.00^{Ac}	100.00 ± 0.0^{Aa}
	KF80	1.04 ± 0.01^{Ab}	75.67 ± 0.58^{Cc}
	KF200	0.67 ± 0.02^{Ae}	61.52 ± 1.32^{Ae}
	NaF80	0.94 ± 0.00^{Ad}	71.96 ± 0.47^{Bd}
	NaF200	NA	NA
	NaCl80	1.01 ± 0.01^{Ac}	87.96 ± 0.95^{Bb}
	NaCl200	1.22 ± 0.00^{Aa}	85.56 ± 0.76^{Bb}
Probability level of significance (ANOVA)	Genotype (A)	*	**
	Treatment (B)	***	***
	A*B	***	***

GR% and TI were calculated according to Eqs. 2 and 3 (see *Materials and methods* section); data are compared to the control (F0) results (TI=1, GR=100%); $^{a-e}$different lowercase letters indicate significant differences at P<0.01 according to the Tukey *post-hoc* test based on treatment within each genotype; $^{A-D}$different capital letters indicate significant differences at P<0.01 according to the Tukey post-hoc test based on genotype within each treatment; *NA, not available plants.

R (Table 4). Under KF treatments, JES accumulated the lowest F amount at KF 200 when compared to F0 (73.3 mg kg^{-1}) in the AP, while LYA 90 accumulated the highest F content (107.3 mg kg^{-1}). On the other hand, LYA 90 and JES took up the lowest F quantity in their R when comparing KF200 to F0 (+32.8 mg kg^{-1} and +43.6 mg kg^{-1}, respectively). Instead, LYA 85 accumulated +62.2 mg kg^{-1} of F with KF200 compared to F0 in R (Table 4).

Concerning NaF, the only available data is the treatment with 80 mg kg^{-1} since higher concentrations of salt inhibited germination. Indeed, BOR accumulated the greatest quantity of F in AP compared to the other genotypes, reaching a value of 37.2 mg kg^{-1}. All African bean genotypes generally accumulated less F in both organs compared to BOR, probably absorbing less F from the enriched (NaF) substrate used. Conversely, LYA 85 accumulated the lowest amount of F compared to the other bean genotypes (17.67 and 10.98 mg kg^{-1} in AP and R, respectively) (Table 4).

Based on the TF, all the genotypes studied showed a high ability to translocate F to the aerial part according to the increased concentration of F (KF200). Under treatments supplying 80 mg kg^{-1} of F, JES was the only variety recalcitrant to translocate F to its AP (0.6), while LYA85 and BOR were able to transfer the F to the aerial part (BOR 1.3 and LYA85 1.8) (Figure 1).

Ion's concentrations

The distribution of Na and Ca cations in R and AP tissues were studied in all genotypes. In detail, Na content varied significantly among organs (R and AP) and treatment (P<0.0001). As expected,

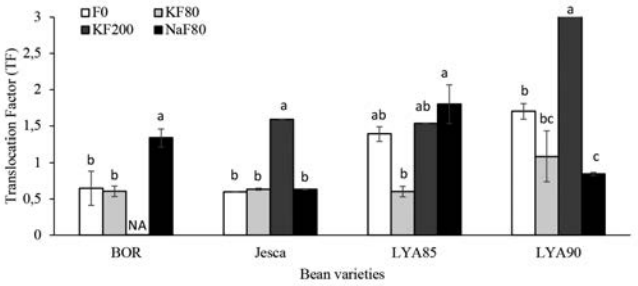

Figure 1. Translocation factor (TF) of four bean ecotypes (BOR, JES, LYA85, and LYA90) under different sources of F (KF and NaF 80 and 200 mg kg^{-1}). TF was evaluated according to Eq. 4 (see *Materials and methods* section). Vertical bars represent standard error. Different letters indicate significant differences at P<0.01 according to the Tukey post-hoc test based on treatments within genotype. NA, not available data.

Table 3. Effect of different salts (KF, NaF, and NaCl) at two different concentrations (80 and 200 mg kg^{-1}) on dry weight (DW) of the aerial part (DW-AP), root (DW-R), and their ratio (R/AP) of four bean varieties: Borlotto, Jesca, Lyamunguru 85, Lyamunguru 90 (BOR, JES, LYA85, and LYA90, respectively) and the salt levels: control (F0), 80 and 200 ppm.

Variety	Treatment (mg kg^{-1})	DW_AP	DW_R	R/AP
BOR	F0	0.22 ± 0.00^{Ac}	0.17 ± 0.00^{Ca}	0.78 ± 0.00^{Ca}
	KF80	0.25 ± 0.00^{Aa}	0.10 ± 0.00^{Bc}	0.40 ± 0.00^{Cc}
	KF200	NA	NA	NA
	NaF80	0.24 ± 0.00^{Ab}	0.08 ± 0.00^{Bd}	0.35 ± 0.00^{Cd}
	NaF200	NA	NA	NA
	NaCl80	0.21 ± 0.00^{Ad}	0.17 ± 0.00^{Ca}	0.79 ± 0.01^{Ca}
	NaCl200	0.24 ± 0.00^{Ab}	0.13 ± 0.00^{Cb}	0.55 ± 0.01^{Cb}
JES	F0	0.21 ± 0.00^{Bb}	0.30 ± 0.01^{Aa}	1.47 ± 0.01^{Aa}
	KF80	0.20 ± 0.00^{Bb}	0.18 ± 0.01^{Aabc}	0.92 ± 0.02^{Bb}
	KF200	0.26 ± 0.00^{Aa}	0.15 ± 0.10^{Abc}	0.59 ± 0.40^{Abc}
	NaF80	0.18 ± 0.00^{Cd}	0.06 ± 0.00^{Dc}	0.35 ± 0.01^{Cc}
	NaF200	NA	NA	NA
	NaCl80	0.19 ± 0.00^{Bc}	0.21 ± 0.00^{Bab}	1.05 ± 0.01^{Bab}
	NaCl200	0.20 ± 0.00^{Bb}	0.17 ± 0.00^{Bbc}	0.83 ± 0.01^{Bbc}
LYA85	F0	0.17 ± 0.00^{Cb}	0.23 ± 0.00^{Ba}	1.33 ± 0.02^{Ba}
	KF80	0.19 ± 0.00^{Cb}	0.19 ± 0.00^{Ab}	1.02 ± 0.03^{Ac}
	KF200	0.25 ± 0.07^{Aa}	0.05 ± 0.01^{Ae}	0.22 ± 0.04^{Af}
	NaF80	0.16 ± 0.00^{Db}	0.07 ± 0.00^{Cd}	0.44 ± 0.00^{Be}
	NaCl80	0.19 ± 0.00^{Cb}	0.22 ± 0.01^{Ba}	1.19 ± 0.03^{Ab}
	NaCl200	0.18 ± 0.00^{Ab}	0.17 ± 0.00^{Ac}	0.91 ± 0.01^{Ad}
LYA90	F0	$0.21\pm0.00A^{Bb}$	0.30 ± 0.01^{Aa}	1.43 ± 0.04^{Aba}
	KF80	0.21 ± 0.00^{Bb}	0.18 ± 0.00^{Ad}	0.88 ± 0.02^{Bc}
	KF200	0.23 ± 0.01^{Aa}	0.08 ± 0.00^{Af}	0.35 ± 0.01^{Ae}
	NaF80	0.21 ± 0.00^{Ab}	0.16 ± 0.00^{Ae}	0.75 ± 0.01^{Ad}
	NaF200	NA	NA	NA
	NaCl80	0.21 ± 0.00^{Cb}	0.24 ± 0.00^{Bb}	1.13 ± 0.01^{Ab}
	NaCl200	0.23 ± 0.00^{Aa}	0.21 ± 0.00^{Ac}	0.90 ± 0.00^{Ac}
Probability level of significance (ANOVA)	Genotype (A)	***	NS	*
	Treatment (B)	***	***	***
	A*B	***	***	***
	Coefficient of variation	15%	49%	50%

Data are reported as means and coefficient of variation; $^{a\text{-}f}$different lowercase letters indicate significant differences at P<0.01 according to the Tukey post hoc test among treatment levels within each genotype; $^{A\text{-}D}$different capital letters indicate significant differences at P<0.01 according to the Tukey post-hoc test based on genotype within each treatment; NA, not available plants; NS, non-significant.

under NaF and NaCl, organs showed a higher Na level than F0 and KF treatments. In all genotypes, Na content in both R and AP significantly increased with the increased level of Na supplied through NaF and NaCl. Moreover, Na content under NaF was significantly higher than under NaCl salt, with the highest level reached for JES and LYA85 (500%-516%) under NaF 80. Conversely, under NaCl 80, the highest levels were observed for BOR (199%) and JES (157%) (Figure 2A).

All genotypes showed a significant dose-dependent increase of Na content in AP, with increased NaF and NaCl stress. Using NaF salt plants accumulated more Na than with NaCl treatment (Figure 2): the highest Na content was reached for JES under NaF 80 and NaCl 80 (584% and 433%), respectively (Figure 2B). With KF 80 treatment, we observed a reduction in Na content in AP for BOR, JES, and LYA90 of 43%, 28%, and 15%, and an increase for LYA85 of 12%. In R, Ca content decreased by the same level with the KF80 mg kg^{-1} treatment in BOR, JES, and LYA90 by 34%, 38%, and 31%, respectively, while for LYA85, it decreased by only 11%. In R, Ca content did not change for LYA85 under NaF 80; in contrast, it decreased by 43% for BOR, 44% for JES, and 34% for LYA90. BOR and JES showed a similar trend in Ca content in R under the NaF treatments. Under KF200, JES showed the highest decrease (by 74%). Under NaCl 200, no changes were observed for JES and LYA90, while an increase was recorded for BOR and LYA85 (Figure 3). A similar trend was observed in AP. Both under KF80 and NaF 80, Ca decreased significantly. In more detail, under KF80, Ca decreased by 36% in BOR, 28% in JES, 19% in LYA85, and 29% in LYA90, while under NaF80, Ca content decreased by 52% in BOR, 13% in JES, 30% in LYA85 and 40%

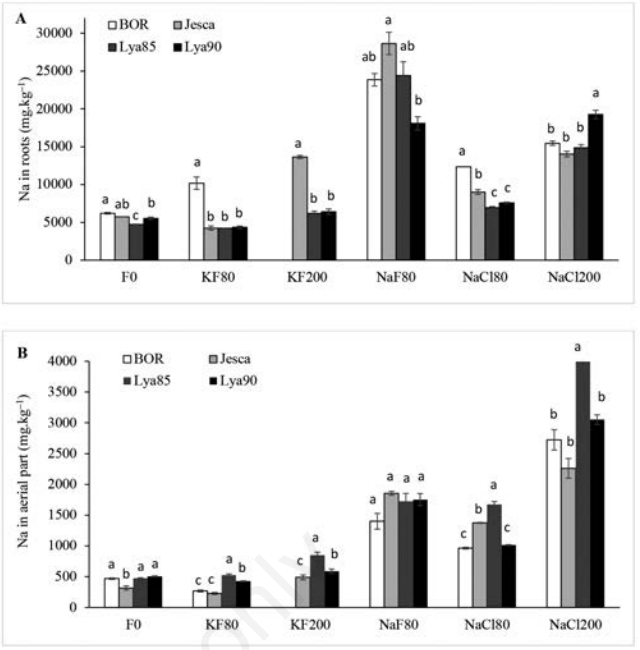

Figure 2. Effect of KF, NaF, and NaCl treatments on the accumulation of Na^+ (mg kg^{-1}) in aerial parts (AP) (A) and roots (R) (B) of the four tested bean genotypes. Standard errors are shown as vertical bars. Different letters indicate significant differences at P<0.01 according to the Tukey post hoc test among genotypes within each treatment.

Table 4. F (ppm) content in aerial part (F-AP) and root (F-R) of BOR, JES, LYA85, and LYA90 (Borlotto, Jesca, Lyamunguru 85, Lyamunguru 90) treated with KF and NaF at 80-200 mg kg^{-1}. Values from the control treatment without fluorine (F0) are also reported.

Treatment (mg kg^{-1})	Genotypes	F (AP)	F (R)
F0	BOR	4.08 ± 1.19^{Cb}	6.75 ± 0.59^{Ba}
	JES	2.40 ± 0.17^{Db}	4.02 ± 0.28^{Cb}
	LYA85	4.17 ± 0.52^{Bb}	3.08 ± 0.58^{Bb}
	LYA90	8.15 ± 0.30^{Ca}	4.81 ± 0.13^{Cab}
KF80	BOR	18.52 ± 1.35^{B}	31.98 ± 6.13^{A}
	JES	15.90 ± 0.35^{C}	25.06 ± 0.02^{B}
	LYA85	9.83 ± 0.31^{B}	16.93 ± 2.38^{B}
	LYA90	14.27 ± 3.62^{BC}	17.50 ± 6.78^{BC}
KF200	BOR	NA	NA
	JES	75.70 ± 3.42^{Ab}	47.63 ± 2.15^{Ab}
	LYA85	100.11 ± 11.57^{Aab}	65.31 ± 7.55^{Aa}
	LYA90	115.44 ± 0.54^{Aa}	37.60 ± 0.26^{Ab}
NaF80	BOR	37.21 ± 3.20^{Aa}	29.93 ± 4.33^{Ab}
	JES	27.93 ± 1.25^{Bab}	43.41 ± 1.94^{Aa}
	LYA85	17.67 ± 3.07^{Bb}	10.98 ± 2.08^{Bc}
	LYA90	21.25 ± 1.88^{Bb}	25.17 ± 2.08^{Abb}
NaF200	BOR	NA	NA
	JES	NA	NA
	LYA85	NA	NA
	LYA90	NA	NA
Probability level of significance (ANOVA)			
	Genotype (A)	NS	NS
	Treatment (B)	***	***
	A*B	***	***
	Coefficient of variation	114%	74%

Data are expressed as average value (coefficient of variation); $^{a-c}$different lowercase letters indicate significant differences at P<0.01 according to the Tukey post hoc test among genotypes within the treatment; $^{A-C}$different capital letters indicate significant differences at P<0.01 according to the Tukey post-hoc test comparing different treatments within genotype; NA, not available plants; NS, non-significant.

LYA90. LYA 85 was again the genotype with the highest Ca content in AP under F treatments. However, Ca content in the AP showed a continuous increase with NaCl treatment (44% BOR, 99% JES, 43% LYA85, and 65% LYA90 at NaCl80 (Figure 3). The Ca/Na ratio in AP decreased in all genotypes and treatments except for BOR and JES under KF 80. The lowest ratio was 0.88 for LYA85 under KF200 (Table 4). All genotypes exhibited a low Ca/Na ratio in AP, with the lowest content under KF200 and NaF80. As a consequence of a more significant increase in Na and decreased K concentration under NaCl and NaF treatment, the K/Na ratio in AP decreased in response to the increased salt level. Indeed, the lowest decrease was observed for LYA85 under KF200 and NaCl200. Under NaF, no difference in terms of genotypes was recorded. In roots, JES showed an increase in K/Na compared to the other African bean genotypes under KF200, while JES showed the lowest ratio under NaF 80 (Table 5).

The bean genotypes with the highest Ca/Na ratios were the most tolerant to F stress, and the most sensitive showed the lowest K/Na ratios. Our results showed that the K/Na ratio decreased (especially in R) through salt-treated plants, and Na was more concentrated in R than in AP. Moreover, JES showed the highest K/Na ratio in AP. However, according to the Ca/Na in the roots, LYA85 and LYA90 showed the highest ratio under high F concentration. Therefore, based on the stability of the tolerance to salinity from germinative to seedlings stage, the commercial variety BOR was identified as the most sensitive to F. Indeed, BOR showed a high accumulation of F and Na in R and AP with the lowest calcium content under high KF and NaF concentration. Among the African bean genotypes, LYA 90 translocated F to shoots. The results indicated that JES and LYA85 could accumulate less Na and F in their R and AP, although JES was more susceptible than LYA85. In addition, JES showed a higher reduction in biomass and R length under high NaF concentration than LYA85. However, LYA85 was the most tolerant variety to F exposure among the tested genotypes.

Discussion

The effect of F treatment on germination, growth and F content in plants is comparatively unexplored in the scientific literature.

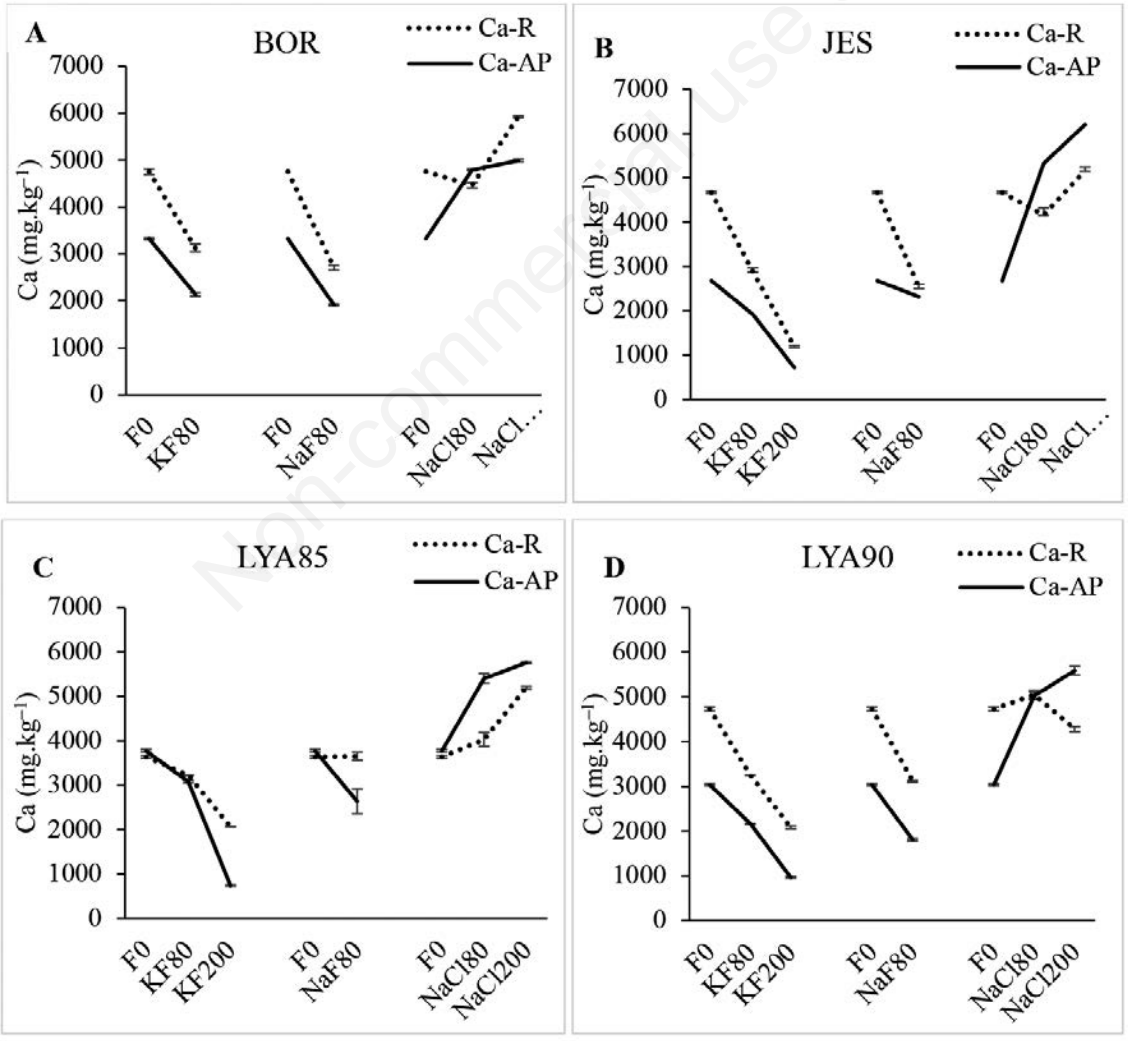

Figure 3. Effect of KF, NaF, and NaCl (mg kg^{-1}) on the accumulation of Ca^{2+} (mg kg^{-1}) in roots (R) and aerial parts (AP) of the four tested bean genotypes: A) BOR; B) JES (B); C) LYA85; and D) LYA90. Standard errors are shown as vertical bars.

Plant F tolerance could be associated with several factors such as plant species, genotype, and environmental conditions. To the best of our knowledge, this is the first study exploring the tolerance of the bean (*P. vulgaris*) to F, considering morpho-physiological parameters and the content of Ca Na and K in the biomass during the early stages of growth. Excess F in the substrate caused reduced seedling development and unbalanced nutrient uptake in the four bean genotypes under investigation. High concentrations of F (200 mg kg^{-1}) deeply altered germination. This behaviour could be associated with the impact of F on plant metabolism. Gadi *et al.* (2016) reported two possible negative effects of F on plants by: i) interfering with active plant metabolism, thus reducing the amylase activity and causing a lower rate of cell division and expansion; and by ii) inducing the gibberellic acid (GA) degradation in seeds deterring the endosperm saccharide metabolism during germination. In this study, partial inhibition of germination was observed in African bean genotypes under KF200, while a stronger effect was observed under NaF200, where even germination was completely inhibited. Similar results describing a more toxic effect of NaF than KF on bean growth were reported by Chahine *et al.* (2021). The higher toxicity of NaF than KF could be explained by a possible synergistic effect of Na and F together, causing the inhibition of vital systems in plants. As already described, NaF causes an inhibitory effect on DNA synthesis in germinating mung bean seeds, leading to decreased RNA and protein synthesis and reduced cell division and cell elongation (Nitsan and Lang, 1965).

Moreover, F affected root growth more strongly than the aerial part of the plant. Such a phenomenon may be due to increased absorption of F by roots as indicated by the total F content found in roots. Indeed, abnormal seedling development and unbalanced nutrient uptake due to F interference were reported in other plant species belonging to the Leguminosae family [*e.g.*, *Cicer arietinum* (Datta *et al.,* 2012)].

In plants, Ca is a ubiquitous secondary messenger involved in multiple signalling cascades in the plant system (Roychoudhury and Banerjee, 2017). Indeed, Ca content in the root cell walls acts as a buffer against F, thus determining plant F sensitivity, while F^- is complexed with Ca^{2+} present in the root cell walls. Thus, species with high Ca in the root control more effectively the absorption of F^- (Stevens *et al.,* 1998). It could be hypothesised that F^-, as an anion, would follow the pathway normally taken by chloride. Due to its high electronegativity, most F probably moves extracellularly from roots cell walls, using the apoplastic pathway to the stele, while only a low F^- takes the symplastic transport (cell membrane,

Table 5. Root (R) and aerial part (AP) K/Na and Ca/Na [K/Na (R), K/Na (AP), Ca/Na (R), Ca/Na (AP)] ratios of the bean genotypes studied (Borlotto, Jesca, Lyamunguru 85, Lyamunguru 90; BOR, JES, LYA85, and LYA90, respectively) grown in KF, NaF and NaCl sand without (F0) and with F (80 and 200 mg kg^{-1}).

Treatment (mg kg^{-1})	Genotypes	K/Na (AP)	K/Na (R)	Ca/Na (AP)	Ca/Na (R)
F0	BOR	51.49±0.64^{b}	1.97±0.00^{a}	7.11±0.14^{ab}	0.77±0.00^{c}
	JES	89.68±10.25^{a}	1.60±0.05^{c}	8.66±1.03^{a}	0.81±0.01^{b}
	LYA85	53.84±1.78^{b}	1.80±0.03^{b}	7.99±0.23^{a}	0.77±0.00^{c}
	LYA90	45.90±1.20^{b}	1.41±0.04^{d}	6.02±0.06^{b}	0.86±0.02^{a}
KF80	BOR	104.88±5.11^{b}	1.85±0.12^{c}	8.04±0.58^{a}	0.31±0.02^{b}
	JES	140.30±8.94^{a}	2.67±0.04^{b}	8.52±0.62^{a}	0.68±0.04^{a}
	LYA85	58.04±1.79^{c}	3.44±0.01^{a}	5.84±0.14^{b}	0.76±0.00^{a}
	LYA90	64.66±0.33^{c}	2.82±0.24^{b}	5.05±0.05^{b}	0.75±0.03^{a}
KF200	BOR	NA	NA	NA	NA
	JES	52.00±0.00^{a}	3.17±0.00^{a}	1.40±0.00^{b}	0.08±0.00^{b}
	LYA85	32.56±0.00^{c}	1.73±0.06^{b}	0.88±0.00^{c}	0.35±0.02^{a}
	LYA90	43.61±1.39^{b}	1.41±0.05^{c}	1.64±0.08^{a}	0.35±0.02^{a}
NaF80	BOR	18.21±2.20^{a}	0.27±0.01^{ab}	1.40±0.20^{a}	0.11±0.01^{b}
	JES	16.16±0.54^{a}	0.15±0.01^{c}	1.25±0.00^{a}	0.09±0.00^{b}
	LYA85	17.02±1.47^{a}	0.24±0.01^{b}	1.57±0.29^{a}	0.15±0.01^{a}
	LYA90	15.74±0.51^{a}	0.29±0.1^{a}	1.04±0.07^{a}	0.17±0.01^{a}
NaF200	BOR	NA	NA	NA	NA
	JES	NA	NA	NA	NA
	LYA85	NA	NA	NA	NA
	LYA90	NA	NA	NA	NA
NaCl80	BOR	28.17±0.68^{a}	0.45±0.00^{b}	4.97±0.10^{a}	0.36±0.00^{d}
	JES	21.36±0.21^{c}	0.40±0.00^{c}	3.87±0.02^{b}	0.46±0.00^{c}
	LYA85	16.10±0.58^{d}	0.63±0.01^{a}	3.24±0.16^{c}	0.58±0.01^{b}
	LYA90	25.40±0.23^{b}	0.47±0.02^{b}	4.96±0.05^{a}	0.67±0.00^{a}
NaCl200	BOR	9.22±0.04^{b}	0.22±0.00^{d}	1.83±0.03^{b}	0.38±0.00^{a}
	JES	13.09±0.84^{a}	0.25±0.00^{c}	2.77±0.19^{a}	0.37±0.00^{a}
	LYA85	6.71±0.37^{c}	0.31±0.00^{a}	1.45±0.08^{c}	0.35±0.01^{b}
	LYA90	8.68±0.18^{b}	0.27±0.00^{b}	1.83±0.02^{b}	0.22±0.00^{c}
Probability level of significance (ANOVA)					
	Genotype (A)	NS	NS	NS	NS
	Treatment (B)	***	***	***	***
	A*B	***	***	***	***
	Coefficient of variation	32%	19%	21%	19%

$^{a-d}$Different letters indicate significant differences at P<0.01 according to the Tukey post hoc test among genotypes within the treatment; NA, not available plants; NS, non-significant.

plasmalemma, or tonoplast) (White and Broadley, 2001).

Moreover, F acts as a metabolic inhibitor in plants (Iram and Khan, 2016). At the molecular level, a large number of Ca transporters, sensor/decoder elements, and calcium-dependent transcription factors are known to be regulated by Ca at different levels by direct binding of Ca calmodulin or other kinases/phosphatases. In addition, Ca is also essential for K-Na selectivity, markedly reducing K efflux in salt-stressed plants (Munns and Tester, 2008). However, a high Na/Ca ratio has a deleterious effect on the function of membranes within cells (Cramer *et al.*, 1985).

Several studies have reported the role of Ca in the salinity stress response, with little information available about its role as a mediator of stress caused by F. The present study showed an opposite trend of Ca response to NaCl and F stresses, which revealed that low Ca increased membrane permeability, leading to an increase in passive F and Na transport. This response became noticeable at high external ion concentrations, *i.e.*, NaF compared to KF. In addition, the comparison of the Ca content in both the roots and the aerial part of the four tested bean genotypes indicated that LYA 85 had the lowest reduction of Ca content under KF80 and NaF 80 compared to the control and, at the same time it accumulated the lowest content of F in its organs, thus indicating that it was more tolerant to F than the other genotypes.

Our results showed a significantly higher F translocation from the R into AP under a high concentration of F (KF200) for all the bean genotypes. The highest translocation factor (TF) was found in LYA90 under KF200. Conversely, JES appeared to be able to accumulate less F compared to LYA85 and LYA90 under NaF and KF treatments since it showed the lowest translocation rate at 80 mg kg^{-1}. BOR accumulated more F compared to the African bean genotypes. Variable F accumulation in the AP biomass between cultivars had been previously reported. This phenomenon was related to the variable root-shoot translocation efficiency determining F accumulation in AP (Mondal, 2017).

Moreover, F accumulation in leaves is mainly in the form of free F anions or in connection with aluminium (Al), Ca, and magnesium (Mg) (Weinstein and Davison, 2004). Ion regulation is an essential factor regulating plant salt tolerance. The studies using NaCl as the selective agent revealed the ability of specific plant genotypes to maintain higher levels of K and Ca and low levels of Na and Cl as one of the critical mechanisms contributing to the expression of high salt tolerance. In the screening of plant genotypes for salt tolerance, K/Na and Ca/Na ratios in the aerial part and tissues Na concentration have often been proposed as a useful screening tool to determine the salt tolerance of different plant species (Shabala and Cuin, 2008).

High Na content in the soil solution can inhibit the uptake of other nutrients because Na interferes with various transporters in the root plasma membrane, such as K-selective ion channels, and constraints root growth (Tester and Davenport, 2003). Although Na transport to AP is largely unidirectional through the xylem, it can only return to roots via the phloem, a limited process that results in progressive accumulation of Na as leaves age (Tester and Davenport, 2003). The toxic effect of Na is due to its tendency to replace K in key enzymes of the cytosol and organelles and to trigger the accumulation of reactive oxygen species (Munns *et al.,* 2016). High levels of Na may also adversely affect plants' nutritional status by interfering with Ca's absorption, resulting in NaCl toxicity in plant tissues caused by low Ca/Na ratios (Kent and Lauchli, 1985).

Conclusions

The contamination of soil with F proved to be particularly serious for salt-sensitive crops such as beans that feed many people worldwide. The results obtained in this study provided new evidence on the key physiological responses that underlie F tolerance in *Phaseolus vulgaris* at a very early stage of growth. The high sensitivity shown by this crop has compromised plant survival and yield. In summary, the observed patterns of mineral and morpho-physiological changes in the four bean genotypes exposed to increasing F concentrations supplied through different salts revealed that: i) the most resistant genotypes, LYA85 and JES, showed a reduced F uptake and translocation from roots to the aerial part; ii) LYA85 showed the lowest F content in roots and its sequestration with Ca; iii) F contamination affects the mobility of minerals and their translocation to growing organs, which enhanced Na uptake and reduced K and Ca absorption in root and shoot of all the bean genotypes studied; iv) BOR was more sensitive to F toxicity than the other genotypes; v) F inhibited shoot elongation, and the root system proved to be more sensitive than the aerial part to the F stress.

This study has revealed the central role of Ca as a key secondary messenger in regulating plant growth and development under F stress. High throughput genetic analyses should be performed to identify quantitative traits to be exploited to generate F-tolerant characters in susceptible crops. Further research is needed to ascertain rates of plant uptake of F under a wide range of soil pH values and the potential impacts of elevated F levels on microbiological processes such as nitrogen fixation and nitrification.

References

Aktas H, Abak K, Cakmak I, 2006. Genotypic variation in the response of pepper to salinity. Sci. Hort. 110:260-6.

Baker AJM, 1986. Metal tolerance. New Phytol 106:93-111.

Chahine S, Giannini V, Roggero PP, Melito S, 2021. First insight of exogenous addition of proline and glycinebetaine to mitigate fluorine toxicity effects on common bean seedlings. Ital. J. Agron. 16:1754.

Choubisa SL, Choubisa D, 2016. Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. Environ. Sci. Pollut. Res. 23:7244-54.

Cramer GR, Läuchli A, Polito VS, 1985. Displacement of Ca 2+ by Na + from the Plasmalemma of Root Cells. Plant. Physiol. 79:207-11.

Cronin SJ, Neall VE, Lecointre JA, Hedley MJ, Loganathan P, 2003. Environmental hazards of Fuoride in volcanic ash: a case study from Ruapehu volcano. J. Volcanol. Geotherm. Res. 121:271-91.

Datta J, Maitra A, Mondal N, Banerjee A, 2012. Studies on the impact of fluoride toxicity on germination and seedling growth of gram seed (Cicer arietinum L. cv. Anuradha). J. Stress Physiol. Biochem. 8:194-202.

Dey U, Mondal NK, Das K, Datta JK, 2012. Dual effects of fluoride and calcium on the uptake of fluoride, growth physiology, pigmentation, and biochemistry of Bengal Gram seedlings (Cicer arietinum L.). Fluoride 45:389-93.

Elloumi N, Ben Abdallah F, Mezghani I, Rhouma A, Boukhris M, 2005. Effect of flouride on almond seedlings in culture solution. Fluoride 38:193-8.

FAOSTAT, Food and Agriculture Organization (of the United Nations); 2014. Online database.
Fornasiero RB, 2003. Fluorides effects on Hypericum perforatum plants: first field observations. Plant Sci. 165:507-13.
Frencken J, 1992. Endemic fluorosis in developing countries: causes, effects and possible solutions. Publication no. 91.082. NIPG-TNO, Leiden, The Netherlands.
Gadi BR, Bhati K, Goswami B, Rankawat R, Kumar S, Kumar R, Ram A, Pooja V, Laxmi V, Singariya P, 2016. Sources and phytotoxicity of fluorides in the environment. In: B.R. Bamniya, B.R. Gadi (Eds.), Environmental impact on biodiversity. Today & Tomorrow's Printers and Publishers, New Delhi, India, pp. 251-66.
Galkovskyi T, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR, Fang S, Harer J, Benfey PN, Weitz JS, 2012. GiA Roots: Software for the high throughput analysis of plant root system architecture. BMC Plant. Biol. 12:116.
George F, Ramachandran KM, 2011. Estimation of parameters of johnson's system of distributions. JMASM 10:494-504.
Gupta S, Banerjee S, Mondal S, 2009. Phytotoxicity of fluoride in the germination of paddy (Oryza sativa) and its effect on the physiology and biochemistry of germinated seedlings. Fluoride 42:142-6.
International Programme on Chemical Safety, United Nations Environment Programme, International Labour Organization, World Health Organization & IPCS Task Group on Environmental Health Criteria for Fluorine and Fluorides, 1984. Fluorine and fluorides. World Health Organization, Geneva, Switzerland. Available from: https://apps.who.int/iris/handle/10665/37288
Iram A, Khan TI, 2016. Effect of sodium fluoride on seed germination, seedling growth and biochemistry of Abelmoschus esculentus. J. Plant Bioch. Physiol. 4:170.
Jha SK, Nayak AK, Sharma YK, 2008. Response of spinach (Spinacea oleracea) to the added fluoride in an alkaline soil. Food Chem. Toxicol. 46:2968-71.
Kamaluddin M, Zwiazek JJ, 2003. Fluoride inhibits root water transport and affects leaf expansion and gas exchange in aspen (Populus tremuloides) seedlings. Physiol. Plant 117:368-75.
Katungi E, Farrow A, Chianu J, Sperling L, Beebe S, 2009. Common bean in Eastern and Southern Africa : a situation and outlook analysis. International Centre for Tropical Agriculture, 1–44.
Kent LM, Lauchli A, 1985. Germination and seedling growth of cotton: salinity-calcium interactions. Plant Cell Environ. 8:155-9.
Loganathan P, Hedley MJ, Grace ND, Lee J, Cronin SJ, Bolan NS, Zanders JM, 2003. Fertiliser contaminants in New Zealand grazed pasture with special reference to cadmium and fluorine - a review. AJSR 41:501-32.
Maggio A, Reddy MP, Joly RJ, 2000. Leaf gas exchange and solute accumulation in the halophyte Salvadora persica grown at moderate salinity. Environ. Exp. Bot. 44:31-8.
Mondal NK, 2017. Effect of fluoride on photosynthesis, growth and accumulation of four widely cultivated rice (Oryza sativa L.) varieties in India. Ecotoxicol. Environ. Saf. 144:36-44.
Munns R, James RA, Gilliham M, Flowers TJ, Colmer TD, 2016. Tissue tolerance: an essential but elusive trait for salt-tolerant crops. Funct. Plant Biol. 43:1103-13.
Munns R, Tester M, 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59:651-81.
Nitsan J, Lang A, 1965. Inhibition of cell division and cell elongation in higher plants by inhibitors of DNA synthesis. Dev. Biol. 12:358-76.
Rizzu M, Tanda A, Canu L, Masawe K, Mtei K, Deroma MA, Roggero PP, Seddaiu G, 2020. Fluoride uptake and translocation in food crops grown in fluoride-rich soils. J. Sci. Food Agric. 100:5498-509.
Roychoudhury A, Banerjee A, 2017. Abscisic acid signaling and involvement of mitogen activated protein kinases and calcium-dependent protein kinases during plant abiotic stress. Mechanism of plant hormone signaling under stress, 1:197-241.
Shabala S, Cuin TA, 2008. Potassium transport and plant salt tolerance. Physiol. Plant 133:651-69.
Stevens DP, Mclaughlin MJ, Alston AM, 1998. Phytotoxicity of the fluoride ion and its uptake from solution culture by Avena sativa and Lycopersicon esculentum. Plant Soil. 200:119-29.
Tester M, Davenport R, 2003. Na+ tolerance and Na+ transport in higher plants. Ann. Bot. 91:503-27.
Weinstein H, Davison A, 2004. Uptake, transport and accumulation of inorganic fluorides by plants and animals. In: L.H. Weinstein, A. Davison (Eds.), Fluorides in the environment: effects on plants and animals. CABI Publishing, London, UK, pp. 21-55.
White PJ, Broadley MR, 2001. Chloride in soils and its uptake and movement within the plant: a review. Ann. Bot. 88:967-88.