Endogenous Calcium Mediated The Seedling Growth And Fluoride Stress Tolerance In Four Bean Genotypes

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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 in F contaminated areas of the world. F tolerance during germination and seedling growth was evaluated for four bean ecotypes: Borlotto nano 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 a potential effect of different Na levels in plant. Total F content and minerals accumulation (Na, K and Ca) in roots and shoots were measured. The translocation factor, growth ratio, 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 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 the plant growth and development under F stress.

Introduction

Fluorine contamination of soil and groundwater represents a serious risk for human health in several countries. The maximum permissible limit of fluoride (F) in drinking water is 1.5 mg L^{-1}, (WHO, 1984). However, there is no stringent threshold limit of F content in soil and plant tissues above which the ingestion is considered to be detrimental to human health. In America, Asia, Middle East, and Africa, people consume water with fluoride concentration greater than 1.5 mg L^{-1} (Frencken, 1992). Contaminated soils pose a threat on human, animal and plant health. Although F is considered an essential element to 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). The excess F in water, air and soils is caused by weathering of volcanic ashes (Cronin et al. 2003), 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, the duration of the exposure to F, over the solubility of the F salt.

High level of F inhibits 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). F absorbed from soil is translocated to the shoots, causing physiological, biochemical and structural damages, depending on its concentration in the substrate and its translocation from roots to shoots (Dey et al., 2012). A high concentration of F adversely affects growth and survival of plants as a consequence of its inhibitory effects on respiration, photosynthetics pigments (Kamaluddin and Zwiazek, 2003), mineral and water uptake and enzymes activity (Fornasiero, 2003).

Sensitivity to F is highly species-dependent (Beast and Haeck, 1983), indeed some plants are able to accumulate F at higher concentrations (up to 4000 μg F g^{-1}) displaying no sign of toxicity (Jha et al., 2008), while other show signs of toxicity at much lower concentration although some species are 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, good carbohydrates and iron among the majority of 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 it happens across many districts of the Rift Valley in East Africa.

While many studies explored the plant response to NaCl salinity, fragmented information is available on plant tolerance to F. Nevertheless, the processes and mechanisms behind NaCl plants' adaptative methods could be useful to paving the way for an investigation into the adaptation strategies of plants to F. Many studies revealed that the ability of plant genotypes to maintaining 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 the screening of plant genotypes for salt tolerance, the shoot K: Na and Ca: Na ratios and tissue Na concentration have often been proposed as a useful screening tool to assess the salt tolerance of different crop species (Aktas et al. 2006; Shabala and Cuin, 2008).

Starting from these milestones, three common bean African genotypes and one commercial variety were selected to compare the effect of different sources of F (NaF and KF) in selected morpho-physiological traits and principal mineral contents. We hypothesized that the effects of excess F content and of other ions (Ca^{2+}, K^{+}, Na^{+}) in the substrate is 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 response of African bean genotypes to F at the seedling level; (ii) the contribution of ion accumulation to the osmotic adjustment and the role of Na, K and Ca homeostasis in the determination of salt tolerance; (iii) 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 on early development stages of different bean genotypes as influenced by increasing exposure to F-based salts. The ultimate aim was to contribute to the rapid identification of promising genotypes which can be used for selection of F-tolerant bean varieties i.e. able to grow in F-rich soils and to minimize the F absorption and translocation in edible plant organs.

Material And Methods

Plant material and experimental conditions

Mature seeds of four bean (*Phaseolus vulgaris* L.) genotypes were used for the germination experiments. 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, 18h 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 were reported as salt name and level (i.e. KF 80 mg kg^{-}, is reported as KF80) The choice of salt concentration levels was based according to 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) contain only silica sand. Plastic containers (58 x 72 mm) containing two seeds each were used for each salt* level* variety combination, 15 replicates (containers) were used for each treatment. experiment was repeated twice.

During the experiment, irrigation was performed adding 5 mL of water in each box every 24 hours. The F and Na^{+} contents in the enriched sand were monitored with 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

The germination was recorded daily. Seedlings with a hypocotyl-radicle axis > 3 cm were considered as germinated. The proportion of germination 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 the 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, oven-dried at 65ºC 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):

$$\%G = \frac{no.seedsgerminated}{Totalno.seeds} * 100 \text{ (Eq. 1)}$$

$$GR = \frac{Plantbiomasswithsalt}{Plantbiomasswithoutsalt} * 100 \text{ (Eq. 2)}$$

$$TI = \frac{Rootlengthwithsalt}{Rootlengthwithoutsalt} \text{ (Eq. 3)}$$

Determination of F content in root and shoot

The total F content was estimated with an acid digestion method and subsequently quantified by 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). The digestion was applied at least in duplicate to each of the samples analysed.

Translocation factor (TF) was calculated for F as:

$$TF = \frac{Fconcentrationinshoot}{Fconcentrationinroot} \text{ (Eq. 4)}$$

Macro and Micro-nutrients 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 randomized design with fifteen replicates. After having checked the main assumption of ANOVA, 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 to 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 of germination percentage (G%) was observed for all genotype as a consequence of the F treatment 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, the germination was significantly affected for all the 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 beans' genotypes were more tolerant to KF than NaF, and a significant effect of the treatment level was observed ($P < 0.0001$). Beside the treatment, also the genotype differentially responded to the F treatments ($P < 0.01$). BOR was the most susceptible variety, and TI value was computable until 80 mg kg^{-1} (0.72, and 0.76 respectively for KF and NaF). Indeed, BOR seeds did not germinate under 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, also GR% was significantly affected by the treatments and genotypes ($P = 0.0091$; $P < 0.0001$, respectively). GR% for BOR followed the same trend observed for 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 the AP with highly significant differences ($P < 0.0001$) (Table 3). DW-R decreased drastically with F salt concentration (Table 3). The effect of F treatments (80 mg kg^{-1}) were much more negative on roots than on the aerial part. 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 not 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 the treatments except for NaCl 80 in BOR and JES compared to the control. This indicated that the R/AP of all genotypes

responded in a similar manner 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 salts concentrations (treatment levels) had a significant impact on 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 in terms of F accumulation among all the genotypes for both AP and 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 quantity of F in their R when comparing KF200 to F0 (+32.8 mg kg^{-1} and +43.6 mg kg^{-1} respectively). LYA 85 instead accumulated +62.2 mg kg^{-1} of F with KF200 compared to F0 in R. (Table 4).

About NaF, the only data available is the treatment with 80 mg kg^{-1}, since higher concentration of salt inhibited the germination. BOR was able to accumulate the greatest quantity of F in AP compared to the other genotypes, reaching the value of 37.2 mg kg^{-1}. In general, all African bean genotypes accumulated less F in both organs compared to BOR, absorbing probably less F from the enriched (NaF) substrate used. 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 accordingly 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) (Fig. 1).

Ion's concentrations

Na and Ca cations distribution in R and AP tissues were studied in all genotypes. Na content varied significantly among organs (R and AP) and treatment ($P < 0.0001$). As expected, under NaF and NaCl, organs showed a higher level of Na compared to 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. 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%) (Fig. 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 (Fig. 2): the highest Na content was reached for JES under NaF 80 and NaCl 80 respectively 584% and 433% (Fig. 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 KF80 mg kg^{-1} treatment in BOR, JES and LYA90 by 34%, 38% and 31% respectively while for LYA85 it decreased only by 11%. Ca content in roots 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 NaF treatment. 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 (Fig. 3).

A similar trend was observed in AP. Both under KF80 and NaF 80, Ca decreased significantly. More in detail, under KF80 it decreased by 36% in BOR, 28% in JES, 19% in LYA85 and 29% in LYA90, while under NaF80 it decreased by 52% in BOR, 13% in JES, 30% in LYA85 and 40% 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 (Fig. 3).

The Ca/Na ratio in AP decreased in all genotypes and treatment 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 greater increase in Na and decreased K concentration under both 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 root, 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 which showed 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 K/Na ratio decreased (especially in roots) through salt-treated plants and Na was more concentrated in roots than in the aerial part. JES showed the highest K/Na ratio in AP, thus revealing higher capability of storing the toxic ions as Na in the roots. 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. 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 thus mimiking the behaviour of a hyperaccumulator. The results indicated that JES and LYA85 were able to accumulate less Na and F in

their R and AP, altough JES was more susceptible than LYA85. In addition, JES showed a higher reduction in biomass and R length under high NaF concentration compared to LYA85. However, among the tested genotypes, LYA85 proved to be the most tolerant variety to F exposure.

Discussion

The effect of F treatment on germination, growth, and F content in plants is still few investigated in the scientific literature. Plant F tolerance could be associated with several factors such as plant species, genotype and environmental conditions. To the best of our knowledge, this was the first study exploring the bean (*P. vulgaris*) tolerance to F, taking into account 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 seedlings development and unbalanced nutrient uptake in the four beans genotypes under investigation.

High concentration 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 the germination (Gadi et al. 2016). 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 about a more toxic effect of NaF than KF on bean growth have been reported by Chahine et al. (2021). The higher toxicity of NaF than KF could be justified 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 to reduced cell division and cell elongation (Nitsan and Lang, 1965).

F affected more strongly root growth 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 was reported in another plant species belonged to Leguminosae family, such as *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). Ca content in the root cell walls acts as a buffer against F, thus determining plants' F sensitivity. F^- is complexed with Ca^{2+} present in the root cell walls. Thus, species with high Ca in the root better control the absorption of F^- (Stevens et al. 1998). It could be hypothesised that F^-, as an anion, would follow the pathway taken normally by chloride. Due to its high electronegativity, most of F probably moves extracellularly from roots cell walls, using the apoplastic pathway to the stele, while only a low F content take the symplastic transport (cell membrane, plasmalemma, or tonoplast) (White and Broadley, 2001).

Therefore, F acts as 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 either by direct binding of Ca, or calmodulin or by other kinases/phosphatases. Ca is also essential for K-Na selectivity, markedly reducing K efflux in salt-stressed plants (Munns and Tester, 2008). A high Na/Ca ratio, however, has a deleterious effect on the function of membranes within cells (Cramer et al. 1985) .

Several studies have reported the role of Ca in salinity stress response, and little information is available about its role as 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 the 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 significant higher F translocation from the R into AP under high concentration of F (KF200) for all the bean genotypes studied. The highest Translocation Factor (TF) was found in LYA90 under KF200. 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 key 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 crop 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 constrains 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 which is a limited process and thus 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 the nutritional

status of plants by interfering with the absorption of Ca, resulting in NaCl toxicity in plant tissues caused by low Ca/Na ratios (Kent and Lauchli, 1985).

Conclusions

The soil contamination with F proved to be particularly serious for the salt-sensitive crops that feed many people around the world, like bean. 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 showed 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

1. the most resistant genotypes, LYA85 and JES, showed a reduced F uptake and translocation from roots to the aerial part,
2. the lowest F content in roots and its sequestration with Ca was shown by LYA85,
3. 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,
4. BOR was more sensitive to F toxicity than the other genotypes,
5. F inhibited shoot elongation, and the root system proved to be more sensitive than aerial part to the F stress.

This study has unfolded the central role of Ca, as a key secondary messenger in regulating the 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 also 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.

Declarations

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Author Contributions

GS, SM, PPR conceived and designed the research; SC, performed the experiments and analysed the data; VG, GS and PPR, analysed the data and revised the manuscript, PPR, GS and SM discussed the data; SC wrote the manuscript with the contributions from the other authors.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Tables

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

Treatment	Genotype			
(mg kg^{-1})	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}

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). GR% and TI were calculated according to Eq 2 and 3 (see material and methods). Data are compared to the control (F0) results (TI = 1, GR = 100%). Different lowercase letters indicate significant differences at p <0.01 according to the Tukey post-hoc test based on treatment within each genotype. Different capital letters indicate significant differences at p <0.01 according to the Tukey post-hoc test based on genotype within each treatment.

Genotype	Treatment	TI	GR%
	mg kg^{-1}		
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±0.00 ^{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		***	***

*NA: not available plants

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 aerial part (DW-AP), root (DW-R) and their ratio (R/AP) on 4 bean varieties: Borlotto, Jesca, Lyamunguru 85, Lyamunguru 90 (BOR, JES, LYA85 and LYA90, respectively) and the salt levels: control (F0), 80 and 200 ppm. In the table data are reported as means and coefficient of variation. Different lowercase letters indicate significant differences at $p<0.01$ according to Tukey post hoc test among treatments levels within each genotype; 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

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}. In table are reported also the control treatment, without fluorine (F0). Data are expressed as average value (coefficient of variation). Different lowercase letters indicate significant differences at $p<0.01$ according to Tukey post hoc test among genotypes within treatment. Different capital letters indicate significant differences at $p<0.01$ according to the Tukey post-hoc test comparing different treatment within genotype.

Variety	Treatment	DW_AP	DW_R	R/AP
	mg kg^{-1}			
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±0.00^{ABb}	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)		***	n.s.	*
Treatment (B)		***	***	***
A*B		***	***	***
Coefficient of Variation		15%	49%	50%

Treatment mg kg^{-1}	Genotypes	F (AP)	F (R)
F0	BOR	4.08 ± 1.19^{Cb}	$6.75\pm0,59^{Ba}$
	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}
	BOR	NA	NA
NaF200	JES	NA	NA
	LYA85	NA	NA
	LYA90	NA	NA
Probability level of significance (ANOVA)			
Genotype (A)		NsS	NS
Treatment (B)		***	***
A*B		***	***
Coefficient of Variation		114%	74%

NA: not available plants

NS: non-significant

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}). Different letters indicate significant differences at $p<0.01$ according to Tukey post hoc test among genotypes within treatment.

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}
	BOR	NA	NA	NA	NA
NaF200	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)		n.s.	n.s.	n.s.	n.s.
Treatment (B)		***	***	***	***
A*B		***	***	***	***
Coefficient of Variation		32%	19%	21%	19%

Figures

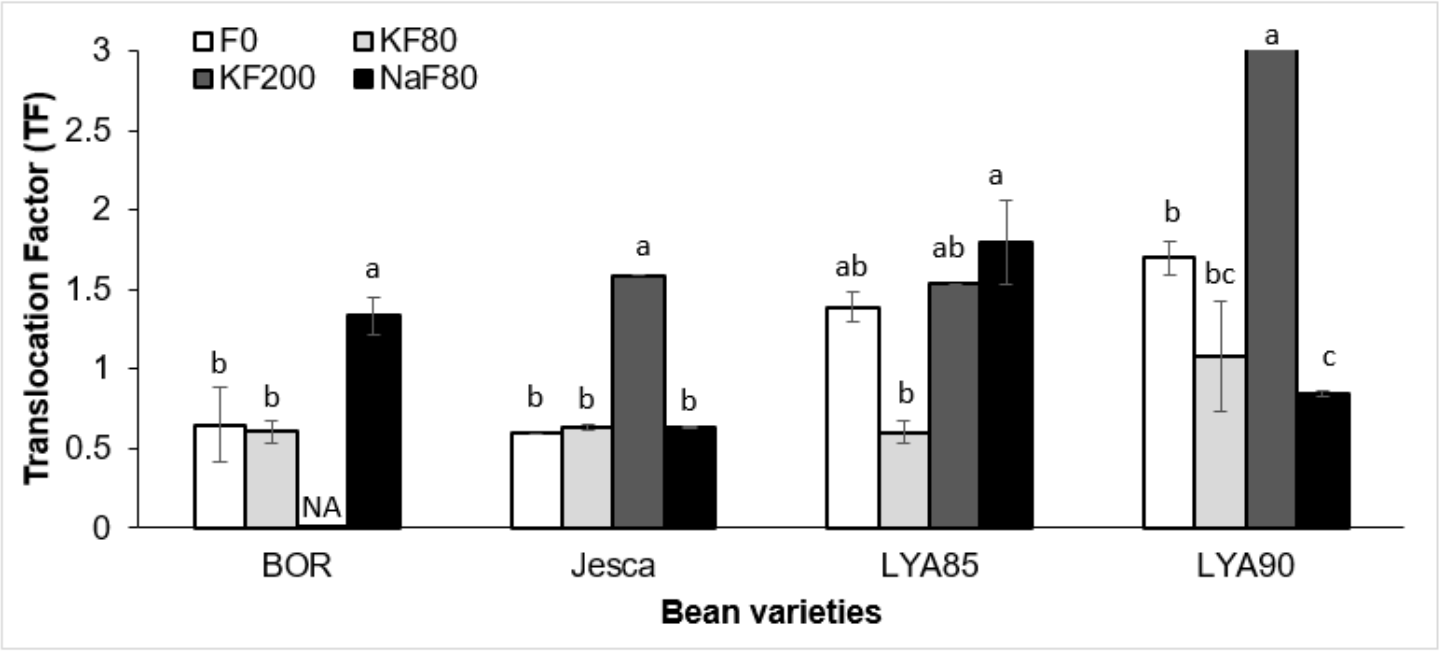

Figure 1

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 material and methods). 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

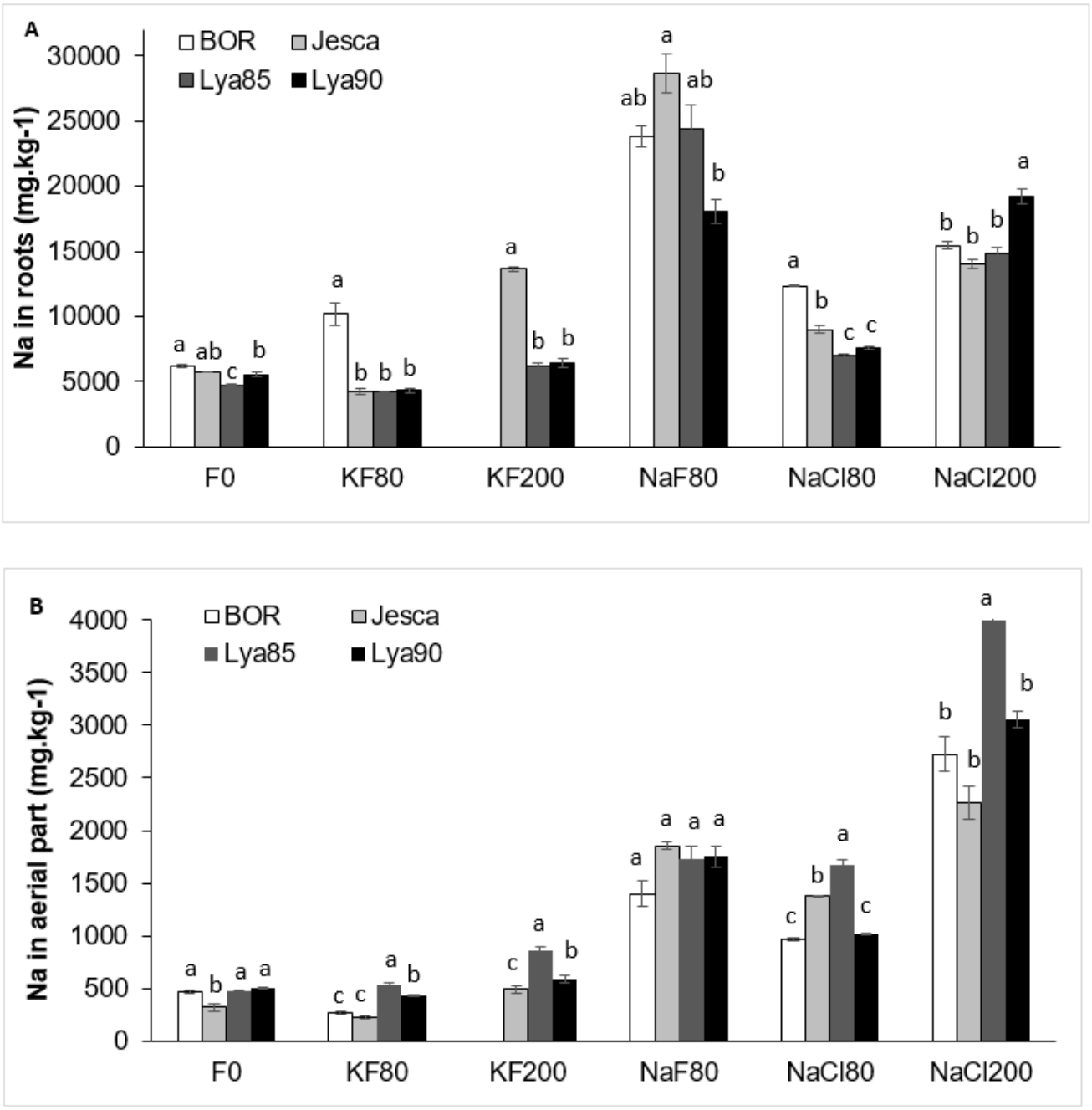

Figure 2

Effect of KF, NaF, and NaCl treatments on the accumulation of Na+ (mg kg-1) in aerial part (AP) (A) and root (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 Tukey post hoc test among genotypes within each treatment.

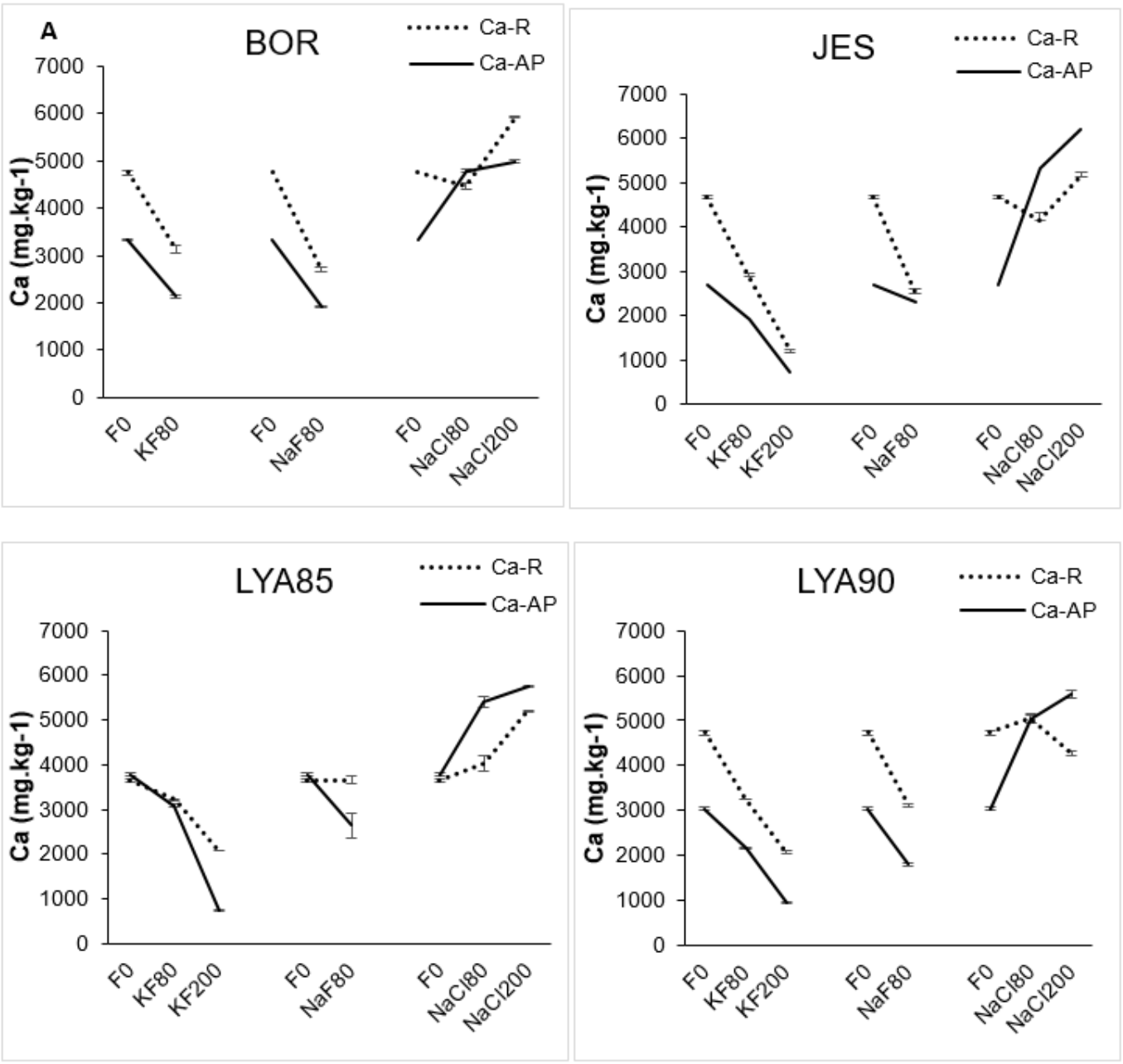

Figure 3

Effect of KF, NaF, and NaCl (mg kg -1) on the accumulation of Ca2+ (mg kg-1) in root (R) and aerial part (AP) of the four tested bean genotypes: BOR (A), JES (B), LYA85 (C) and LYA90 (D). Standard errors are shown as vertical bars.