Division – Soil Use and Management | Commission – Soil pollution, Remediation and Reclamation of Degraded Areas

Oxidative stress as markers in identification of aluminum-tolerant peach tree rootstock cultivars and clonal selections

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ABSTRACT: Peach rootstock and scion cultivars are selected in breeding programs considering resistance to pests and diseases, salt tolerance, drought tolerance, and vigor. However, rootstock tolerance to aluminum (Al), which is markedly present in tropical and subtropical soils of the world, is not considered. Thus, it is essential to define potential markers that can contribute to the selection of Al-resistant or Al-tolerant peach rootstocks. The objective of this study was to identify Al-tolerant peach tree rootstock cultivars and clonal selections using physiological and oxidative stress variables. A completely randomized experimental design was used in a 13 (rootstock cultivars and clonal selections) × 2 (with and without Al) factorial arrangement, with three replications. Nursery peach trees of own-rooted 'BRS Mandinho' (without rootstock) and nursery trees of 'BRS Mandinho' grafted on different rootstock cultivars and clonal selections were grown in a hydroponic system, consisting of two treatments, with and without 100 mg L^{-1} of Al. Dry biomass, photosynthetic variables, pigment concentration, hydrogen peroxide content, membrane lipid peroxidation, and activity of the antioxidant enzymes were evaluated. The total dry matter production of the own-rooted 'BRS Mandinho' trees and the SS-CHI-09-39 and SS-CHI-09-40 selections is affected by the Al presence, representing a decline of 35.4, 37.2, and 24.4 %, respectively, compared to the treatment with Al. The highest total dry matter production in Al presence was observed for the 'Sharpe' rootstock. 'Capdeboscq', DB-SEN-09-23, FB-ESM-09-43, JB-ESM-09-13, JAH-MAC-09-77, SAS-SAU-09-71, and VEH-GRA-09-55 rootstock selections are tolerant to Al. The use of physiological and biochemical variables shows potential for the selection of clonal rootstocks tolerant or resistant to Al.

Keywords: aluminum toxicity, antioxidant enzymes, biochemical markers, physiological variables, *Prunus*.

INTRODUCTION

Rio Grande do Sul State (RS), in southern Brazil, has the largest peach tree [*Prunus persica* (L.) Batsch] growing area in the country (Dini et al., 2021; IBGE, 2022). This has become possible mainly because of the development of dozens of scion cultivars adapted to climatic conditions (Raseira et al., 2014, 2019). However, peach scion cultivars were not selected for tolerance to aluminum (Al) in the soil and, for that reason, their seeds should not be used to produce rootstocks (Mayer et al., 2014). Aluminum can be toxic and is present in high levels in some soils, especially in tropical and subtropical regions (Kochian et al., 2015). Aluminum may be one of the factors that intensifies the incidence of the Peach Tree Short Life (PTSL) syndrome (Mayer et al., 2015; Ueno et al., 2019). In the peach orchards of Rio Grande do Sul, this syndrome causes mortality of up to 90 % of peach trees in the hardest hit orchards (Mayer et al., 2009), reducing orchard longevity and yield, currently standing at only 12.95 Mg ha^{-1}, which is lower than the world average (16.47 Mg ha^{-1}) (FAO, 2022). Therefore, it has become necessary to select genotypes for rootstocks with Al-resistant functions and including markers, such as photosynthetic or oxidative stress-related variables, that can contribute to this selection.

Aluminum in solution can rapidly inhibit root growth in acidic soils, which are characterized as those with a pH value below 5.5, resulting in a reduced and atrophied root system (Shetty et al., 2021). Disorganized meristematic tissue has frequently been described in plants under stress caused by Al toxicity, and this is one of the explanations for decreased/inhibited root growth and a smaller number of lateral roots (Ciamporová, 2002). Also, Al can interact with nucleic acids, inhibiting mitosis in the root apex and binding to cell wall components. This increases the rigidity of the cell wall, which may also contribute to reduced root growth. Reduction in the root system causes less soil exploitation, impairing water and nutrient uptake (Kochian et al., 2015; Smirnov et al., 2020). In the symplast, Al toxicity can negatively affect membrane integrity and function, DNA synthesis, elongation, and cellular metabolism (Bojórquez-Quintal et al., 2017); and it induces oxidative stress through the formation of reactive oxygen species (ROS) (Ranjan et al., 2021), resulting in oxidative damage in lipids, proteins, and DNA (Choudhury and Sharma, 2014).

Thus, photosynthetic activity may have negative effects, such as damage to photosynthetic pigments, reduced stomatal conductance, and increased fluorescence rates (Cárcamo et al., 2019). In plants, induced production of ROS is one of the most important biochemical signals of Al toxicity (Possebom et al., 2018). Also, a gradual increase in the activity of antioxidant enzymes involved in a chain of ROS neutralization reactions is understood as an adaptive biochemical response to Al stress (Smirnov et al., 2020). Some genes may encode antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidases (POD, EC 1.11.1.7) induced by Al toxicity (Di Ferdinando et al., 2012), but this has not been sufficiently investigated in peach rootstocks.

Some plant species have developed adaptation mechanisms in response to soils with high Al content (Rahman et al., 2018) to counteract the high toxicity of Al to plants. Two main types of Al resistance mechanisms are reported in the literature: Al exclusion, which prevents the metal from entering the root apex; and Al tolerance, in which the metal enters the plant and is sequestered/detoxified (Kochian et al., 2015). In Al-resistant plants, an important strategy to prevent Al from reaching sensitive targets in the root tips is the exudation of organic and phenolic acids, which can form highly stable non-toxic complexes with Al in the apoplast and rhizosphere (Pereira and Ryan, 2019). Aluminum resistance also involves intense regulation of gene expression. The Al can induce the expression of a number of Al resistance genes associated with different mechanisms (Kochian et al., 2015). Thus, studies on Al-resistant plants, such as on peach trees, are essential to understanding the phytotoxic effects of this metal associated with plant

resistance, determining the resistance potential of species and their establishment in Al-rich environments (Cárcamo et al., 2019).

Our hypothesis is that selecting Al-tolerant or Al-resistant plants among clonal rootstock genotypes, through physiological and biochemical variables obtained from methodologies already known and used in laboratories is a suitable strategy to reduce peach tree death and decrease expenses on soil acidity correctives and fertilizers. This study aimed to identify Al-tolerant peach tree rootstock cultivars and clonal selections using physiological and oxidative stress variables.

MATERIALS AND METHODS

Plant material

Nursery trees of the 'BRS-Mandinho' flat peach [*Prunus persica* var. platycarpa (L.) Batsch] scion (Raseira et al., 2016) were produced in an alternative system using *citropots* (3.78 L capacity) on benches in an agricultural greenhouse equipped with fertigation. During the nursery production period, which was around 18 months, the average temperature in the greenhouse was 24 °C. Nine clonal rootstock genotypes from Embrapa Clima Temperado (Pelotas, Rio Grande do Sul, Brazil), selected as tolerant to the PTSL syndrome (Mayer et al., 2009; Mayer and Ueno, 2021), were used as rootstocks for 'BRS Mandinho'. The genotypes were propagated by rooting of softwood cuttings under an intermittent mist system from shoots collected in mother trees managed with drastic winter pruning (Mayer et al., 2020). Additionally, the cultivars 'Capdeboscq', 'Okinawa', and 'Sharpe' were included as clonal rootstocks, as well as the own-rooted 'BRS Mandinho' (without rootstock), all of them also propagated by softwood cuttings (Table 1). Although 'Capdeboscq' is a scion cultivar for processing, it was included in the trial because it was widely used in the past, especially from the 1960s to 1980s, for rootstock production, due to its relative ease of seed germination, adaptation, and vigor. The rootstock 'Okinawa', the most used in the Southeast region of Brazil, provides greater tolerance to PTSL compared to trees on no-selected genotypes as rootstocks (seed mixtures of scion peaches for canning) (Mayer et al., 2014; Mayer and Ueno, 2021). The rootstock 'Sharpe', released in the United States, is the only rootstock tolerant to PTSL available in Brazil (Beckman et al., 2008; Mayer et al., 2015). Own-rooted 'BRS Mandinho' nursery trees were included to verify what effect the absence of the rootstock (and, consequently, the absence of a grafting point) would have on the variables analyzed.

Location of the experiment

The study was conducted in a greenhouse at the Federal University of Santa Maria (UFSM) in Santa Maria, Rio Grande do Sul (RS), in the South region of Brazil (29° 42' 56.35" S and 53° 43' 12.64" W). A completely randomized experimental design was used, composed of a 13 (own-rooted trees, rootstock cultivars and clonal selections) × 2 (with Al and without Al) factorial arrangement, with three replications of each cultivar. All nursery trees of the 13 types (Table 1) were removed from the *citropotes*.

Roots were washed with tap water until complete removal of the substrate. Then, each seedling was placed in a 6-L capacity vessel containing the complete nutrient solution of Hoagland and Arnon (1950). A sheet of Styrofoam was added on the surface of each vessel, with one center hole to allow the plant to pass through. The Styrofoam sheet allowed the fixation of the plants, and the evaporation of the solution contained in each vessel was reduced.

Seedlings were acclimatized for seven days in the nutrient solution of Hoagland and Arnon (1950) at 100 % of its original concentration. After acclimatization, two treatments were applied, in which seedlings received a new solution with pH 4.5, with or without 100 mg L^{-1} of Al (in the form of $AlCl_3$). The nutrient solution used was that of Hoagland

Table 1. Own-rooted 'BRS Mandinho' nursery peach trees (without rootstock) and 'BRS Mandinho' nursery trees grafted on 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' (*Prunus angustifolia* Marsh.) x *Prunus* spp.]
'BRS Mandinho'/JB-ESM-09-13	*Prunus persica*
'BRS Mandinho'/DB-SEN-09-23	*Prunus persica*
'BRS Mandinho'/SS-CHI-09-39	*Prunus persica*
'BRS Mandinho'/SS-CHI-09-40	*Prunus persica*
'BRS Mandinho'/FB-ESM-09-43	*Prunus persica*
'BRS Mandinho'/VEH-GRA-09-55	*Prunus persica*
'BRS Mandinho'/PRBO-SAU-09-62	*Prunus persica*
'BRS Mandinho'/SAS-SAU-09-71	*Prunus persica*
'BRS Mandinho'/JAH-MAC-09-77	*Prunus persica*
'BRS Mandinho'/Capdeboscq	*Prunus persica*
'BRS Mandinho'/Okinawa	*Prunus persica*
Own-rooted 'BRS Mandinho'	*Prunus persica* var. platycarpa

x/x indicates the scion cultivar/rootstock; Genotype without (/) refers to the identification of the own-rooted scion cultivars (without rootstock). The rootstock selections are coded according to the initial letters of the peach grower who owns the original orchard where the selection took place, the initial letters of the scion cultivar where the rootstock was selected, the year of selection, and the number of the rootstock selection.

and Arnon (1950), only modifying the amount of P, due to its complexation with Al. Thus, we used only 50 µM of P. Aeration of the solution in each vessel was performed by PVC microtubes connected to an air compressor. Microtubes were inserted into the solution through the Styrofoam sheet present over each vessel.

After Al application, the plants were exposed to the treatments for 14 days, and during that period, the nutrient solution in each vessel was replaced three times a week, and pH levels were adjusted daily to 4.5 ± 0.1, with HCl 1.0 mol L^{-1} or NaOH 1.0 mol L^{-1}.

Growth Variables

On day 14, the plants were collected and separated into shoots and roots, which were washed in distilled water and dried in an oven with forced air circulation at 65 °C until constant weight. From the results, shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) (TDW = SDW + RDW) were calculated.

Photosynthetic variables

Physiological variables related to the photosynthetic apparatus in each plant were measured on the thirteenth day of exposure to the treatments. The analysis was made during the period from 8 am to 11 am using an infrared radiation gas analyzer [Infrared gas analyzer (IRGA), Li-COR® Mod. 6400 XT]. At that time, the following variables were determined: net CO_2 assimilation rate / photosynthetic rate (A), stomatal water vapor conductance (Gs), internal CO_2 concentration (Ci), transpiration rate (E), water use efficiency (WUE), and Rubisco carboxylation efficiency (A/Ci) at an ambient CO_2 concentration of 400 µmol mol^{-1} at 20-25 °C, 50 ± 5 % relative humidity, and photon flux density of 1500 µmol m^{-2} s^{-1}.

Biochemical variables

Fresh leaf and root samples were frozen (-80 °C) and subsequently macerated with liquid N for determination of photosynthetic pigment (total chlorophyll and carotenoids) concentration, hydrogen peroxide content, membrane lipid peroxidation, and activity of the guaiacol peroxidase (POD) and superoxide dismutase (SOD) enzymes. Chlorophylls *a* and *b* and carotenoids were extracted according to the method of Hiscox and Israelstan (1979) and estimated using the equation of Lichtenthaler (1987). Total chlorophyll was obtained from the sum of chlorophyll *a* and chlorophyll *b*. In this method, macerated leaf

samples (0.05 g) were incubated at 65 °C with dimethyl sulfoxide (DMSO) until complete pigment extraction occurred. After that, the absorbances of the solution were measured in a spectrophotometer at 663, 645, and 470 nm for chlorophyll *a*, chlorophyll *b,* and carotenoids, respectively. Hydrogen peroxide content was determined according to Loreto and Velikova (2001), and the concentration of H_2O_2 was expressed as µmol g^{-1} fresh weight. Lipid peroxidation was determined by malondialdehyde (MDA) concentration following the method of El-Moshaty et al. (1993) and expressed as nmol of MDA mg^{-1} of protein. For quantification of guaiacol peroxidase and superoxide dismutase enzyme activity, root and leaf samples (0.5 g) were homogenized in 3.0 mL of 0.05 mol L^{-1} sodium phosphate buffer (pH 7.8). The homogenate was then centrifuged, and the supernatant was used to determine enzyme activity (Zhu et al., 2004). The activity of the guaiacol peroxidase enzyme was determined according to Zeraik et al. (2008), using guaiacol as a substrate. Results were expressed as units of enzyme per mg protein (U mg^{-1} protein). Superoxide dismutase (SOD) activity was determined based on the spectrophotometric method described by Giannopolitis and Ries (1977). The SOD unit was defined as the amount of enzyme that inhibits NBT photoreduction by 50 % (Beauchamp and Fridovich, 1971).

Statistical analysis

Analysis of variance (ANOVA) was carried out on the tree growth, photosynthetic, and biochemical variables, considering the interaction between the rootstock: Al treatment factors. The normality of residuals was tested using the Shapiro-Wilk test, and the homogeneity of variances of errors using Bartlett's test to verify if any transformation was necessary. Whenever the null hypothesis (equal means) was rejected with alpha equal to 0.05, the means were compared by Tukey's test (p<0.05). ANOVA was performed using the R package 'ExpDes.en' (R Development Core Team, 2022).

In addition, multivariate principal component analysis (PCA) was performed to examine the variance of the data, allowing identification of more complex interactions between variables, as well as to verify the similarity/dissimilarity between rootstocks in relation to the treatments. Factor analysis was previously used in the selection of variables with greater weight in explaining the variance of the phenomena (cutoff criterion of 50 % of the explained variance), and PCA analysis was subsequently performed. The contribution of each variable in explaining the variance of the principal component data was also quantified. The PCA analysis was performed using the 'FactoMineR' packages of the R statistical environment (R Development Core Team, 2022).

RESULTS

Dry biomass

'Sharpe' showed the highest total dry matter production in treatments with Al and without Al (Figure 1). The highest dry matter production was observed in the selection PRBO-SAU-09-62 with Al. In contrast, the lowest dry matter productions were observed in the own-rooted trees and the selections SS-CHI-09-39 and SS-CHI-09-40 with Al (Figure 1).

Photosynthetic variables

The highest means for photosynthetic rate (A) in Al presence were found in the JAH-MAC-09-77, 'Okinawa', SAS-SAU-09-71, and SS-CHI-09-39 genotypes (Figure 2a). In contrast, the lowest photosynthetic rate values were seen in the genotypes FB-SM-09-43 and JB-ESM-09-13 with Al. The lowest photosynthetic rate (A) values were observed in JB-ESM-09-13 and 'Sharpe' without Al (Figure 2a). In comparison to the presence or absence of Al within each rootstock, JB-ESM-09-13 showed higher photosynthetic rates in the presence of Al. In the own-rooted trees and those grafted on 'Okinawa' and 'Sharpe', the Al presence reduced photosynthetic rates (Figure 2a). Higher values of stomatal water
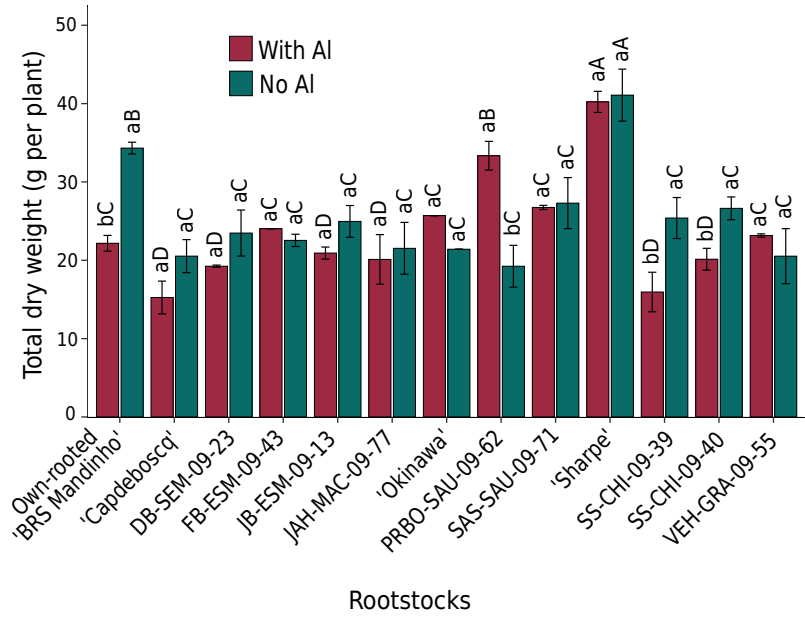
Figure 1. Mean values of total dry weight (TDW) in clonal peach rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.
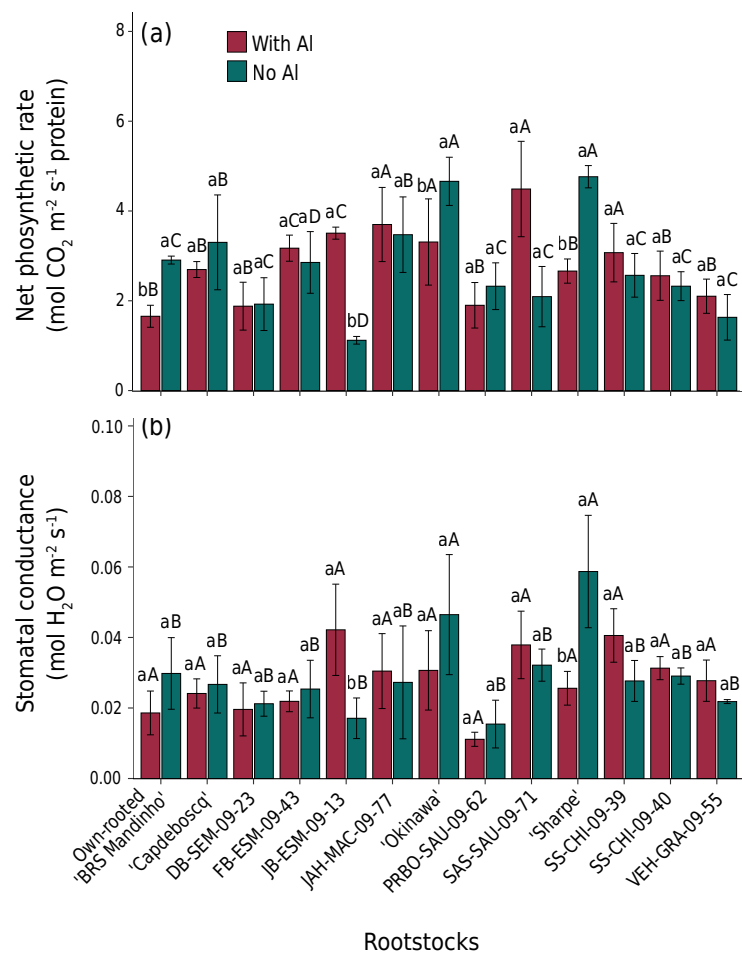

Figure 2. Mean values of net CO_2 photosynthetic rate (A) (a) and stomatal conductance of water vapor (Gs) (b) in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock, and uppercase letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.

vapor conductance (Gs) in the selections JB-ESM-09-13 and 'Sharpe' were observed without and with Al, respectively (Figure 2b). The Gs values did not differ among rootstocks with Al. However, higher Gs values were observed on 'Okinawa' and 'Sharpe' rootstocks without Al (Figure 2b).

Values of internal CO_2 concentration (Ci) with Al differed among rootstocks only in SAS-SAU-09-71 and 'Sharpe' (Figure 3a). The highest Ci values without Al were observed in 'Capdeboscq', JB-ESM-09-13, JAH-MAC-09-77, SAS-SAU-09-71, SS-CHI-09-40, 'Sharpe', and VHE-GRA-09-55 (Figure 3a). The Al presence affected the internal CO_2 concentration only in the rootstock selection DB-SEN-09-23, leading to an increase in the internal CO_2 concentration (Figure 3a).

In Al absence, the highest average for transpiration rate was found in 'Okinawa' and 'Sharpe', differing significantly from the other genotypes (Figure 3b). With Al, SAS-SAU-09-71 showed the highest transpiration rate value, and the lowest values were seen for DB-SEN-09-23 and PRBO-SAU-09-62 (Figure 3b). Comparing values in presence or Al absence within each rootstock, JB-ESM-09-13, SAS-SAU-09-71 and SS-CHI-09-39 showed higher transpiration rates in the Al presence, whereas, the Al presence led to a reduction in transpiration rates for the 'Okinawa' and 'Sharpe' rootstocks (Figure 3b).
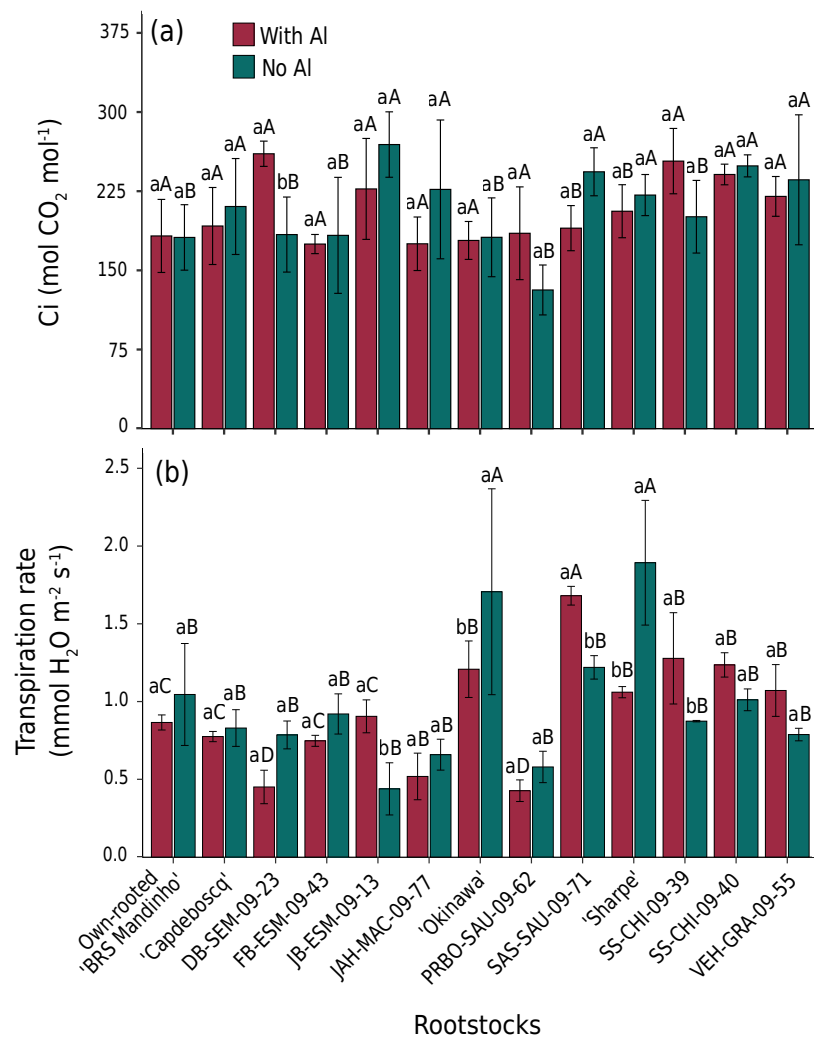
Figure 3. Mean values of intercellular CO_2 concentration (Ci) (a) and transpiration rate (E) (b) in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.

Biochemical variables

For total chlorophyll, the own-rooted 'BRS Mandinho', DB-SEN-09-23, PRBO-SAU-09-62, SAS-SAU-09-71, SS-CHI-09-40, 'Sharpe', and VEH -GRA-09-55 showed the highest means in the presence of Al, differing from the other genotypes (Figure 4a); and the lowest values were seen in JAH-MAC-09-77, SS-CHI-09-39, and 'Sharpe' (Figure 4a). In comparison between treatments within each rootstock, the own-rooted trees, DB-SEN-09-23, SAS-SAU-09-71, and SS-CHI-09-40 showed higher values of total chlorophylls with Al. The presence of Al decreased total chlorophyll values in the rootstocks 'Capdeboscq', JAH-MAC-09-77, SS-CHI-09-39, and 'Sharpe' (Figure 4a).

'Capdeboscq', FB-ESM-09-13, and JHA-MAC-09-77 showed the lowest values for carotenoid content in Al presence, significantly differing from the other genotypes (Figure 4b). Without Al, the lowest carotenoid values were observed in the own-rooted trees, DB-SEN-09-23, and SS-CHI-09-40 (Figure 4b). Between the treatments within each nursery tree type, own-rooted trees and SS-CHI-09-40 showed higher carotenoid values with Al. However, the presence of Al in the 'Capdeboscq' rootstock decreased the carotenoid values (Figure 4b).

The 'Okinawa', PRBO-SAU-09-71, and 'Sharpe' genotypes showed the lowest averages in Al presence for superoxide dismutase (SOD) enzyme activity in the shoots (Figure 5). In Al absence, the lowest values of SOD activity were observed in the own-rooted trees,
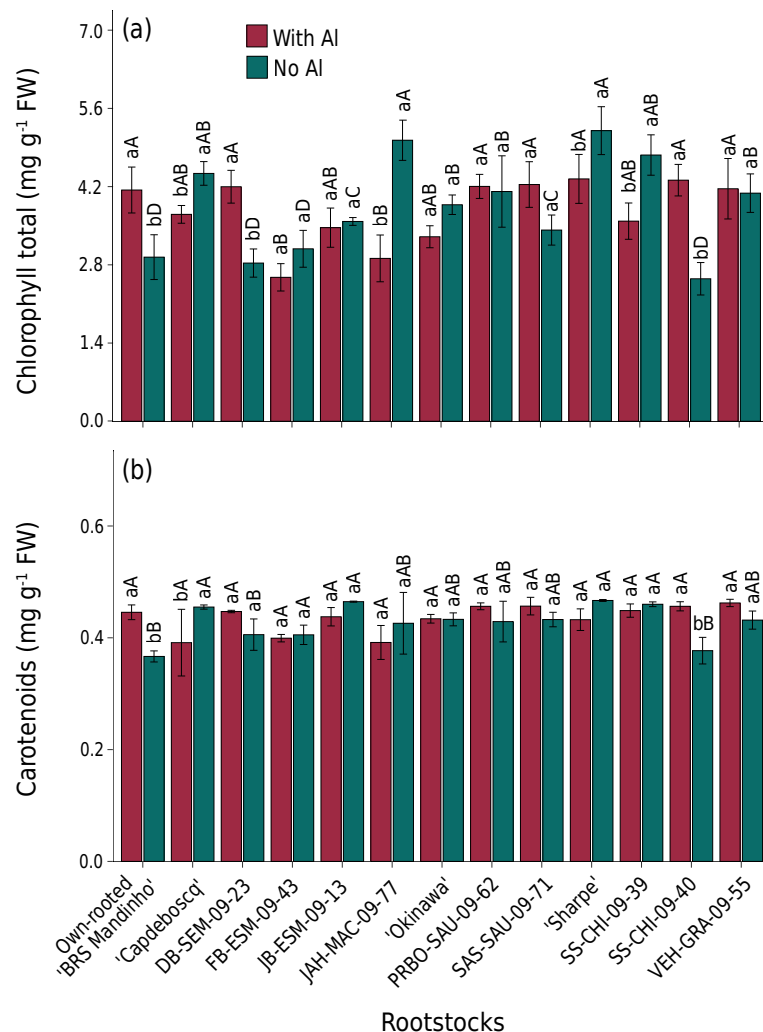
Figure 4. Mean values of total chlorophyll (a) and carotenoids (b) in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.

FB-ESM-09-43, JAH-MAC-09-77, SAS-SAU-09-71, and VEH-GRA-09-55 (Figure 5). In the comparison between treatments within each tree type, the own-rooted trees, FB-ESM-09-43, JAH-MAC-09-77, SAS-SAU-09-71, SS-CHI 09-39, and VEH-GRA-09-55 showed higher SOD activities in the shoots in Al presence. Yet, in the rootstocks JB-ESM-09-13, 'Okinawa', and 'Sharpe', the Al presence decreased SOD activity (Figure 5).

For SOD activity in the roots, the highest averages were observed in JB-ESM-09-13 in Al presence, followed by 'Capdeboscq' and PRBO-SAU-09-62 (Figure 5), while in 'Okinawa', the Al presence decreased SOD activity. The POD activity in the plant shoots, in turn, showed the highest values in 'Capdeboscq' and 'Sharpe' with Al, and the lowest values were seen in DB-SEN-09-23, FB-ESM-09-13, JAH-MAC-09-77, and SAS-SAU-09-71 (Figure 6). In Al absence, the highest values of POD activity were observed in the rootstock 'Okinawa' (Figure 6). Between the treatments, the own-rooted trees, 'Capdeboscq', PRBO-SAU-09-71, and 'Sharpe' showed higher POD activities in the shoots in Al presence, while in the rootstocks JAH-MAC-09-77 and 'Okinawa', the Al presence decreased POD activity (Figure 6). The highest values of POD activity in the roots with Al were observed in JB-ESM-09-13, but in Al absence, the highest values were obtained in FB-ESM-09-43 (Figure 6). Between the treatments within each rootstock, 'Capdeboscq', JB-ESM-09-13, and PRBO-SAU-09-62 showed higher POD activities in roots in Al presence.
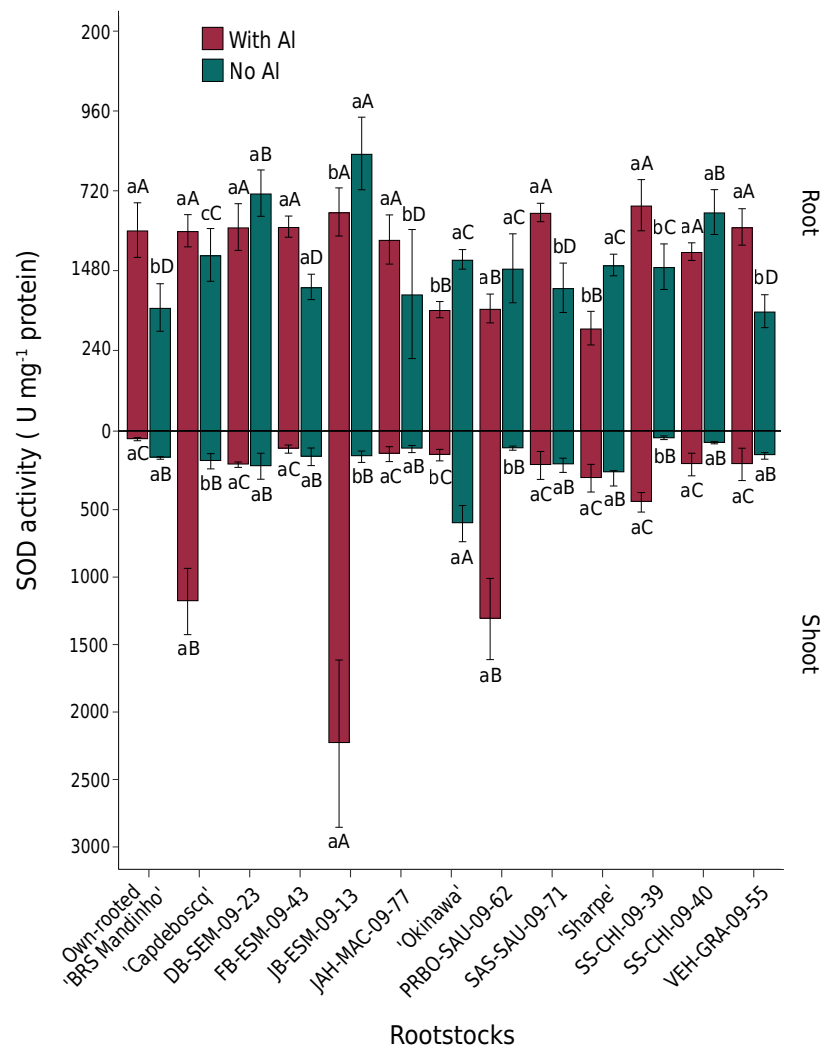
Figure 5. Mean values of superoxide dismutase (SOD) enzyme activity in shoot and roots in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.
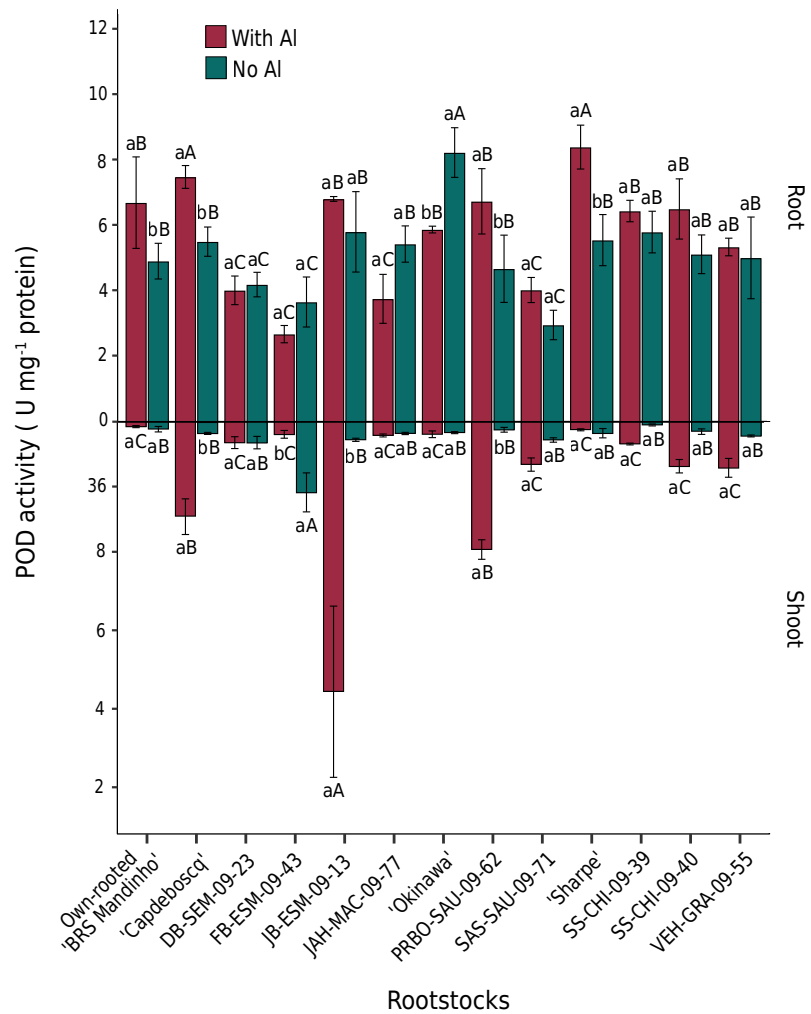
Figure 6. Mean values of guaiacol peroxidase (POD) enzyme activity in shoot and roots in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.

For hydrogen peroxide (H_2O_2) content in the shoots in the presence of Al, the highest values were observed in DB-SEN-09-23 and FB-ESM-09-4, and the lowest values were seen in 'Capdeboscq', PRBO-SAU-09-62, 'Sharpe', and SS-CHI-09-40 (Figure 7). In the absence of Al, the highest H_2O_2 content was observed in the rootstock JB-ESM-09-13 (Figure 7). Between the treatments, the own-rooted trees, DB-SEN-09-23, FB-ESM-09-43, and VEH-GRA-09-55 showed higher content of H_2O_2 in the shoots in Al presence (Figure 7). But in the rootstocks JB-ESM-09-13, 'Okinawa', SS-CHI-09-39, and SS-CHI-09-40, the Al presence decreased the H_2O_2 content (Figure 7).

Genotypes DB-SEN-09-23, JB-ESM-09-13, JAH-MAC-09-77, 'Okinawa', 'Sharpe', and VEH-GRA-09-55 with Al showed the highest content of H_2O_2 in the roots (Figure 7). In the absence of Al, the highest H_2O_2 content in the roots was observed in 'Capdeboscq', JAH-MAC-09-77, and VEH-GRA-09-55 (Figure 7). Between the treatments within each rootstock, 'Sharpe' showed higher H_2O_2 content in roots in Al presence, whereas the Al presence decreased the H_2O_2 content in the rootstock 'Capdeboscq' (Figure 7).

For shoots, the highest values of malondialdehyde (MDA) were found in 'Capdeboscq', FB-ESM-09-43, JB-ESM-09-13, PRBO-SAU-09-62, SS-CHI-09-39, and VEH-GRA-09-55 with Al (Figure 8). Between the treatments within each rootstock, most of genotypes showed difference in MDA values in relation to the absence or Al presence; and own-rooted trees, 'Capdeboscq', JB-ESM-09-13, PRBO-SAU-09-62, SS-CHI-09-39, and VEH-GRA-09-55 had

especially prominent differences. The roots of PRBO-SAU-09-62 with Al showed the highest MDA content, and a difference was also observed in relation to the presence or Al absence (Figure 8).

Principal component analysis

The PCA was performed after the prior selection of variables by factorial analysis, extracting only the first two components, as the sum of components PC1 (51.2 %) and PC2 (38.6 %) explained about 90 % of the original data variance (Figure 9). We emphasize that there is a difference between some rootstocks under the presence or Al absence, especially JB-ESM-09-13, PRBO-SAU-09-62, and 'Capdeboscq' in Al presence, and 'Okinawa' and 'Sharpe' in the absence of Al, which appeared in separation from the other rootstocks. The variables that most contributed to explain the variance of the data are related to stomatal conductance of water vapor (Gs), transpiration rate (T), and photosynthetic rate (A) in the rootstocks 'Okinawa' and 'Sharpe' without the presence of Al (Figure 9). The variables that contributed the most in explaining the variance are related to Tbars in the shoots of the rootstock PRBO-SAU-09-62 in Al presence, and the variables POD and SOD in the roots related to the rootstock JB-ESM-09-13 in Al presence.
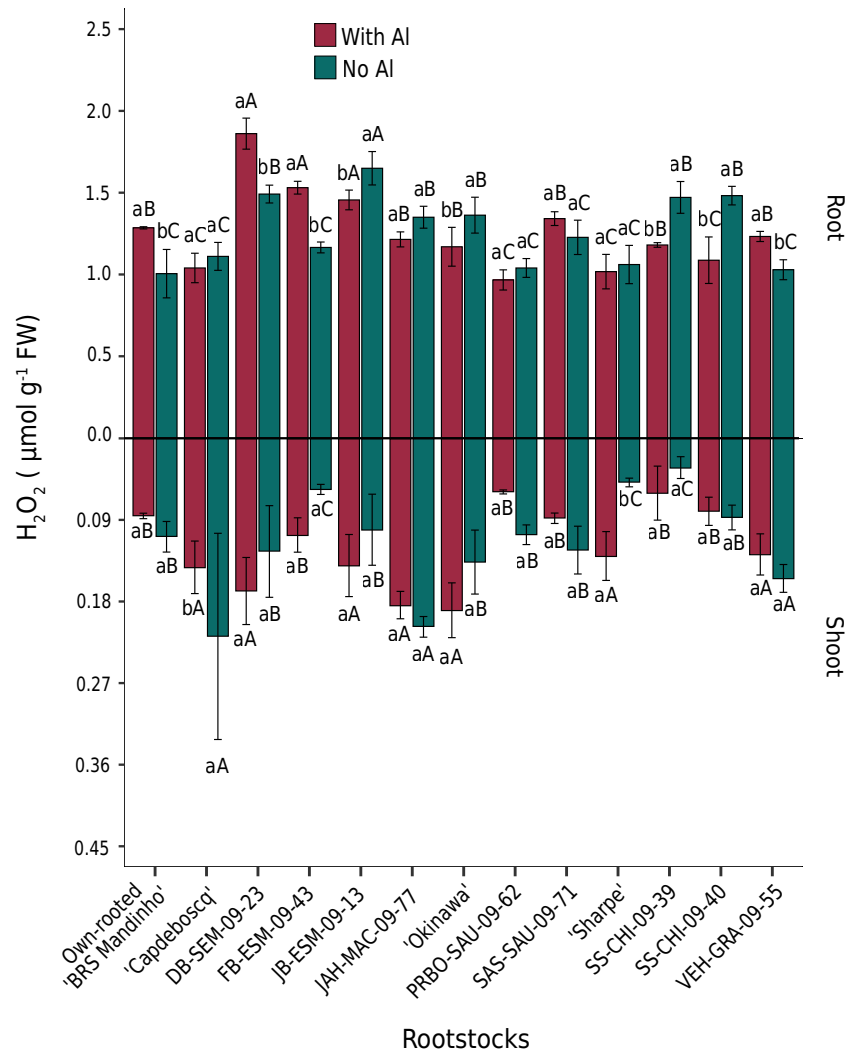
Figure 7. Mean values of hydrogen peroxide (H_2O_2) content in shoot and roots in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test ($p<0.05$). Bars represent mean ± standard deviation.
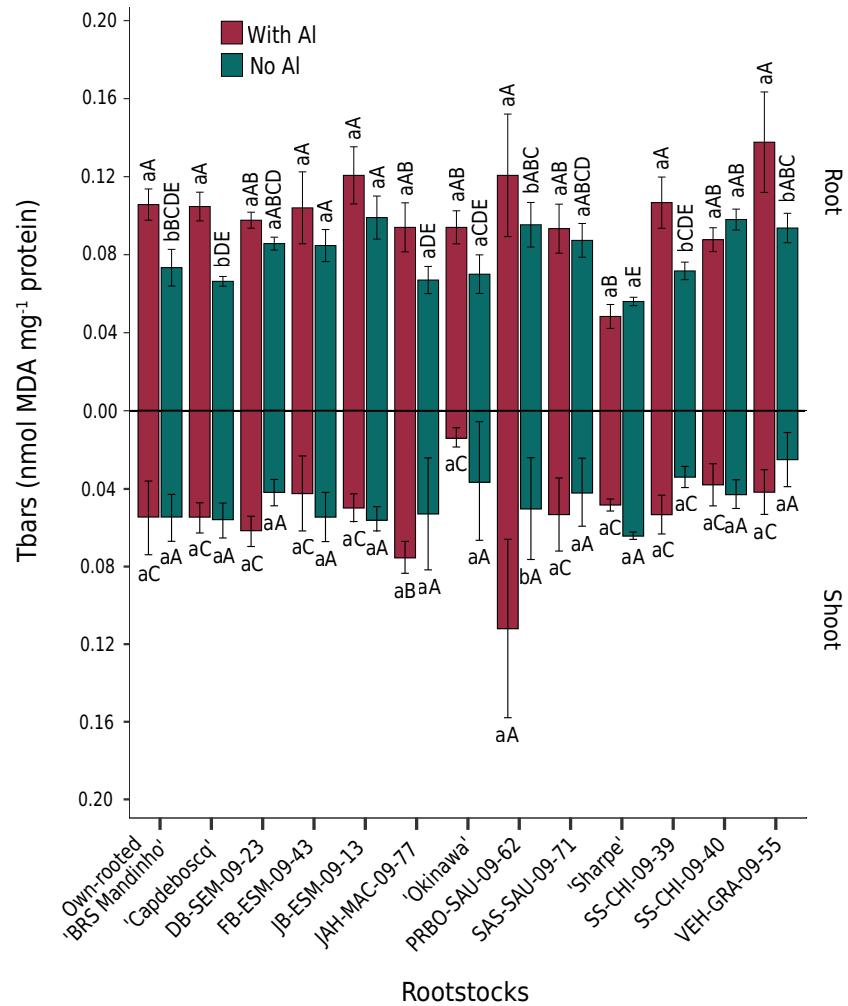
Figure 8. Mean values of membrane lipid peroxidation in shoot and roots in peach clonal rootstocks cultivated with Al and without Al in a hydroponic system. Rootstock: treatment interaction not significant, effects of factors analyzed in isolation. Lowercase letters compare means between treatments within the same rootstock and capital letters between rootstocks within the same treatment by Tukey's test (p<0.05). Bars represent mean ± standard deviation.

DISCUSSION

For total dry weight (TDW), the highest values were found in 'Sharpe' in the presence and Al absence, and in PRBO-SAU-09-62 in Al presence (Figure 1). This may have occurred because these rootstocks have tolerance and/or resistance mechanisms to reduce Al bioavailability. These mechanisms may include the exclusion of Al from uptake sites (Al resistance, apoplastic mechanisms) or internal detoxification mechanisms (Al tolerance, symplastic mechanisms) (Chauhan et al., 2021). Apoplastic mechanism restricts Al entry into the cell. Molecules such as citrate and malate are able to bind to Al in the apoplasm, decreasing the ability of Al to bind to the cell wall. Symplastic tolerance to Al is achieved by Al complexation with various organic compounds, such as citrate, malate, and oxalate. These Al complexes can be secreted through various transport proteins located in the membrane or sequestered and stored in the vacuole for a prolonged period, helping the plant to prevent damage caused by Al toxicity (Grevenstuk and Romano, 2013). In this respect, organic acids may be involved in both Al tolerance and resistance mechanisms.

In contrast, Al decreased the TDW of own-rooted 'BRS Mandinho', SS-CHI-09-39, and SS-CHI-09-40. This may have occurred due to the lack of Al resistance and/or tolerance mechanisms (Yang et al., 2015) in these trees. Thus, Al may be negatively affecting a wide range of physical, cellular, and molecular processes in these genotypes. That is because Al toxicity initially damages the root apex, rapidly inhibiting its elongation and root hair
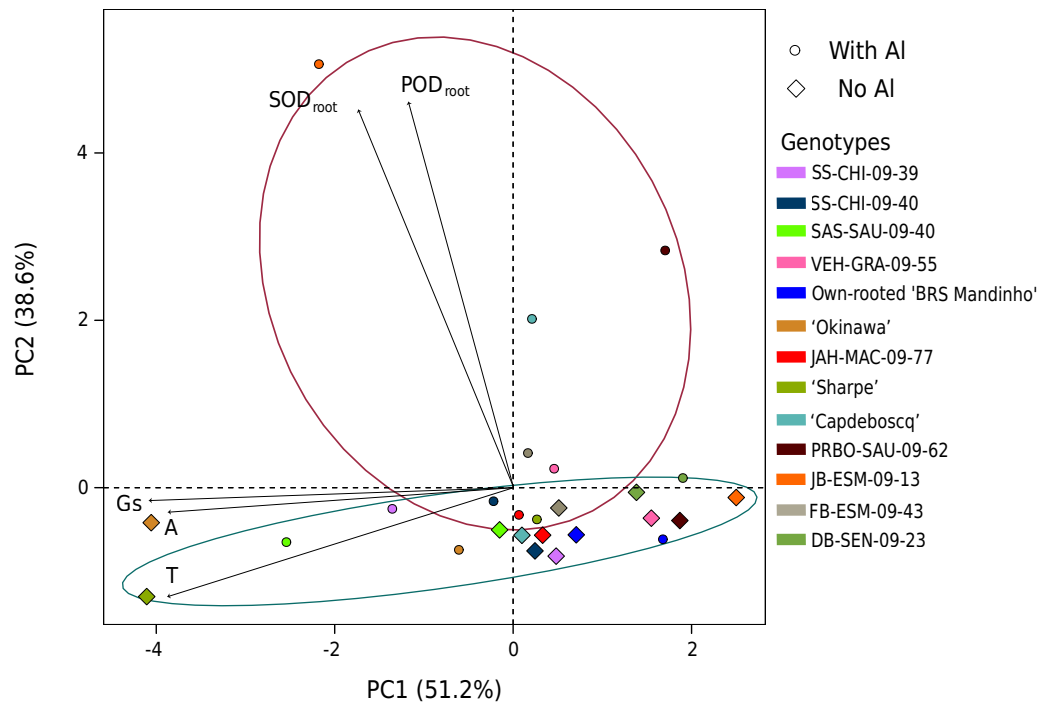
Figure 9. Relationship between principal component 1 (PC1) and principal component 2 (PC2), for growth, photosynthetic and biochemical variables in relation to rootstock genotypes cultivated with Al and without Al in a hydroponic system. Photosynthetic rate (A), transpiration rate (T), stomatal conductance of water vapors (Gs), activity of the enzymes guaiacol peroxidase (POD) and superoxide dismutase (SOD).

production, mainly through damage to the cell wall and plasma membrane (Yamamoto, 2019). Also, Al negatively affects cell division and alters the stability, association, and polymerization of microtubules and microfilaments, which ultimately limits root growth (Ciamporová, 2002). As a result, plants may show lower water and nutrient uptake, which contributes to decreased dry matter production (Figure 1) (Possebom et al., 2018). The Al transported from the roots to the shoot may also have negatively affected the formation and growth of these organs, which could trigger a reduced photosynthetic rate and consequently contribute to reduction in biomass in these plants (Guo et al., 2018).

In Al presence, 'Okinawa' and 'Sharpe' showed the lowest values for photosynthetic rate (Figure 2a). That may occur because Al damages the thylakoid membranes and the electron transport chain, directly repressing the CO_2 assimilation rate in several species (Mendes et al., 2018). In contrast, the genotype JB-ESM-09-13 led to higher photosynthetic rates in Al presence. This result may be related to the higher stomatal conductance this genotype shows in Al presence (Figure 2b). This higher photosynthetic activity in Al presence was not reflected in higher biomass production, since Al had no significant effect on TDW. The genotypes 'Capdeboscq', DB-SEN-09-23, FB-ESM-09-43, JB-ESM-09-13, JAH-MAC-09-77, SAS-SAU-09-71, and VEH-GRA-09-55 showed tolerance to Al, because photosynthetic activity and TDW were not affected by the presence of the metal.

The presence of Al decreased the stomatal conductance (Gs) in the rootstock 'Sharpe' (Figure 2b). That may have occurred because of ultrastructural modifications and reduced stomatal opening, which, consequently, reduces stomatal conductance (Gavassi et al., 2020) and decreases the photosynthetic rate. Thus, Al-induced stomatal closure is at least partially responsible for the decreased net CO_2 assimilation rate (Guo et al., 2018). In addition, an Al-activated malate transporter in guard cells may also be involved in stomatal closure, suggesting a direct effect of Al on stomatal movements (Sun et al., 2018).

For transpiration rate, comparing the treatments with and without Al within each rootstock, higher values were observed in JB-ESM-09-13, SAS-SAU-09-71, and SS-CHI-09-39 with Al (Figure 3b). However, in the rootstocks 'Okinawa' and 'Sharpe', the Al presence led to a reduction in transpiration rates (Figure 3b). This decrease observed in transpiration rate may be related to a change in stomatal behavior (Gavassi et al., 2020). The stress caused by Al results in stomatal changes, which directly interfere with stomatal closure, resulting in lower transpiration (Smirnov et al., 2014). This lower transpiration likely occurred because of increased leaf tissue stiffness and reduced porosity induced by Al in leaves; a reduced transpiration rate increases leaf resistance to Al stress (Yang et al., 2015). Although the rootstocks 'Okinawa' and 'Sharpe' led to reduced transpiration, the plants showed adequate growth even in the presence of Al. That may have occurred because of higher efficiency of these genotypes in using the absorbed/fixed CO_2 (Dorneles et al., 2019).

Comparison of the treatments within each rootstock shows that higher values of total chlorophyll were observed in the own-rooted trees and those grafted on DB-SEN-09-23 and SS-CHI-09-40 in Al presence (Figure 4a). This higher pigment concentration may provide greater absorption of light energy, contributing to higher biomass production (Dorneles et al., 2019). With increased pigments, plants can absorb more light radiation and convert it into carbohydrates, which results in increased biomass (Roca et al., 2018). However, the higher chlorophyll concentration in Al presence in these rootstocks was not reflected in higher TDW (Figure 1); in fact, Al reduced (own-rooted 'BRS Mandinho' and SS-CHI-09-40) or did not significantly affect (DB-SEN 09-23) TDW in these rootstocks.

The lower total chlorophyll values in the rootstocks 'Capdeboscq', JAH-MAC-09-77, and 'Sharpe' in Al presence may have occurred because Al can compete with Mg, which is an integral part of the chlorophyll molecule, in binding to binding sites in the plasma membrane of roots, interfering with Mg uptake and transport (Yang et al., 2015; Possebom et al., 2018). Thus, higher Al concentrations can cause lower chlorophyll content, which limits the metabolic potential of the plant (Mendes et al., 2018).

For carotenoid content, in comparison between treatments within each rootstock, higher values were observed in the own-rooted trees and those grafted on SS-CHI-09-40 (Figure 4b). That may be because carotenoids play an essential role in protecting the photosynthetic apparatus from the harmful effects of light and reactive oxygen species (ROS) by dissipating excess light in the form of heat in the antenna pigment complexes (Sun et al., 2018). Thus, increased carotenoid content in plants is generally related to increased tolerance to oxidative stress from toxic metals (Cunha Neto et al., 2020).

In the presence of Al, SOD enzyme activity in the roots was highest in JB-ESM-09-13 (Figure 5 and 9), followed by 'Capdeboscq', PRBO-SAU-09-62, and SS-CHI-09-39. This may indicate that the superoxide anion is being neutralized, resulting in high accumulation of H_2O_2, which is less reactive (Schmitt et al., 2020). The SOD is involved in the dismutation of the superoxide free radical into H_2O_2, and it is effective in preventing cell damage. This was observed especially in the rootstocks JB-ESM-09-13, 'Capdeboscq', and PRBO-SAU-09-62 (Figure 9), since, in those rootstocks, Al did not lead to a reduction in TDW. The observations of increased activity of antioxidant enzymes are in agreement with the fact that they remain active for ROS elimination (Berghetti et al., 2022). However, the lowest values for SOD activity were observed in the shoots of trees grafted on JB-ESM-09-13 and 'Sharpe', and in the roots and shoots of 'Okinawa' in Al presence. This may have occurred because the SOD enzyme requires metal ions, such as Fe, Mn, Zn, and Cu; and Al can interfere with the uptake or binding of these ions to the active site of the enzyme, resulting in lower values for SOD activity (Dorneles et al., 2019).

In a comparison of treatments within each rootstock, higher values for POD enzyme activity in the shoots and roots were observed in 'Capdeboscq' in the presence of Al. This may have occurred because of the higher activity of SOD, resulting in high accumulation

of H_2O_2; POD is then also triggered to establish a balance and control the concentration of cellular ROS, such as H_2O_2 and O_2, preventing OH from being produced (Bernardy et al., 2020). Furthermore, Al led to an increase in POD activity in the own-rooted trees, in trees grafted on 'Sharpe' and SAS-SAU-09-71 in the shoots, and in JB-ESM-09-13 and PRBO-SAU-09-71 in the roots (Figures 6 and 9). According to Dorneles et al. (2019), who studied Al stress tolerance in potato genotypes grown with silicon, the most Al-tolerant plants increase POD activity in Al presence. This increase in POD enzyme activity occurs in the plants in an attempt to reduce Al-induced oxidative stress. However, the increase in POD activity was not enough to avoid oxidative stress, since lipid peroxidation was significantly increased in some of the rootstocks exposed to Al.

The highest content of hydrogen peroxide (H_2O_2) in the shoots in the presence of Al was observed in the own-rooted trees and trees grafted on DB-SEN-09-23, FB-ESM-09-43, and VEH-GRA-09-55 (Figure 7). This was because of activation of the SOD enzyme, since the production of H_2O_2 in the shoots showed a very similar response to SOD activity for the same tree organ (Schmitt et al., 2020). In contrast, the Al presence led to lower content of H_2O_2 in the rootstocks JB-ESM-09-13, 'Okinawa', and SS-CHI-09-39. This response was indicated by lower SOD activity, and consequently, there was lower content of H_2O_2 accumulated in the tissues of these rootstocks. For H_2O_2 content in the roots, Al brought about an increase in 'Sharpe' and a decrease in 'Capdeboscq' (Figure 7). The increase in H_2O_2 production may be related to the fact that the antioxidant system was not able to overcome the toxicity caused by the higher levels of Al (Kuinchtner et al., 2021). That can result in negative effects, such as lipid peroxidation, which affects the oxidation of membrane proteins and can cause a decrease in plant growth (Possebom et al., 2018). Even with the increased concentrations of H_2O_2 in the rootstocks DB-SEN-09-23, FB-ESM-09-43, and VEH-GRA-09-55, there was no reduction in TDW in these rootstocks, indicating tolerance of these genetic materials to Al.

Comparison between the treatments within each tree type showed higher malondialdehyde (MDA) content in the shoots in the own-rooted trees and trees grafted on 'Capdeboscq', PRBO-SAU-09-62, SS-CHI-09-39, and VEH-GRA-09-55 in Al presence. In contrast, the increase in MDA content only in PRBO-SAU-09-62 (Figure 8) may be a direct effect of Al toxicity, indicating oxidative stress in this rootstock, which may cause irreversible damage to the long-term development and function of plant tissue (Kuinchtner et al., 2021). This result may also be due to Al prompting ROS formation, since rootstocks with characteristics of Al tolerance (mainly in relation to TDW and photosynthetic rate – 'Capdeboscq' and VEH-GRA-09-55, for example) and those with characteristics of Al sensitivity (own-rooted 'BRS Mandinho' and SS-CHI-09-39, for example) had high malondialdehyde (MDA) values. This response is manifested because Al can alter the arrangement of membrane lipids, facilitating lipid peroxidation, which can cause changes in membrane permeability (Chen et al., 2017). The MDA is a result of lipid peroxidation in cells, and this product remains an important indicator of oxidative stress in various plant studies (Daud et al., 2021). In rootstocks with Al tolerance characteristics ('Capdeboscq' and VEH-GRA-09-55, for example), the higher values of MDA production did not interfere with the TDW of the plants.

The PCA indicates that some rootstocks show differences in the presence or absence of Al, especially JB-ESM-09-13, PRBO-SAU-09-62, and 'Capdeboscq' in Al presence (Figure 9). Moreover, the variables that contributed most were related to POD, SOD, and Tbars in roots and shoots, most prominently POD and SOD in the roots of the rootstock DB-SEN-09-23.

The ability to maintain the proper balance of ROS generation by the network of ROS generating and eliminating enzymes, associated with an increased photosynthetic rate, may have been a contributing factor in forming the more complex set of processes underlying Al tolerance in peach rootstocks, and this outcome confirms our initial hypothesis. Therefore, in tolerant trees, even in the presence of toxic levels of Al,

enzymes were able to reestablish homeostasis, indicating the existence of efficient antioxidant defense systems against Al (Ribeiro et al., 2012). To resist and survive under such stressful conditions, plants may also have developed some defense mechanisms, such as external and internal exclusion of Al, and exudation of organic acids that help them grow in the presence of Al (Chauhan et al., 2021). The Al exclusion also occurs as a tolerance mechanism through the plant secreting ligands that chelate and detoxify Al ions externally and prevent their uptake into the cytosol of root cells (Brunner and Sperisen, 2013).

Physiological and oxidative stress variables can be used in the selection of genetic materials tolerant to Al toxicity, which can be an appropriate strategy to reduce the death of peach trees and reduce expenses with soil acidity correctors. We also highlight that peach plants, even when grown in soils with a history of limestone application, can produce roots in uncorrected soil layers. This reinforces the importance of selecting Al-tolerant genotypes.

Regarding the expected Al concentrations in the soil pore solution, this response varies with pH, soil type, soil particle size (proportion of clay, sand and silt), and also depends on the amount of organic matter present in the soil. Thus, the use of a nutrient solution allows us to observe the direct effect of Al on plants, without the presence of interactions that could affect its bioavailability. In the nutrient solution, Al is readily available and in direct contact with the plants' root system, facilitating the absorption of Al. Therefore, the nutrient solution (hydroponic system) does not hide or mitigate the toxicity caused by Al, since in this system the Al is readily available for uptake by plants. On the other hand, in soil the toxicity effect of Al would probably not be very evident, because of the adsorption of Al to functional groups of reactive particles or even its complexation, followed by precipitation in the solution.

CONCLUSION

Physiological and biochemical variables show potential for selection of Al-tolerant or Al-resistant peach clonal rootstocks, especially the variables photosynthetic rate, SOD, POD, and Tbars. Total dry matter production of the own-rooted 'BRS Mandinho' and trees grafted on SS-CHI-09-39 and SS-CHI-09-40 is affected by the presence of Al. The highest total dry matter production in the presence or absence of Al was observed for trees on the 'Sharpe' rootstock. Photosynthetic rate of the own-rooted 'BRS Mandinho' and trees grafted on 'Okinawa' and 'Sharpe' is negatively affected by the presence of Al. 'Capdeboscq', DB-SEN-09-23, FB-ESM-09-43, JB-ESM-09-13, JAH-MAC-09-77, SAS-SAU-09-71, and VEH-GRA-09-55 are tolerant to Al.

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