

# Tolerance of cultivars and clonal selections of peach rootstocks to excess aluminum

## Tolerância de cultivares e seleções clonais de porta-enxertos de pessegueiro ao excesso de alumínio

Marcos Vinícius Miranda Aguiar<sup>1\*</sup>; Jean Michel Moura Bueno<sup>2</sup>; Newton Alex Mayer<sup>3</sup>; Gilberto Nava<sup>3</sup>; Gustavo Brunetto<sup>4</sup>; Luciane Almeri Tabaldi<sup>5</sup>

### Highlights

Root morphological variables can indicate the degree of Al tolerance in peach trees.

Six rootstock selections were tolerant to aluminum.

The critical level of Al in the roots of peach rootstock was 1400 mg Al kg<sup>-1</sup>.

The range of toxicity in the roots was between 1200 and 1500 mg Al kg<sup>-1</sup>.

### Abstract

Forms of aluminum (Al) present in the solution of tropical and subtropical soils can cause toxicity in rootstocks and peach cultivars, impairing growth and productivity. This can be minimized by growing Al-tolerant rootstocks and cultivars. However, this is not sufficiently known, especially because plant breeding programs do not always consider tolerance as a selection variable for genetic materials. The study aimed to (a) select cultivars and clonal selections of Al-tolerant peach rootstocks, (b) identify variables that confer Al tolerance for use in genetic improvement programs, and (c) propose critical levels (NC) and ranges of toxicity (TF) of Al in relation to morphological variables of the root system. The experimental design was completely randomized, comprising a factorial of 13 (cultivars and clonal selections) x 2 (with and without Al) with three replications. Own-rooted 'BRS Mandinho' peach seedlings (without rootstock) and grafted seedlings of 'BRS Mandinho' on different cultivars and clonal rootstock selections were cultivated in a hydroponic system, composing two levels for the Al factor (absence and presence at 100 mg L<sup>-1</sup>). The morphological variables of the canopy and root system, Al accumulation in tissues, translocation factor, and the critical level (NC) and toxicity range (TF) of Al in the roots were evaluated. Rootstocks FB-SM-09-43, JB-ESM-09-13, SAS-SAU-09-71, SS-CHI-09-40, 'Sharpe' and VEH-GRA-09-55 were tolerant at high Al concentrations. The NC of Al in the roots in relation to the root

<sup>1</sup> Doctoral Student in the Postgraduate Program in Forest Engineering, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brazil. E-mail: aguilmarcos2009@hotmail.com

<sup>2</sup> Prof. Dr., Universidade de Cruz Alta, UNICRUZ, Cruz Alta, RS, Brazil. E-mail: bueno.jean1@gmail.com

<sup>3</sup> Drs., Researchers, Brazilian Agricultural Research Corporation, Embrapa Temperate Climate, Pelotas, RS, Brazil. E-mail: alex.mayer@embrapa.br; gilberto.nava@embrapa.br

<sup>4</sup> Prof. Dr., Soil Science Department, UFSM, Santa Maria, RS, Brazil. E-mail: brunetto.gustavo@gmail.com

<sup>5</sup> Prof<sup>a</sup> Dr<sup>a</sup>, Biology Department, UFSM, Santa Maria, RS, Brazil. E-mail: lutabaldi@yahoo.com.br

\* Author for correspondence

surface area of peach rootstocks was 1400 mg Al kg<sup>-1</sup>, and the FT was between 1200 and 1500 mg Al kg<sup>-1</sup>.

**Key words:** Aluminum resistance. Aluminum toxicity. *Prunus*. Root morphology. Tissue aluminum.

## Resumo

Formas de alumínio (Al) presentes na solução de solos tropicais e subtropicais podem causar toxicidade em porta-enxertos e cultivares de pessegueiro, prejudicando o crescimento e a produtividade. Isso pode ser minimizado pelo cultivo de porta-enxertos e cultivares tolerantes ao Al. Porém, isso não é suficientemente conhecido, principalmente porque os programas de melhoramento vegetal nem sempre consideram a tolerância como uma variável de seleção de materiais genéticos. O estudo teve como objetivo (a) selecionar cultivares e seleções clonais de porta-enxertos de pessegueiro tolerantes ao Al, (b) identificar variáveis que confirmam tolerância ao Al para uso em programas de melhoramento genético e (c) propor níveis críticos (NC) e faixas de toxicidade (FT) do Al em relação às variáveis morfológicas do sistema radicular. O delineamento experimental foi inteiramente casualizado, compreendendo fatorial 13 (cultivares e seleções clonais) x 2 (com e sem Al) com três repetições. Mudanças de pessegueiro 'BRS Mandinho' autoenraizadas (sem porta-enxerto) e mudas enxertadas de 'BRS Mandinho' em diferentes cultivares e seleções de porta-enxertos clonais foram cultivadas em sistema hidropônico, compondo dois níveis para o fator Al (ausência e presença a 100 mg L<sup>-1</sup>). Foram avaliadas as variáveis morfológicas da parte aérea e do sistema radicular, acúmulo de Al nos tecidos, fator de translocação, NC e FT do Al nas raízes. Os porta-enxertos FB-SM-09-43, JB-ESM-09-13, SAS-SAU-09-71, SS-CHI-09-40, 'Sharpe' e VEH-GRA-09-55 foram tolerantes a altas concentrações de Al. O NC do Al nas raízes em relação à área radicular dos porta-enxertos de pessegueiro foi de 1400 mg Al kg<sup>-1</sup>, e o FT ficou entre 1200 e 1500 mg Al kg<sup>-1</sup>.

**Palavras-chave:** Alumínio em tecidos. Morfologia radicular. *Prunus*. Resistência do alumínio. Toxicidade de alumínio.

## Introduction

Peach (*Prunus persica* L.) is the most cultivated stone fruit species of the Rosaceae family, with more than 3000 species in approximately 110 genera distributed throughout the world (Eldem et al., 2012). Rootstock cultivars are selected in breeding programs considering the induction of productivity of canopy cultivars, fruit quality, and resistance to biotic and abiotic soil factors, among other factors (Reighard & Loreti, 2008; Betemps et al., 2020; Mayer et al., 2020; Mayer & Ueno, 2021). However,

tolerance to excess aluminum (Al) is usually not considered, which is necessary in tropical and subtropical soils (Mayer et al., 2015). This is because these soils naturally have low pH values and high Al contents (Ranjan et al., 2021; Shetty et al., 2021). Acidification and an increase in Al levels are natural processes in soils, but they have been increased in peach orchards by the application of fertilizers, especially nitrogenous ones (Nava et al., 2022; Paula et al., 2021). Therefore, there is a need to select cultivars and clones tolerant of excess Al.

Al<sup>3+</sup> in the solution can impair cell division and elongation, increasing root diameter, decreasing growth and, consequently, volume and surface area (Bahmani et al., 2020; Dorneles et al., 2016; Yan et al., 2021). Thus, the roots are likely to reduce water and nutrient uptake (Reis et al., 2018), which may slow the growth of young peach trees and delay the onset of fruit production (Mayer & Ueno, 2021). However, peach cultivars and clones may present tolerance mechanisms, such as higher Al accumulation in plant tissues, increased root length and surface area and higher dry weight production. Thus, the plants can adapt to soils with a higher Al content, which is desired.

Cultivars and clonal selections that are more tolerant to Al can also be selected considering critical levels (NC) and toxicity ranges (FT) (Alva et al., 1986; Baquy et al., 2017). Thus, adapted plants that present higher NC or FT can be grown in soils with higher Al content. However, NC and FT are not well known for peach trees. This can be achieved through the use of machine learning techniques combined with a database containing soil nutritional information and plant growth variables (Padarian et al., 2020). In this scenario, the existing biological relationships between plants and nutrients or toxic elements, such as the response to growth variables, are related through Bayesian models (Theobald & Talbot, 2002). This technique has been used to support the development of criteria for decision making in fertilizer recommendation systems (Kyveryga et al., 2013; Theobald & Talbot, 2002). In addition, the translocation factor is an interesting variable to be analyzed in studies of tolerance of genetic materials to

toxic metals, as it calculates the ability of plants to transfer metals from the root to the canopy (Raj et al., 2020).

Our hypothesis was that morphological variables of the root system could be used in the selection of genetic materials tolerant to Al toxicity. In addition, cultivars and clonal selections more tolerant to Al can also be selected considering critical levels, toxicity ranges and the translocation factor. This study aimed to (a) select cultivars and clonal selections of Al-tolerant peach rootstocks, (b) identify variables that confer Al tolerance for use in genetic improvement programs, and (c) propose critical levels (NC) and ranges of toxicity (TF) to Al in relation to morphological variables of the root system.

## Materials and Methods

### *Plant material*

Peach seedlings of the cultivar BRS-Mandinho, which produces flat or platycarp fruit (Raseira et al., 2016), were produced in a container (citropotes) with a capacity of 3.78 L. The containers were conditioned on benches in an alternative system equipped with fertigation in agricultural greenhouses. During the seedling production period, which was around 18 months, the internal temperature of the greenhouse was around 24°C. Nine genotypes from Embrapa Clima Temperado (Pelotas, Rio Grande do Sul, Brazil), selected as early death-tolerant rootstocks of the peach tree, were used in the present study (Mayer et al., 2009). The genotypes were propagated by rooting herbaceous cuttings under intermittent mist from parent plants managed with drastic

pruning (Mayer et al., 2020). Additionally, as rootstocks, cultivars 'Okinawa,' 'Capdeboscq' and 'Sharpe' were included, in addition to the own-rooted cultivar BRS Mandinho (without rootstock and without grafting). The cultivars were also propagated by rooting herbaceous cuttings (Table 1). The rootstock 'Okinawa' confers greater tolerance to early death compared to plants grafted on seed mixtures from the canning industry. The crown cultivar 'Capdeboscq' was also widely used, mainly between the 1960s and 1980s for the production of rootstocks, due to its ease of seed germination, adaptation and vigor. The 'Sharpe' rootstock from the United States is the only rootstock available in Brazil, tolerant to early death syndrome (PTSL) (Beckman et al., 2008; Mayer et al., 2015). Own-rooted seedlings of 'BRS Mandinho' were included to verify the effect of the absence of the grafting point (and, consequently, of the rootstock) on the variables analyzed.

### *Conducting the experiment*

The experiment was carried out in the greenhouse of the Federal University of Santa Maria (UFSM) in Santa Maria, State of Rio Grande do Sul, southern Brazil (29°42'56.35"S and 53°43'12.64"W). The experimental design was completely randomized, comprising a factorial of 13 (cultivars and clonal rootstock selections) x 2 (presence and absence of Al), with three replications per treatment. The peach seedlings (Table 1) were removed from the citropots, and the roots were washed with water until the substrate was completely

removed. The seedlings were then transferred to the hydroponic system, where they remained for 7 days in acclimatization in the complete nutrient solution. After this period, 100 mg L<sup>-1</sup> Al (in the form of AlCl<sub>3</sub>) was added, composing two levels for the Al factor (presence and absence). The nutrient solution used was from Hoagland and Arnon (1950), with changes only in the amount of P due to the complexation with Al. Thus, only 50 µM of P was used. After applying Al, the plants were exposed to the treatments for 14 days, the nutrient solution was changed every three days and the pH was adjusted to 4.5±0.1 with 1.0 mol L<sup>-1</sup> HCl or 1.0 mol L<sup>-1</sup> NaOH.

### *Growth variables*

At 14 days after the plants were exposed to the treatments, they were collected and separated into canopy and roots, which were washed in distilled water and dried until a constant weight in an oven with forced air circulation at 65 °C. With the results, the root dry weight (RDW), canopy dry weight (CDW) and total dry weight (TDW = CDW + RDW) were calculated. The morphological characterization of the roots was obtained from digitized images using WinRhizo Pro 2013 software, coupled to the EPSON Expression 11000 scanner equipped with additional light (TPU), with a resolution of 600 DPI. After scanning the images, the root surface area (cm<sup>2</sup> plant<sup>-1</sup>), total root length (cm plant<sup>-1</sup>), root volume (cm<sup>3</sup> plant<sup>-1</sup>) and mean root diameter (mm) were determined.

**Table 1**

**Own-rooted 'BRS Mandinho' peach seedlings (without rootstock) and grafted seedlings of 'BRS Mandinho' on different cultivars and clonal rootstock selections produced in an alternative system using citropots**

Scion/rootstock combinations and own-rooted tree	Rootstock or own-rooted tree species
'BRS Mandinho'/'Sharpe'	['Chicasaw' ( <i>Prunus angustifolia</i> Marsh.) x <i>Prunus</i> spp.]
'BRS Mandinho' autoenraizado	<i>Prunus persica</i>
'BRS Mandinho'/'Capdeboscq'	<i>Prunus persica</i>
'BRS Mandinho'/DB-SEN-09-23	<i>Prunus persica</i>
'BRS Mandinho'/FB-ESM-09-43	<i>Prunus persica</i>
'BRS Mandinho'/JB-ESM-09-13	<i>Prunus persica</i>
'BRS Mandinho'/SS-CHI-09-39	<i>Prunus persica</i>
'BRS Mandinho'/SS-CHI-09-40	<i>Prunus persica</i>
'BRS Mandinho'/JAH-MAC-09-77	<i>Prunus persica</i>
'BRS Mandinho'/'Okinawa'	<i>Prunus persica</i>
'BRS Mandinho'/PRBO-SAU-09-62	<i>Prunus persica</i>
'BRS Mandinho'/SAS-SAU-09-71	<i>Prunus persica</i>
'BRS Mandinho'/VEH-GRA-09-55	<i>Prunus persica</i>

Note 1: x / x indicates the scion/rootstock cultivar; Note 2: Genotype without ( / ) refers to the identification of the own-rooted crown cultivar (without grafting). Note 3: The rootstock selections are coded according to the initial letters of the fruit grower who owned the orchard where selection took place, the initial letters of the crown cultivar where the rootstock was selected, the year of selection and the number of the rootstock selection.

### *Aluminum analysis of plant tissue*

After drying, the canopy and root samples were ground in a Wiley mill and passed through a sieve with a mesh size of 2 mm. The plant tissue was subjected to nitroperchloric digestion (3.0 mL of HNO 65% PA and 1 mL of HClO 70% PA) (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2009). The total concentration of Al was analyzed using an atomic absorption spectrophotometer (AAS, Perkin Elmer Analyst 200, USA). Subsequently, the Al accumulated in the aerial parts and roots of the plants was calculated. The translocation factor (TF) was calculated according to equation 1 (Raj et al., 2020):

$$TF = \frac{\text{(Al concentration in the canopy)}}{\text{(Al concentration in the roots)}} \text{ Equation 1}$$

### *Statistical analysis*

The variables were submitted to analysis of variance (ANOVA) considering the interaction between the rootstock and treatment factors. Residual normality was tested using the Shapiro-Wilk test and homogeneity of error variances were tested using the Bartlett test. Whenever the null hypothesis (equal means) was rejected with an alpha equal to 0.05, the means were compared using the Tukey test ( $p \leq 0.05$ ). ANOVA was performed using the R package 'ExpDes.pt' (R Core Team [R], 2022).



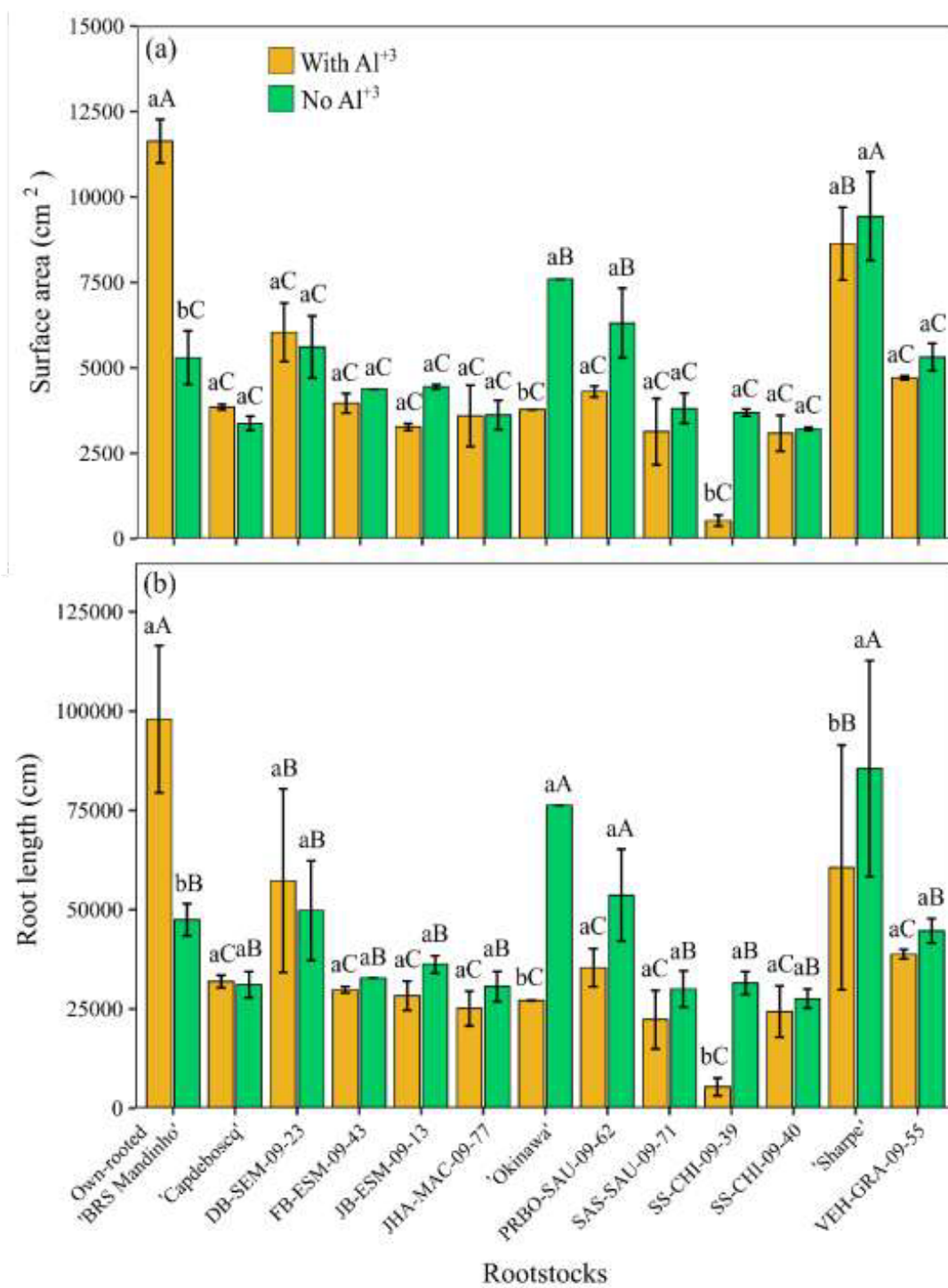
In addition, multivariate analysis of principal components (PCA) was carried out to explore the variance in the data, allowing the identification of more complex interactions between the variables, as well as the verification of the similarity/dissimilarity between the rootstocks in relation to the treatments. The contribution of each variable in explaining the variance of the principal component data was also quantified. PCA was performed using the 'FactoMineR' packages from the R statistical environment (R, 2022).

For the development of NC and FT estimation models, models were developed through plateau regression to quantify the relationship between the dependent variables (morphological variables of roots) and the concentration of Al in the roots. Hierarchical Bayesian analysis was used to adjust the regression models. In this step, a Monte Carlo simulation with Markov chains (MCMC) (Gelman & Hill, 2007) was performed using the Gibbs sampling algorithm with 20,000 random drawings after a warm-up period of 10,000 iterations. The sampling step was performed according to the normal distribution based on the a posteriori distribution of nutrient concentrations. Modeling was implemented using the 'rjags' package (Plummer, 2016) of the R statistical environment (R, 2022).

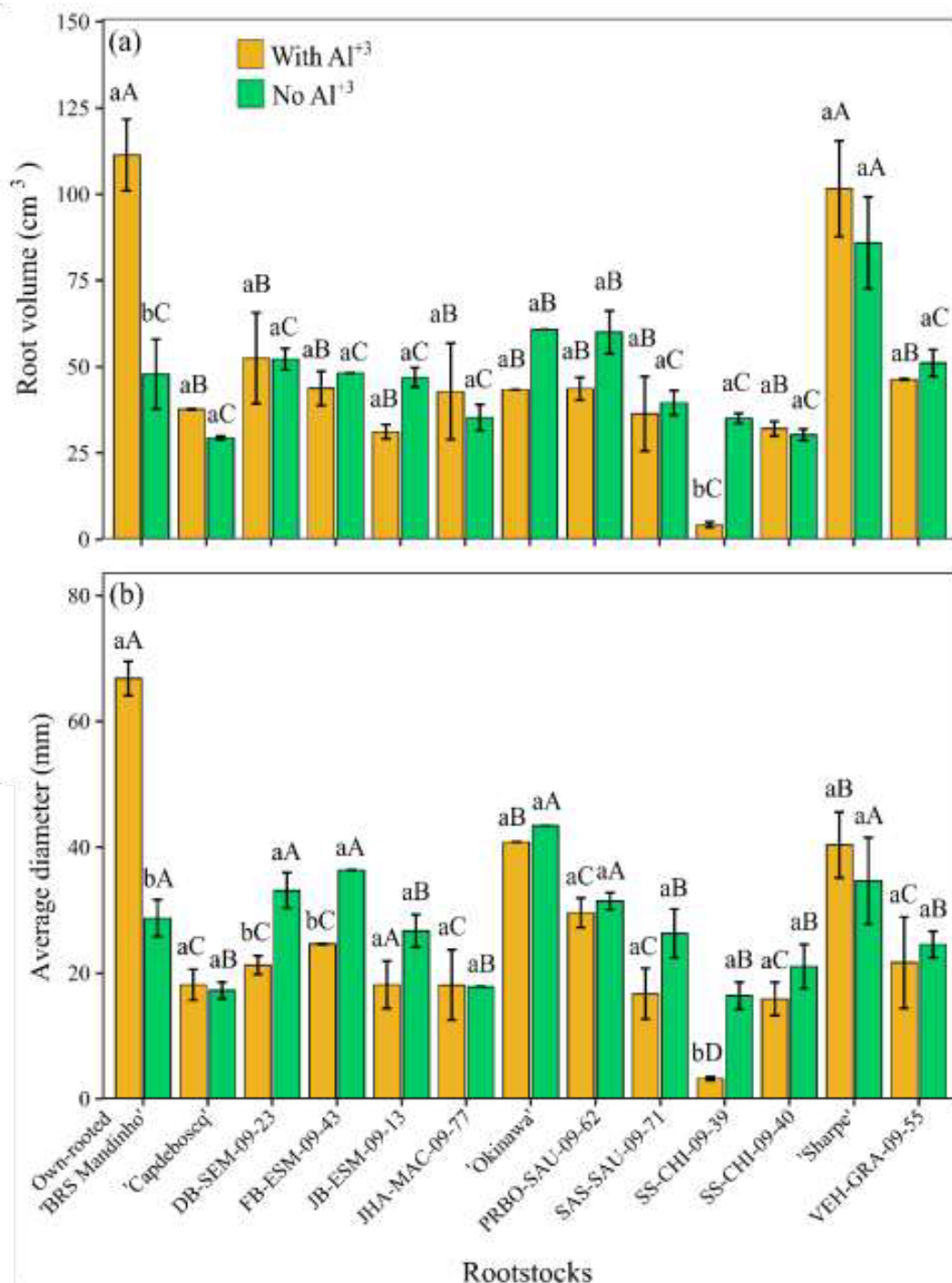
## Results and Discussion

The decrease in root length and surface area of the 'Okinawa' and SS-CHI-09-39 rootstocks (Figure 1a and 1b) and in the root volume of the SS-CHI-09-39 genotype in the solution with Al (Figure 1c) may have occurred because Al can bind strongly to negatively charged carboxylic groups in the cell wall of cortical and epidermal root cells, altering the binding and distribution of ions in the apoplast, which directly influences the growth of the organ (Ranjan et al., 2021). Plants with a smaller root system caused by Al toxicity tend to absorb less water and nutrients, which slows their growth (Sun et al., 2020).

The presence of Al increased the surface area (Figure 1a), root length (Figure 1b), and root volume (Figure 2a) of own-rooted 'BRS Mandinho'. However, the presence of Al promoted a reduction in the production of root biomass and total biomass in this genotype (Figure 3). This indicates that greater Al tolerance in the morphological variables of roots was not maintained for the production of dry matter in the plants. In addition, the highest Al concentration in the roots was also observed in own-rooted 'BRS Mandinho' with Al (Figure 4b). These results indicate that some root system variables were less sensitive to Al, suggesting that this greater tolerance was based on internal chelation and compartmentalization of Al rather than restriction of absorption (Yan et al., 2021).



**Figure 1.** Mean values recorded for root surface area (a), root length (b) in peach clonal rootstocks cultivated in the presence and absence of Al<sup>3+</sup> in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test. Bars represent mean  $\pm$  standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

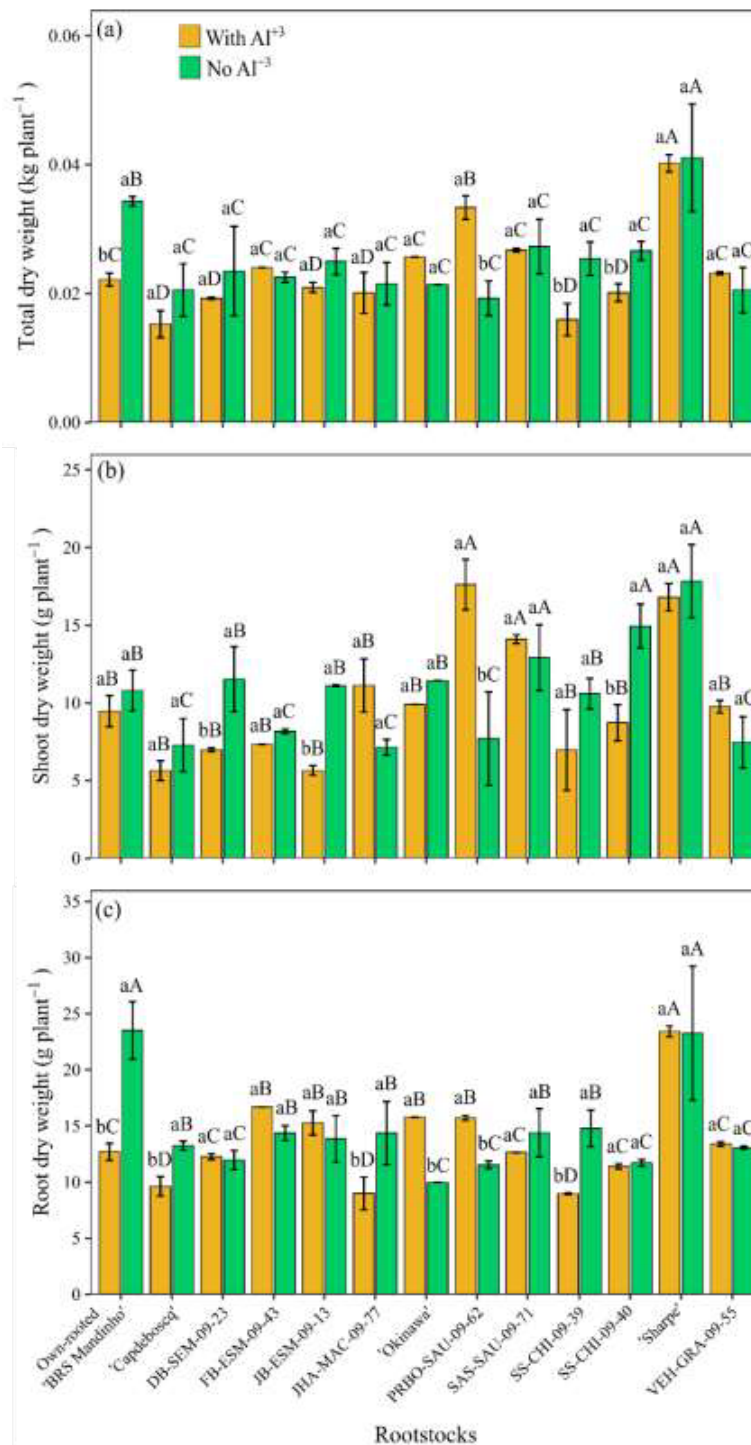


**Figure 2.** Mean values recorded for root volume (a) and root diameter (b) in peach clonal rootstocks cultivated in the presence and absence of Al<sup>3+</sup> in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test. Bars represent mean ± standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



The highest total dry weight (TDW) of PRBO-SAU-09-62 was observed in the presence of Al, while 'Sharpe' showed the highest production of TDW with and without Al (Figure 3a). Furthermore, it was observed that the 'Sharpe' and SAS-SAU-09-71 genotypes showed the highest values for canopy dry weight both in the presence and absence of Al, but did not differ from the PRBO-SAU-09-62 genotype in the presence of Al (Figure 3b). This is because the Al<sup>3+</sup> that enters the cell can be redistributed to non-toxic or relatively less toxic areas, which is an effective way to release Al in plants (Yan et al., 2021). Al entering the cytoplasm can be transferred to vacuoles via transporters in the tonoplast (detoxification of Al toxicity), which are areas of low Al toxicity (Zhu et al., 2019), consequently contributing to the increase in dry weight.

However, Al promoted a reduction in canopy dry weight in rootstocks JB-ESM-09-13, DB-SEN-09-23 and SS-CHI-09-40 (Figure 3b). This response was evidenced because Al transported from the roots to the canopy may have negatively influenced leaf formation and growth, causing a decrease in photosynthetic rate and dry weight production (Banhos et al., 2016). Thus, the effects of Al on canopy growth are considered a consequence of root damage, causing a decrease in water and nutrient absorption (Reis et al., 2018).



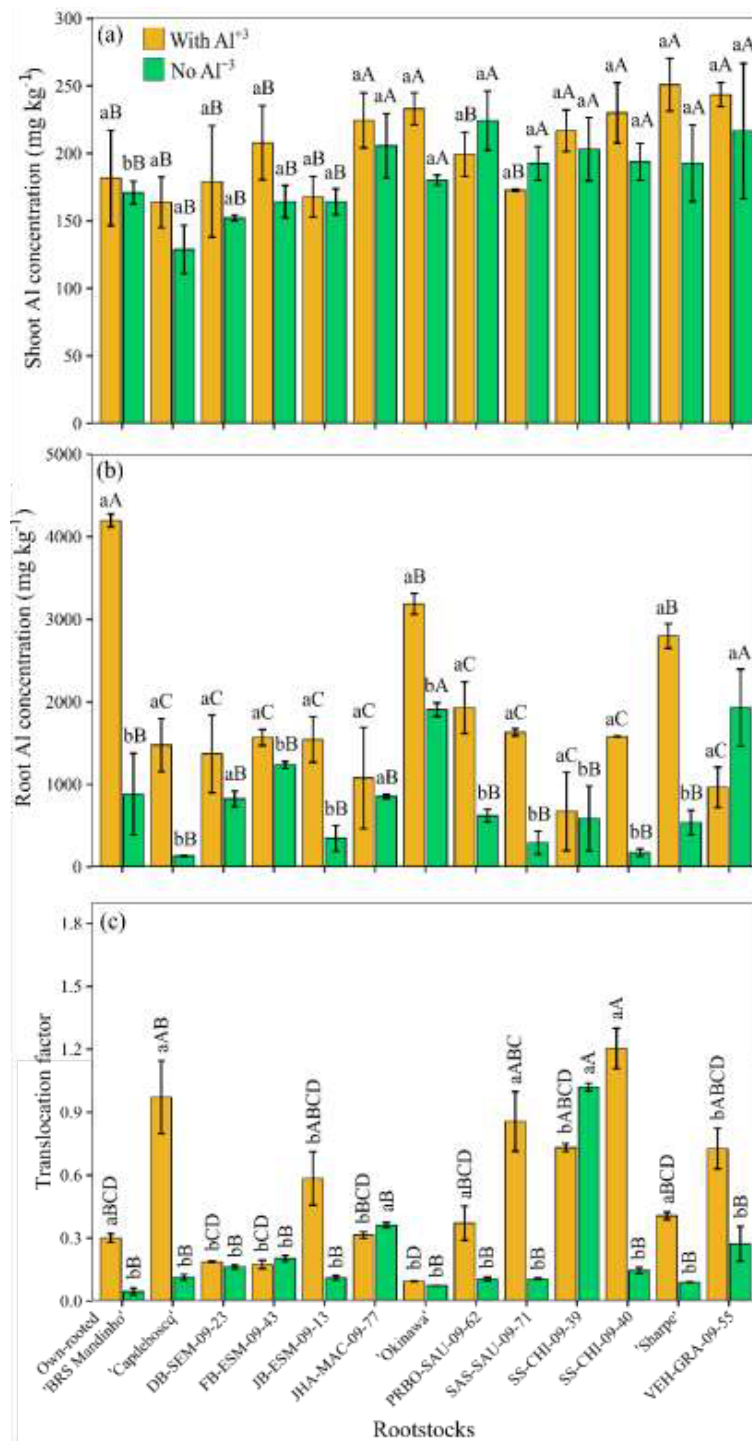
**Figure 3.** Mean values recorded for total dry weight (TDW) (a), canopy dry weight (SDW) (b) roots dry weight (RDW) (c) in peach clonal rootstocks cultivated in the presence and absence of Al<sup>3+</sup> in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test. Bars represent mean  $\pm$  standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Comparing the genotypes, within each level of Al, it was observed that Al did not have a toxic effect on the morphological variables of the root system and biomass production of the genotypes FB-ESM-09-43, JB-ESM-09-13, SAS-SAU-09-71, SS-CHI-09-40, 'Sharpe' and VEH-GRA-09-55, indicating a tolerance to Al. The greater tolerance of genotypes to Al may have occurred because, under Al stress conditions, plants present some adaptations, such as external exclusion and/or internal tolerance to detoxify excess Al, contributing to increased relative growth from the roots (Badia et al., 2020; Du et al., 2020). Internal tolerance mainly includes Al absorption and sequestration in the vacuole and activation of the antioxidant system within cells (He et al., 2019; Ranjan et al., 2021). External exclusion, in turn, prevents Al from entering cells through the Al-induced root exudation of organic compounds, decreasing the cell wall's ability to bind  $Al^{3+}$  (Wei et al., 2021). Thus, some plant roots have the ability to secrete organic acids, such as malate, citrate and oxalate, in response to Al stress, which prevents the  $Al^{3+}$  ion from entering the root cells and causing further damage to this organ (X. Zhang et al., 2019). Thus, the presence of some families of organic acid transporter genes, such as ALMT (Al-activated malate transporter) and MATE (extrusion of multiple drugs and toxic compounds), which confer Al resistance, has been reported and simultaneously induces signaling pathways that promote root growth (Ribeiro et al., 2021). Allied to this, the accumulation of phenol also suggests an Al detoxification strategy and subsequent complexation with these metabolites. Thus, phenolic compounds act as antioxidants in plants under Al stress and play a potential

role in excluding this metal (Rodrigues et al., 2019).

The Al concentration in the canopy, except for own-rooted 'BRS Mandinho,' did not differ between plants cultivated with and without Al (Figure 4a). This is desirable, as it indicates a lower probability of Al translocation to the fruit (H. Zhang et al., 2022).

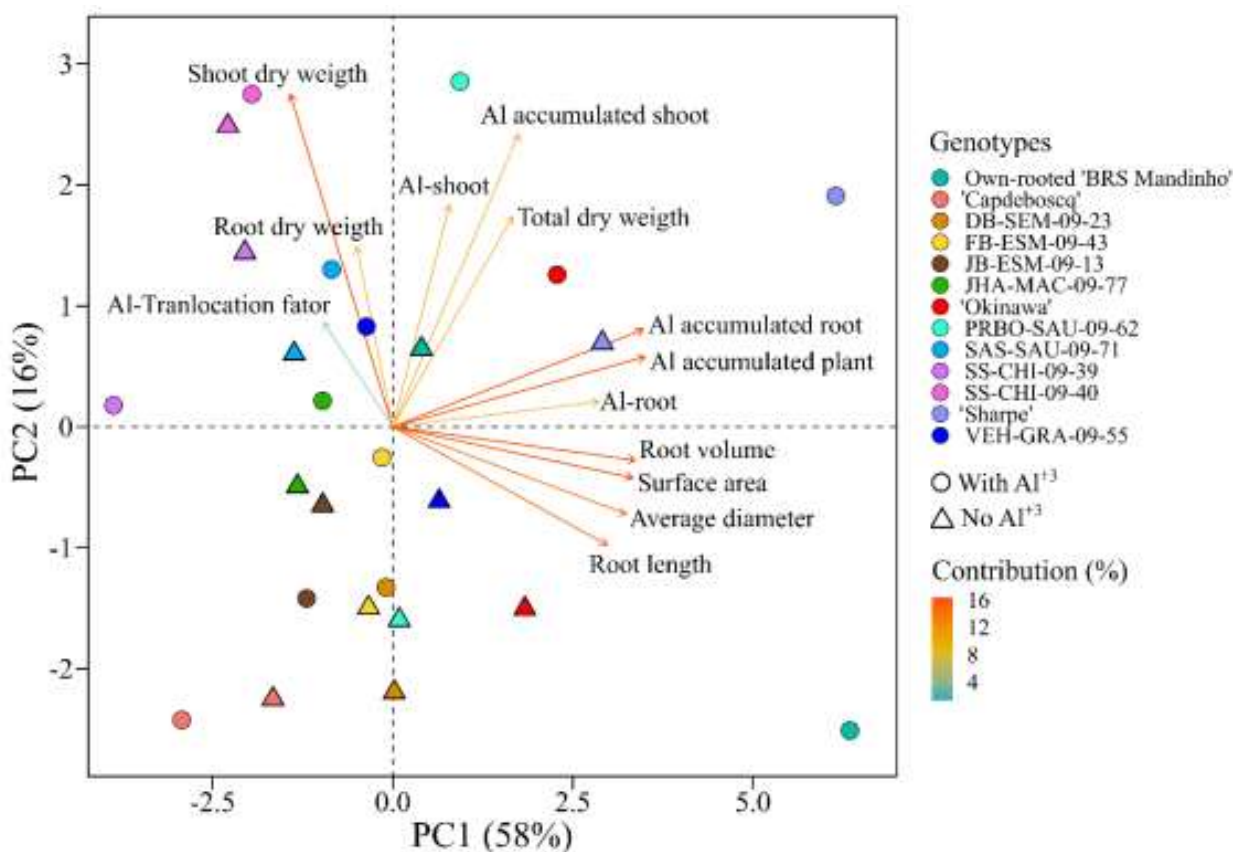
Comparing each rootstock in the presence or absence of Al, it was observed that Al promoted an increase in the translocation factor in 'BRS Mandinho,' 'Capdeboscq', PRBO-SAU-09-62, SAS-SAU-09-71, SS-CHI-09-40 and 'Sharpe', and promoted a decrease in JAH-MAC-09-77 and SS-CHI-09-39 (Figure 4c). Added to this, the highest correlation of the Al translocation factor is observed in the rootstocks SAS-SAU-09-71, SS-CHI-09-39 and SS-CHI-09-40 (Figure 5). Plants with a  $TF > 1$  transfer toxic metals to the canopy (Raj et al., 2020), while plants with a  $TF < 1$  have a lower ability to transfer toxic metal from the roots to the canopy (Mahdavian, 2021). However, higher Al concentrations in the roots and lower concentrations in the canopy suggest that there may be protective mechanisms through which Al translocation from the roots to the canopy is limited (Reis et al., 2018). Regulation of toxic metal uptake by roots and low translocation to canopy are considered tolerance mechanisms when levels of toxic metals are in excess in the environment (Bojórquez-Quintal et al., 2017). Thus, under Al phytotoxicity conditions, the rate of accumulation and translocation to canopy and leaves depends on different plant species, Al speciation, bioavailability and initial concentrations of the medium (Rahman et al., 2018).



**Figure 4.** Mean values recorded for Al concentration in canopy (a) and roots (b), and Al translocation factor (c) in peach clonal rootstocks cultivated in the presence and absence of Al<sup>3+</sup> in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test. Bars represent mean  $\pm$  standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

The PCA was performed by extracting only the first two components since the sum of the components, PC1 (58%) and PC2 (16%), explained 74% of the original variability in the data (Figure 5). The PC1 and PC2 scores confirmed that there was a distinct behavior between the rootstocks when submitted to the presence or absence of Al since the samples were distributed in

all quadrants (Figure 5). The variables with the greatest contribution to data variance were volume, surface area, and root diameter and length, followed by Al accumulation in canopy, plants and roots. These variables showed an inverse correlation with the dry matter of canopy and roots and with the Al translocation factor.



**Figure 5.** Association between principal components 1 (PC1) and 2 (PC2) recorded for morphological variables of the root system, Al concentration in roots and canopy, Al accumulated in roots, canopy and plant, total dry weight, canopy dry weight, roots dry weight, Al-translocation factor based on the 13 peach rootstocks submitted to the presence and absence of Al<sup>3+</sup>. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

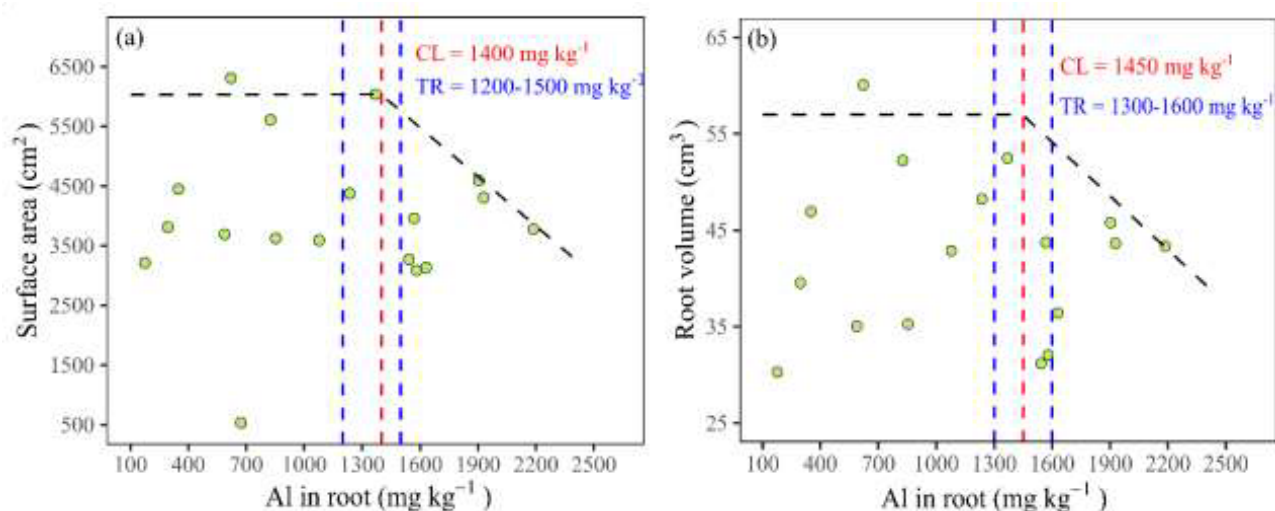


The variables volume, surface area, diameter and length of roots, followed by Al accumulation in the canopy, plant and roots, were sensitive to indicate the degree of tolerance of different peach genotypes to high Al concentrations. This information is of great relevance for genetic improvement programs, especially for those who select genotypes considering only vigor, yield, fruit quality of peach trees and resistance to diseases and pests, without considering the possible damages caused by excess metal in the environment that can cause plants.

The NC and FT values of the Al concentration in roots proposed in this study were obtained through Bayesian modeling, in which the observations collected in experiments served as a subsidy to generate new samples with a posteriori distribution, which can be tested by simulations (Theobald & Talbot, 2002). In addition, this modeling

approach made it possible to obtain the NC and FT of the Al concentration in peach rootstock roots related to root morphological variables, which is an innovative aspect of peach tree culture.

Based on the results, reference values (NC and FT) of Al concentration in roots in relation to surface area and root volume were proposed (Figure 6a and 6b, respectively). The CN of Al in the root in relation to the root surface area of peach rootstocks was 1400 mg Al kg<sup>-1</sup> and the FT was between 1200 and 1500 mg Al kg<sup>-1</sup> (Figure 6a). In relation to root volume, NC and FT were similar, with an NC of 1450 mg Al kg<sup>-1</sup> and an FT between 1300 and 1600 mg Al kg<sup>-1</sup> (Figure 6b). These values indicate that at Al concentrations above the NC, a decrease in surface area and root volume occurred, especially for rootstocks SS-CHI-09-39, SS-CHI-09-40, SAS-SAL-09-71, PRBO-SAU-09-62 and 'Okinawa.



**Figure 6.** Critical level (NC) and toxicity range (FT) of Al concentration in roots in relation to surface area (a) and volume (b) of roots in peach clonal rootstocks cultivated in the presence and absence of Al<sup>3+</sup> in a hydroponic system. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

## Conclusion

The morphological variables of the root system, such as volume, surface area, diameter and length of roots, were sensitive, indicating the degree of tolerance of different genotypes capable of developing in environments with high Al levels. Thus, rootstock selections and cultivars FB-SM-09-43, JB-ESM-09-13, SAS-SAU-09-71, SS-CHI-09-40, 'Sharpe' and VEH-GRA-09-55 were tolerant to high Al concentrations. In addition, own-rooted plants of 'BRS Mandinho' also showed Al tolerance with no reduction in surface area and root volume.

The critical level (NC) of Al in the roots in relation to the surface area of peach rootstocks was  $1400 \text{ mg Al kg}^{-1}$ , and the toxicity range (FT) was between  $1200$  and  $1500 \text{ mg Al kg}^{-1}$ . In relation to the volume of roots, the NC and FT were similar, with an NC of  $1450 \text{ mg Al kg}^{-1}$  and an FT between  $1300$  and  $1600 \text{ mg Al kg}^{-1}$ .

## Acknowledgments

We are grateful to Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Research Support Foundation of the State of Rio Grande do Sul)- FAPERGS, for the financial support (PROGRAMA PESQUISADOR GAÚCHO – PqG; 21/2551-0002280-9).

## References

- Alva, A. K., Edwards, D. G., Asher, C. J., & Blamey, F. P. C. (1986). Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. *Soil Science Society of America Journal*, *50*(1), 133-137. doi: 10.2136/sssaj1986.03615995005000010026x
- Badia, M. B., Maurino, V. G., Pavlovic, T., Arias, C. L., Pagani, M. A., & Andreo, C. S. (2020). Loss of function of Arabidopsis NADP-malic enzyme 1 results in enhanced tolerance to aluminum stress. *The Plant Journal*, *101*(1), 653-665. doi: 10.1111/tpj.14571
- Bahmani, R., Kim, D. G., Modareszadeh, M., Thompson, A. J., Park, J. H., Yoo, H. H., & Hwang, S. (2020). The mechanism of root growth inhibition by the endocrine disruptor bisphenol A (BPA). *Environmental Pollution*, *257*(10), 1-13. doi: 10.1016/j.envpol.2019.113516
- Banhos, O. F. A. A., Brenda, M. D. O., Carvalho, B. M., Veiga, E. B. da, Bressan, A. C. G., Tanaka, F. A. O., & Habermann, G. (2016). Aluminum-induced decrease in CO<sub>2</sub> assimilation in 'Rangpur' lime is associated with low stomatal conductance rather than low photochemical performances. *Scientia Horticulturae*, *205*(1), 133-140. doi: 10.1016/j.scienta.2016.04.021
- Baquy, M. A. A., Li, J. Y., Xu, C. Y., Mehmood, K., & Xu, R. K. (2017). Determination of critical pH and Al concentration of acidic Ultisols for wheat and canola crops. *Solid Earth*, *8*(11), 149-159. doi: 10.5194/se-8-149-2017
- Beckman, T. G., Chaparro, J. X., & Sherman, W. B. (2008). 'Sharpe', a clonal plum rootstock for peach. *HortScience*, *43*(7), 2236-2237. doi: 10.21273/HORTSCI.43.7.2236

- Betemps, D. L., Paula, B. V., Parent, S. E., Galarca, S. P., Mayer, N. A., Marodin, G. A. B., Rozane, D. E., Natale, W., Melo, G. W. B., Parent, L., & Bruneto, G. (2020). Humboldtian diagnosis of peach tree (*Prunus persica*) nutrition using machine-learning and compositional methods. *Agronomy*, *10*(6), 900. doi: 10.3390/agronomy10060900
- Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., & Martínez-Estévez, M. (2017). Aluminum, a friend or foe of higher plants in acid soils. *Frontiers in Plant Science*, *8*(1767), 1-18. doi: 10.3389/fpls.2017.01767
- Dorneles, A. O. S., Pereira, A. S., Rossato, L. V., Possebom, G., Sasso, V. M., Bernardy, K., Sandri, R. Q., Nicoloso, F. T., Ferreira, P. A. A., & Tabaldi, L. A. (2016). Silicon reduces aluminum content in tissues and ameliorates its toxic effects on potato plant growth. *Ciência Rural*, *46*(3), 506-512. doi: 10.1590/0103-8478cr20150585
- Du, H., Huang, Y., Qu, M., Li, Y., Hu, X., Yang, W., Li, H., He, W., Ding, J., Liu, C., Gao, S., Cao, M., Lu, Y., & Zhang, S. (2020). A maize ZmAT6 gene confers aluminum tolerance via reactive oxygen species scavenging. *Frontiers in Plant Science*, *11*(1016), 1-12. doi: 10.3389/fpls.2020.01016
- Eldem, V. C., Akcay, U. C., Ozhuner, E., Bakır, Y., Uranbey, S., & Unver, T. (2012). Genome-wide identification of miRNAs responsive to drought in peach (*Prunus persica*) by high-throughput deep sequencing. *PLoS ONE*, *7*(12), 1-14. doi: 10.1371/journal.pone.0050298
- Empresa Brasileira de Pesquisa Agropecuária (2009). *Manual de análises químicas de solos, plantas e fertilizantes* (2a ed.). EMBRAPA.
- Gelman, A., & Hill, J. (2007). *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press.
- He, H., Li, Y., & He, L. F. (2019). Aluminum toxicity and tolerance in Solanaceae plants. *South African Journal of Botany*, *123*(1), 23-29. doi: 10.1016/j.sajb.2019.02.008
- Hoagland, D. R., & Arnon D. I. (1950). *The waterculture method for growing plants without soil*. (Circ. 347). Agric. Exp. Stn., Univ. Of California.
- Kyveryga, P., Caragea, P. C., Kaiser, M. S., & Blackmer, T. M. (2013). Predicting risk of reduced nitrogen fertilization using hierarchical models and on-farm data. *Agronomy Journal*, *105*(1), 85-94. doi: 10.2134/agronj2012.0218
- Mahdavian, K. (2021). Effect of citric acid on antioxidant activity of red bean (*Phaseolus calcaratus* L.) under Cr<sup>+6</sup> stress. *South African Journal of Botany*, *139*(12), 83-91. doi: 10.1016/j.sajb.2021.02.002
- Mayer, N. A., & Ueno, B. (2021). Peach tree short life in Rio Grande do Sul State, Brazil. *Agrociencia Uruguay*, *25*(395), 1-17. doi: 10.31285/AGRO.25.395
- Mayer, N. A., Ueno, B., & Antunes, L. E. C. (2009). *Seleção e clonagem de porta enxertos tolerantes à morte-precoce do pessegueiro*. (Comunicado Técnico, 209). EMBRAPA Clima Temperado.
- Mayer, N. A., Ueno, B., Rickes, T. B., & Resende, M. V. L. A. (2020). Cloning of rootstock selections and *Prunus* spp. cultivars by softwood cuttings. *Scientia Horticulturae*, *273*(1), 1-11. doi: 10.1016/j.scienta.2020.109609

- Mayer, N. A., Ueno, B., Silva, V. A. L., Valgas, R. A., & Silveira, C. A. P. (2015). A morte precoce do pessegueiro associada à fertilidade do solo. *Revista Brasileira de Fruticultura*, 37(3), 773-778. doi: 10.1590/0100-2945-156/14
- Nava, G., Reisser, C., Jr., Parent, L.-É., Brunetto, G., Moura-Bueno, J. M., Navroski, R., Benati, J. A., & Barreto, C. F. (2022). Esmeralda peach (*Prunus persica*) fruit yield and quality response to nitrogen fertilization. *Plants*, 11(3), 352. doi: 10.3390/plants11030352
- Padarian, J., Minasny B., & McBratney, A. B. (2020). Machine learning and soil sciences: a review aided by machine learning tools. *Soil*, 6(1), 35-52. doi: 10.5194/soil-6-35-2020
- Paula, B. V., Sete, P. B., Trapp, T., Vitto, B., Zalameña, J., Melo, G. W. B., Rozane, D. E., Baldi, E., Toselli, M., & Brunetto, G. (2021). Annual and residual urea nitrogen contribution to the nutrition of peach trees (*Prunus persica* L.) grown under subtropical climate. *Scientia Horticulturae*, 284(10), 1-6. doi: 10.1016/j.scienta.2021.110099
- Plummer, M. (2016). *Rjags: bayesian graphical models using MCMC*.
- R Core Team (2022). *R: a language and environment for statistical computing*.
- Rahman, M. A., Lee, S. H., Ji, H. C., Kabir, A. H., Jones, C. S., & Lee, K. W. (2018). Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: current status and opportunities. *International Journal of Molecular*, 19(10), 1-28. doi: 10.3390/ijms19103073
- Raj, D., Kumar, A., & Maiti, S. K. (2020). Mercury remediation potential of *Brassica juncea* (L.) Czern. For clean-up of flyash contaminated sites. *Chemosphere*, 248(1), 1-9. doi: 10.1016/j.chemosphere.2020.125857
- Ranjan, A., Sinha, R., Sharma, T. R., Pattanayak, A., & Singh, A. K. (2021). Alleviating aluminum toxicity in plants: implications of reactive oxygen species signaling and crosstalk with other signaling pathways. *Physiologia Plantarum*, 173(4), 1-20. doi: 10.1111/ppl.13382
- Raseira, M. C. B., Scaranari, C., Franzon, R. C., Feldberg, N. P., & Nakasu, B. H. (2016). 'BRS Mandinho': the first platycarpa peach cultivar released in Brazil. *Revista Brasileira de Fruticultura*, 38(3), 1-4. doi: 10.1590/0100-29452016616
- Reighard, G. L., & Loreti, F. (2008). Rootstock development. In D. R. Layne, & D. Bassi, *The peach: botany, production and uses* (pp. 193-220). Oxfordshire.
- Reis, A. R., Lisboa, L. A. M., Reis, H. P. G., Barcelos, J. P. de Q., Santos, E. F., Santini, J. M. K., & Lavres, J. (2018). Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. *Plant Physiology and Biochemistry*, 130(1), 377-390. doi: 10.1016/j.plaphy.2018.07.028
- Ribeiro, A. P., Vinecky, F., Duarte, K. E., Santiago, T. R., Casari, R. A. C. N., Hell, A. F., & Molinari, H. B. C. (2021). Enhanced aluminum tolerance in sugarcane: evaluation of SbMATE overexpression and genome-wide identification of ALMTs in *Saccharum* spp. *BMC Plant Biology*, 21(300), 1-15. doi: 10.1186/s12870-021-02975-x

- Rodrigues, A. A., Vasconcelos, S. C., Fº., Müller, C., Rodrigues, D. A., Sales, J. F., Zuchi, J., & Barbosa, D. P. (2019). Tolerance of *Eugenia dysenterica* to aluminum: germination and plant growth. *Plants*, 8(9), 1-15. doi: 10.3390/plants8090317
- Shetty, R., Vidya, C. S., Prakash, N. B., Lux, A., & Vaculík, M. (2021). Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: a review. *Science of the Total Environment*, 65(1), 1-11. doi: 10.1016/j.scitotenv.2020.142744
- Sun, L., Zhang, M., Liu, X., Mao, Q., Shi, C., Kochian, L. V., & Liao, H. (2020). Aluminum is essential for root growth and development of tea plants (*Camellia sinensis*). *Journal of Integrative Plant Biology*, 62(7), 984-987. doi: 10.1111/jipb.12942
- Theobald, C. M., & Talbot, M. (2002). The Bayesian choice of crop variety and fertilizer dose. *Applied Statistics*, 51(1), 23-26. doi: 10.1111/1467-9876.04863
- Wei, Y., Han, R., Xie, Y., Jiang, C., & Yu, Y. (2021). Recent advances in understanding mechanisms of plant tolerance and response to aluminum toxicity. *Sustainability*, 13(4), 1-22. doi: 10.3390/su13041782
- Yan, L., Riaz, M., Liu, J., Yu, M., & Cuncang, J. (2021). The aluminum tolerance and detoxification mechanisms in plants; recent advances and prospects. *Critical Reviews in Environmental Science and Technology*, 52(9), 1-37. doi: 10.1080/10643389.2020.1859306.
- Zhang, H., Li, Xy., Lin, M., Hu, P., Lai, N., Huang, Z., & Chen, L. (2022). The aluminum distribution and translocation in two citrus species differing in aluminum tolerance. *BMC Plant Biology*, 22(93), 1-11. doi: 10.1186/s12870-022-03472-5
- Zhang, X., Long, Y., Huang, J., & Xia, J. (2019). Molecular mechanisms for coping with Al toxicity in plants. *International Journal of Molecular Sciences*, 20(7), 1-16. doi: 10.3390/ijms20071551
- Zhu, C. Q., Cao, X. C., Zhu, L. F., Hu, W. J., Hu, A. Y., Abliz, B., Bai, Z. G., Huang, J., Liang, Q. D., Sajid, H., Li, Y. F., Wang, L. P., Jin, Q. Y., & Zhang, J. H. (2019). Boron reduces cell wall aluminum content in rice (*Oryza sativa*) roots by decreasing H<sub>2</sub>O<sub>2</sub> accumulation. *Plant Physiology and Biochemistry*, 138(10), 80-90. doi: 10.1016/j.plaphy.2019.02.022