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Differential response to calcium-labelled (^{44}Ca) uptake and allocation in two peach rootstocks in relation to transpiration under *in vitro* conditions

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ABSTRACT

Calcium-labelled (^{44}Ca) uptake and transport under *in vitro* GreenTray® temporary immersion bioreactor conditions have been studied related to aeration conditions. For this aim, Rootpac®-20 (RP-20) and Garnem® ($G \times N$) were selected as two main rootstocks used in peach production. Two transpiration conditions, aerated and unaerated, were established for each plant material. ^{44}Ca location, plant development and foliar stomata surface were measured after the *in vitro* culture period. The results showed that aeration improved Ca transport within the shoot, but it did not enhance Ca uptake by the roots. Regarding plant material, $G \times N$ presented a better Ca uptake capacity and concentration. The findings suggest that Ca uptake in the roots is a precise process that is influenced by transpiration. However, it was observed that transpiration and thus the water flux is not the only force promoting Ca uptake by roots. Furthermore, the transport of Ca to the shoot was primarily determined by transpiration, indicating that water flux plays a crucial role in the aboveground movement of Ca. The study also revealed distinct behaviors in Ca uptake and allocation between the different peach rootstocks, emphasizing the importance of considering these factors in the selection process of rootstocks. These findings contribute to our understanding of the mechanisms involved in Ca uptake and transport in peach rootstocks under *in vitro* conditions. They provide valuable insights for rootstock selection processes and highlight the need for further research in this area.

1. Introduction

Calcium (Ca) has traditionally been used as an example of a nutrient with low phloem mobility in plants (White and Ding, 2023). Due to this characteristic, the upward delivery of Ca occurs withing the transpiration stream through the xylem (Hanger, 1979; Montanaro et al., 2010). Therefore, the movement and delivery of Ca is strongly influenced by the plant transpiration rate, which also depends on various environmental and orchard conditions such as soil water availability, vapor pressure deficit (VPD) or light interception (Girona et al., 2002; Ayars et al., 2003). When the transpiration is low, the root pressure strongly influences the xylem flow, and consequently is also affected by soil osmotic potential. Therefore, roots are not only involved in Ca uptake, but also in Ca transport, especially when the transpiration rate is low (Schenk et al., 2021).

A recent study reported that increasing the xylem vessel area is a new putative target in breeding approaches related to enhancing stomatal conductance and improving adaptability to Ca deficiencies in peaches (Aras et al., 2021). By contrast, peach rootstock selection has been traditionally based on plant vigor, fruit quality and soil adaptability (Reig et al., 2016; Iglesias et al., 2019). Additionally, the uptake capacity of mineral nutrients is determined by the rootstock choice, and therefore, its responses under nutritional deficit conditions should be understood and considered in the rootstock selection process (Melgar et al., 2023). In this study, a novel approach to trace Ca uptake and movement in peach rootstocks is described.

Traditionally, plant nutrition studies have employed isotopes to trace nutrients, and therefore, find out easily the uptake, distribution, and partitioning rates. A common technique to investigate Ca uptake is the use of the radioactive isotope ^{45}Ca that permitted to trace Ca by its radioactivity (Bonomelli et al., 2022). Even though this technique has been widely used in agriculture, there are some difficulties related to

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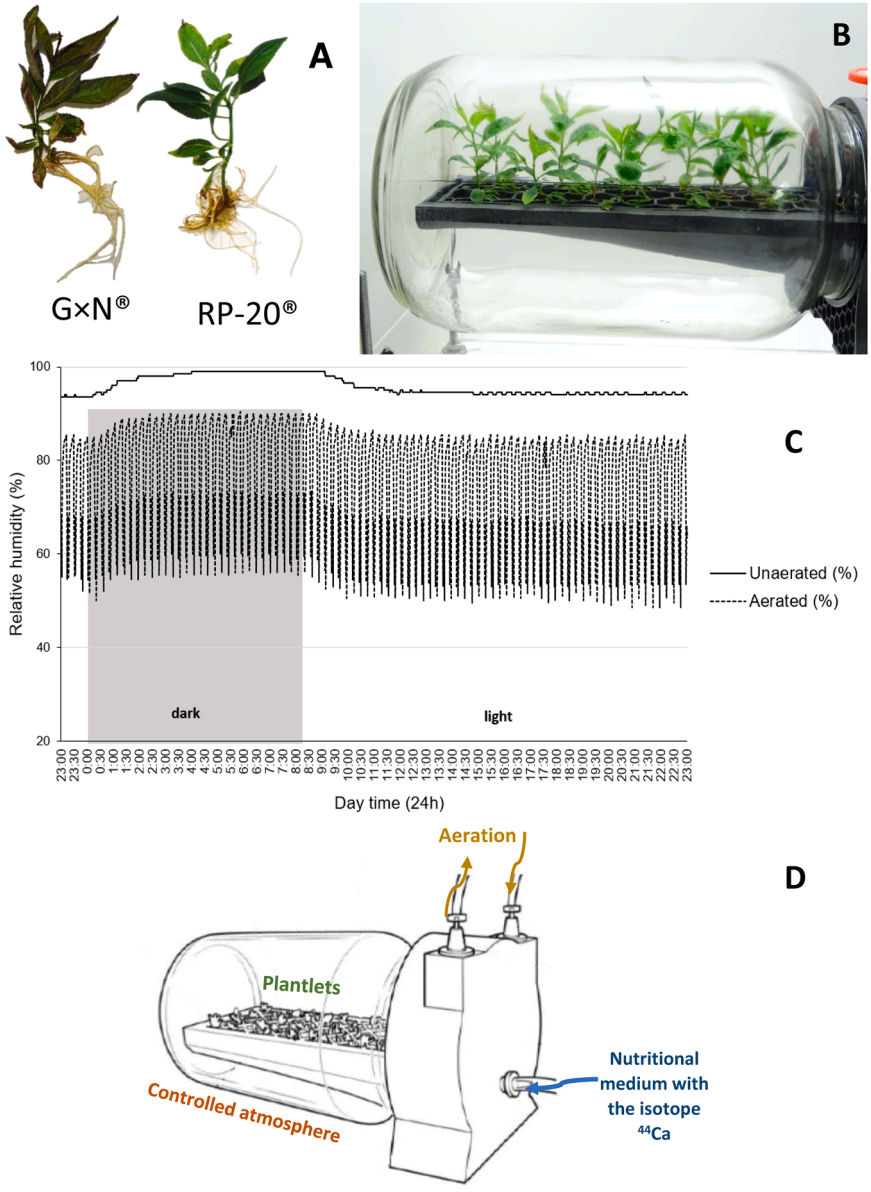

Fig. 1. Plant material (A), GreenTray® (B), air relative humidity inside an unaerated or aerated GreenTray® during a 24 h dark light cycle (C) and GreenTray® operating diagram (D).

human health or environmental risks that promote the development of new approaches. The stable isotope ^{44}Ca, although has not been commonly employed in agronomical studies, can provide an easy and safe way to trace Ca, as shown before in apple (Kalcsits et al., 2019). The natural concentration of ^{44}Ca is sufficiently low (2.086 %), enabling the identification of the majority of ^{44}Ca in plant tissues originating from the applied nutrient solution enriched with a higher concentration of ^{44}Ca. This approach opens new possibilities for gathering insights into calcium nutrition in crops, offering a valuable alternative to radioactive isotopes.

Although the ^{44}Ca use should be theoretically the most suitable option to trace Ca, its high price currently makes this option unfeasible in field assays. As an alternative, the ^{44}Ca trace under *in vitro* laboratory conditions is proposed. For this aim, the recently patented temporary immersion system (TIS) bioreactor GreenTray® (Dolcet-Sanjuan and Mendoza, 2018) brings the optimal platform to trace ^{44}Ca under *in vitro* controlled conditions, providing a good stage to evaluate Ca dynamics and accumulation using Ca-labelled fertilizers. Herein the effect of aeration on Ca uptake and movement, using the stable Ca isotope ^{44}Ca as a tracer, in two different peach rootstocks grown in the bioreactor GreenTray® have been studied. This implies a novel approach in plant nutrition studies that will effectively streamline the assessment of nutrient absorption.

2. Materials and methods

2.1. In vitro plant material

Two different *Prunus* commercial rootstocks were used for the study: Rootpac® 20 (RP-20) and Garnem® (*G* × N) (Fig. 1A). RP-20 rootstock is a hybrid between *Prunus cerasifera* and *Prunus* besseyi and *G* × N rootstock constitutes a hybrid between *Prunus dulcis* and *Prunus persica*.

The study was conducted using *in vitro* propagated shoot-tip cultures of both rootstocks. Both rootstocks were propagated by axillar branching in MS medium (Murashige and Skoog, 1962) with 5 μM benzylaminopurine (BAP), as described by Iglesias et al. (2004). Two-cm-long nodal segments derived from shoot tip cultures in the multiplication phase were used as initiation explants. Shoot rooting was induced in ½-MS supplemented with 10 μM indole-3-acetic acid (IAA), for 7 days

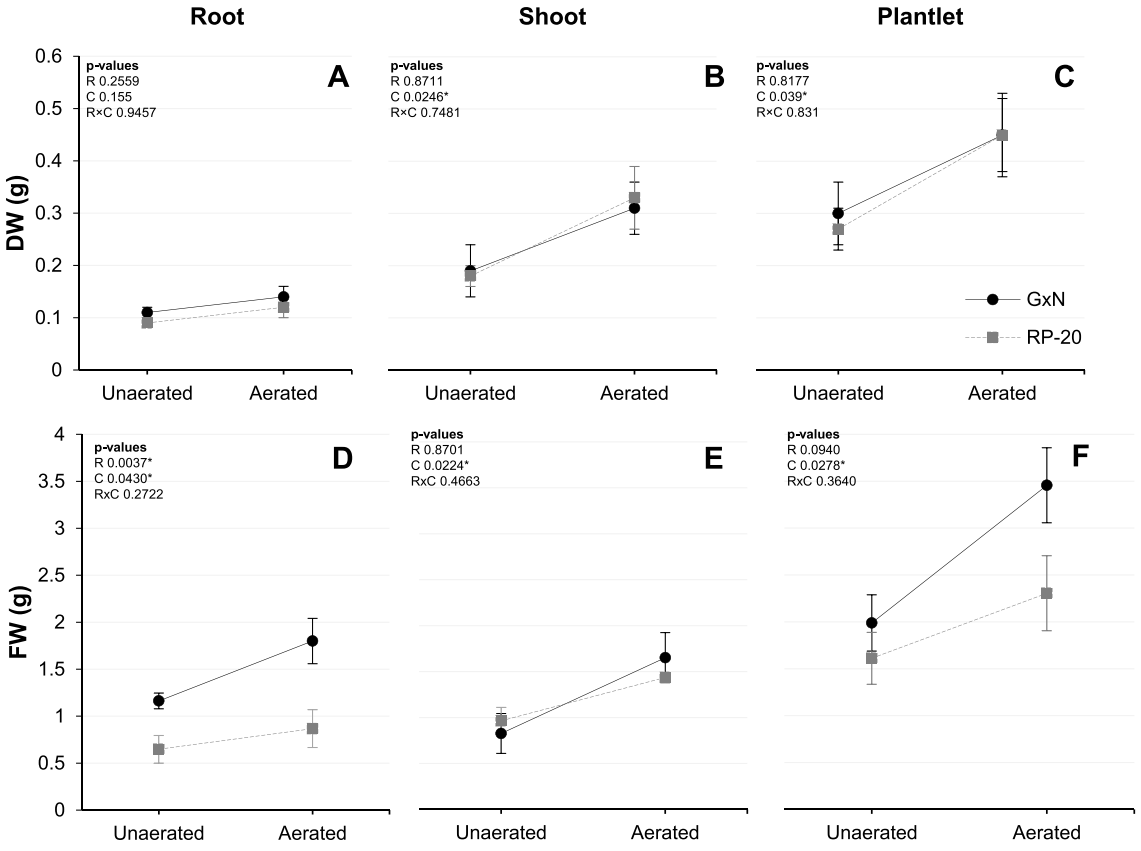

Fig. 2. Differential responses of rootstocks $G \times N$ (black circles and continuous lines) and RP-20 (gray squares and dashed lines), under aerated or unaerated conditions (abscissa axis) for Dry weight for root (A), shoot (B) and the whole plantlet (C) and Fresh weight (FW) for root (D), shoot (E) and the whole plantlet (F). A two-way ANOVA was used to test the main effects of conditions (C) and rootstocks (R). Statistical significance was assumed at $p < 0.05$*.

under 24 °C and dark conditions. Afterwards, shoots were transferred to a ½-MS medium without auxin for root elongation during 7 days under 24 °C and a 16-hour-photoperiod with white-light fluorescents (100–120 μmol m^{-2} s^{-1}). The two ½-MS mediums had 50 % MS-salt and macronutrient concentration and were supplemented with Fe-EDTA (10 mM) and $CuSO_4 \cdot 5H_2O$ (0.1 g L^{-1}). All media were pH-adjusted to 5.7 using NaOH (1 M) before adding agar (8 g L^{-1}) and were then autoclaved at 121 °C for 20 min. Glass tubes, 15 mm of diameter, with 15 ml of medium each were utilized.

2.2. Culture conditions and experimental design

GreenTray® temporary immersion bioreactors (Patent number ES2763637B1) (Dolcet-Sanjuan and Mendoza, 2018) were used for the development of rooted shoots as a system to evaluate the root uptake and plant transport of Ca (Fig. 1B). A modified liquid MS-medium (Murashinge and Skoog, 1962), pH 5.7 and autoclaved at 121 °C for 20 min (without adding sugar or hormones) was used in the GreenTray® bioreactor culture system (Cantabella et al., 2022).

The experiment consisted of two different treatments, combining two bioreactor ventilation conditions to force two plant transpiration situations (aerated and unaerated) on both rootstocks. The bioreactor air phase was renewed in the aeration treatment, while in the unaerated there were not a force air renewal of the aerial part.

Fifteen rooted shoots per bioreactor were used. During the plant material establishment to the GreenTray® culture conditions (3 days), all cultures were kept at 24 ± 1 °C under a photoperiod of 16 h of cool-white fluorescent light (100–120 μmolm^{-2} s^{-1}), and an immersion frequency of 2 min every 3 h. Afterwards, the culture medium was replaced with fresh medium of the same composition but with 38.81 % of $^{44}CaCl_2$ and 61.19 % of $CaCl_2$. $^{44}CaCl_2$ was prepared by dissolving $^{44}CaCO_3$ (enriched 97 %, Cambridge Isotope Laboratories, Inc., USA) in concentrated HCl based on Kalcsits et al. (2017).

Throughout the following 10 days, the culture conditions were the same as in the establishment phase, but one bioreactor for each rootstock were heavily aerated. To study the effect of ventilation and transpiration on Ca accumulation, the bioreactor air phase was renewed in the aeration treatment, while in the unaerated there were not a force air renewal of the aerial part (Fig. 1D). In the aerated regime, a 60-second-long aeration of the GreenTray® culture flask was scheduled every 15 min. While the unaerated culture regime was saturated till 94–99 % of ambient humidity inside the bioreactor, the aerated conditions maintain humidity between 49 % and 91 % (Fig. 1C).

After 10 days of culture on the enriched medium with ^{44}Ca, the bioreactor immersion and aeration were stopped, and several measurements on the plantlets were taken to evaluate the differences in the plant growth and Ca nutrition.

2.3. Morphological measurements

Plants were extracted and total plantlet, root and shoot fresh weight and shoot and root length (cm) were measured shortly after collection in each plant. Vegetative fractions were oven-dried (Memmert, ULP 800), at 65 °C until constant weight and the dry material was ground and homogenised using a mortar and pestle till powder. Dry weight (DW, g) was calculated afterwards. Plantlet, root, and shoot fresh and root/shoot index, and shoot and root length (cm) were measured in each plant. For the root length, the longest root was measured in each plant.

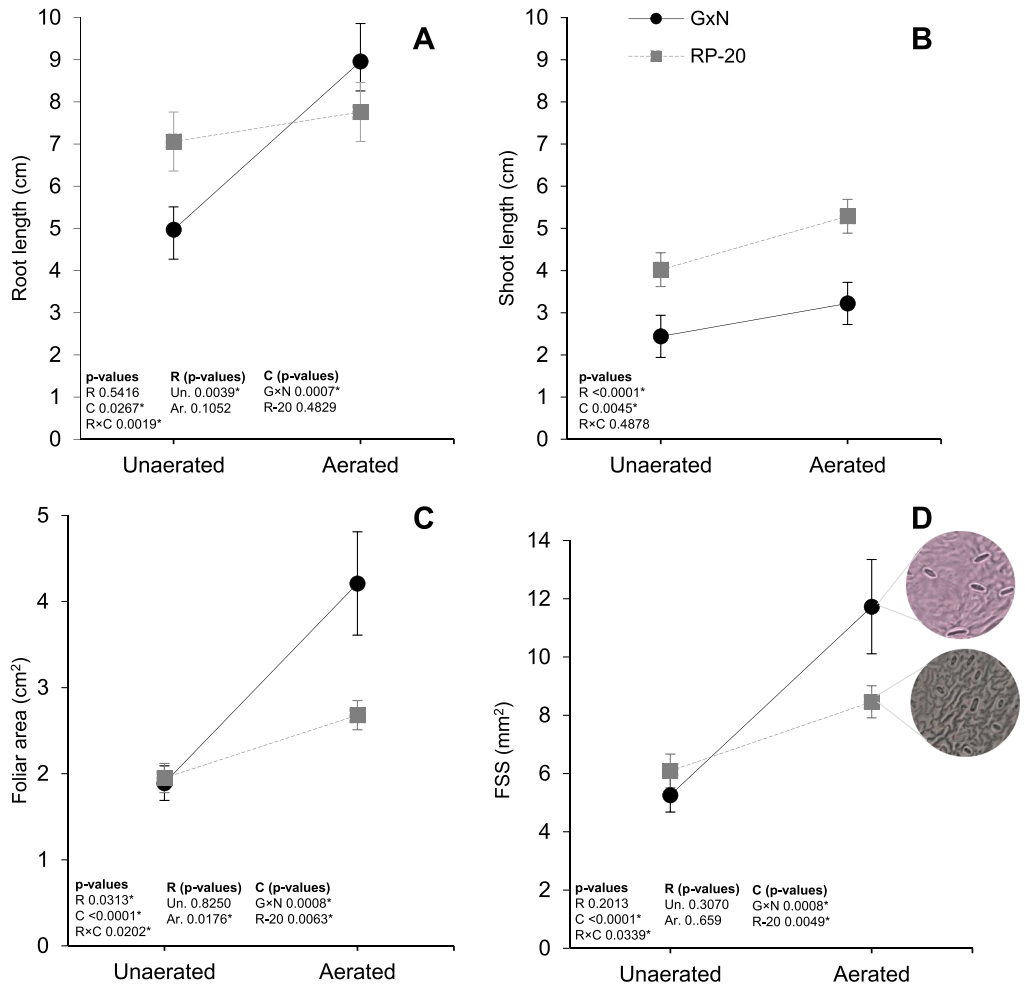

Fig. 3. Root length (cm) (A), Shoot length (cm) (B), Foliar area (cm^2) (C) and Foliar Stomata Surface (FSS) (mm^2) (D) for unaerated and aerated plantlets, in GxN (black circle and continuous lines) and RP-20 (grey square and dashed lines). A two-way ANOVA was used to test the main effects of conditions (C) and rootstocks (R). Statistical significance was assumed at $p < 0.05$*.

2.4. Stomatal density and foliar area

The total foliar stomatal surface (FSS) (mm^2) and total foliar area (cm^2) were determined. Stomatal parameters were measured from the second and fourth totally developed youngest leaf of three plantlets following the methodology described by Zhu et al. (2018). An optical white-light microscope (Leica DM5000B, Leica Microsystems CMS GmbH, Germany) was used to observe the stomatal characteristics under 40 magnifications (40x) and total stomata were counted from three different leaf slides from each leaf. In this experiment, a colourful digital Leica camera (Leica DFC 420) connected with the microscope was used to take images from the different leaf fields and from five individual stomata. Images were analysed using the *Application Suite System LEICA* and the stomata length and stomata width were measured (μm) to calculate the individual stomatal area using the ellipse area formula [(π × sl × sw)], where *sl* is the stomata length and *sw* is the stomata width. The total FSS was determined following the formula [(stomatal density) × (individual stomatal area) × (total foliar area)]. The total foliar area was measured with *LeafByte application*, following the protocol described by Getman-Pickering et al. (2020).

2.5. Calcium analysis

For each bioreactor, three groups of plantlets were established considering their homogeneity and for each group, roots and shoot were separated. Hence, a total of six samples were collected for every bioreactor (3 from shoots and 3 from roots). Mineral content and isotope analysis were measured at the analytical mass spectrometry department (CACTI) of the University of Vigo. Samples were oven-dried, and the dry material was ground and homogenized using a mortar and pestle till powder. Mineral content determination was achieved by nitric digestion and subsequent dilution of the digestion with mili-Q water. The total Ca concentration (mg g^{-1} DW) was measured with an atomic emission spectrometer with an inductively coupled plasma source (ICP-AES iCAP 6000, Thermo Scientific). The ^{44}Ca concentration was determined in a mass spectrometer with a multiple collector and inductively coupled plasma source (MC-ICP MS, Thermo Finnigan Neptune, University of Vigo) following the described methodology by Heuser et al. (2002).

The ^{44}Ca excess (%) of each sample was calculated following the formula:

$$^{44}Ca\ excess\ (\%) = \%^{44}Ca - 2.086\%$$

where 2.086 is the percentage of Ca naturally found in nature.

To calculate the ^{44}Ca (mg) the following formula was applied:

$$^{44}Ca(mg) = {}^{44}Ca\ excess(\%) \times DW(mg) \times Ca\left(mg{\cdot}kg^{-1}\right) \times 10^4$$

The Ca derived from the fertilizer (Cadff), which means the proportion of Ca in the plant that comes from the fertilizer (in this case from the *in vitro* culture medium) was calculated following the formula:

$$Cadff(\%) = \frac{^{44}Ca\ excess}{^{44}Ca\ excess\ culture\ medium} \times\ 100$$

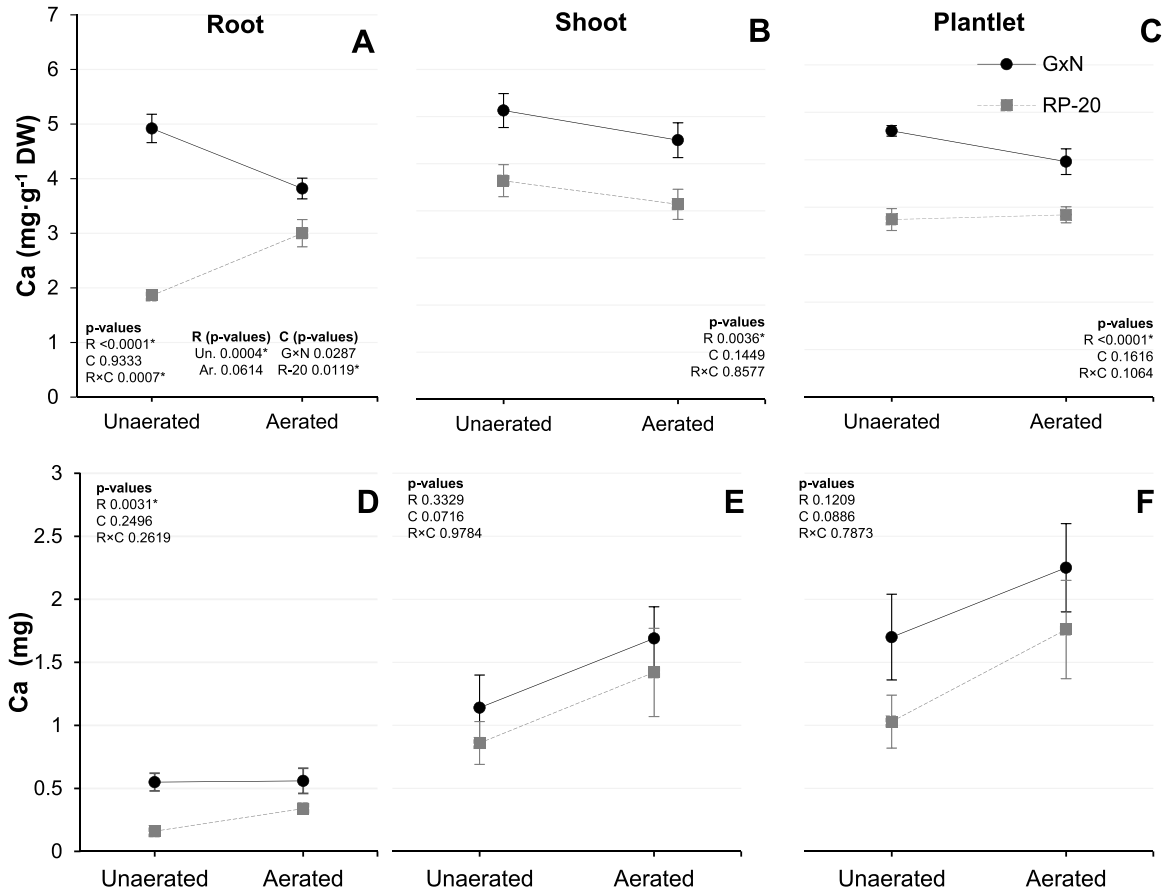

Fig. 4. Differential responses of rootstocks $G \times N$ (black circles and continuous lines) and RP-20 (grey squares and dashed lines), under aerated or unaerated conditions (abscissa axis), for Ca (mg g^{-1} DW) (A, B and C) and Ca (mg) (D, E and F) on roots (A and D), shoots (B and E) and the whole plantlet (C and F). A two-way ANOVA was used to test the main effects of conditions (C) and rootstocks (R). Statistical significance was assumed at $p < 0.05$*.

where ^{44}Ca excess (%) enrichment in culture medium was of 36.836 %.

2.6. Statistical analysis

The data were analysed via analysis of variance (ANOVA) by using JMP Pro-Software (version 16.0, SAS Institute Inc., Cary, NC). A two-way ANOVA was used to test the main effects of conditions (C), aerated and unaerated, and rootstocks (R), $G \times N$ and RP-20, in roots, shoots and the whole plantlet. Statistical significance was assumed at p levels <0.05.

3. Results

3.1. Morphological and physiological plantlet changes

The root DW (g) was independent of the conditions and the rootstocks. On the other hand, the shoot and total plantlet DW was determined by the conditions, but not by the rootstock. Concretely, shoot DW was significantly higher under the aeration conditions (0.32 g for aerated and 0.19 g for unaerated) as well as the total plantlet DW (0.45 g for aerated and 0.3 g for unaerated) (Fig. 2A, B and C).

Regarding FW (g), the rootstock and the conditions had differentially influenced the parts of the plant. On one hand, $G \times N$ presented significantly higher FW values than RP-20 in the roots (1.48 and 0.76 g respectively) and in the whole plantlet (2.72 g for $G \times N$ and 1.95 g for RP-20). On the other hand, the bioreactor aeration significantly increased the FW in the roots (1.35 g for aerated and 0.76 g for unaerated), the shoots (1.54 g for aerated and 0.89 g for unaerated) and the whole plant (2.88 g for aerated and 1.8 g for unaerated) (Fig. 2D, E and F).

Root length (cm) was significantly affected by the interaction between the rootstock and the conditions. While the aeration in $G \times N$ significantly increased root length, the RP-20 aerated and unaerated values were similar. Moreover, only under unaerated conditions there were statistical differences between rootstocks, where the RP-20 presented the longest roots. Apart from that, RP-20 significantly had longer shoots than $G \times N$ (4.65 and 2.83 cm respectively) and shoot growth was enhanced with aeration for both rootstocks (Fig. 3A and B).

What is more, the differences between rootstocks foliar areas (cm^2) were significantly increased by aeration of the GreenTray®, especially with $G \times N$, which doubled the RP-20 leaf surface under aeration conditions (Fig. 3C). Concretely, RP-20 presented a significantly higher stomata density (n° stomata/mm^2) ($p < 0.0001$) than $G \times N$, but $G \times N$ significantly presented a biggest stomata size (μm^2) ($p < 0.0001$) (data not shown). Consequently, $G \times N$ presented a higher FSS (mm^2) than RP-20 under aerated conditions, and under unaerated conditions both rootstocks presented a similar FSS (Fig. 3D).

3.2. Ca concentration and content

Overall, RP-20 presented significantly lower Ca concentration than $G \times N$, especially under unaerated conditions. Concretely in the roots, Ca concentration, expressed as Ca mg g^{-1} DW, of both rootstocks was influenced by the bioreactor aeration conditions. While $G \times N$ roots presented higher values in unaerated than in aerated conditions, the Ca concentrations for roots of RP-20 were higher for aerated conditions than for unaerated. Regarding shoots, $G \times N$ presented a significantly higher Ca (mg g^{-1} DW) concentration than RP-20, without any

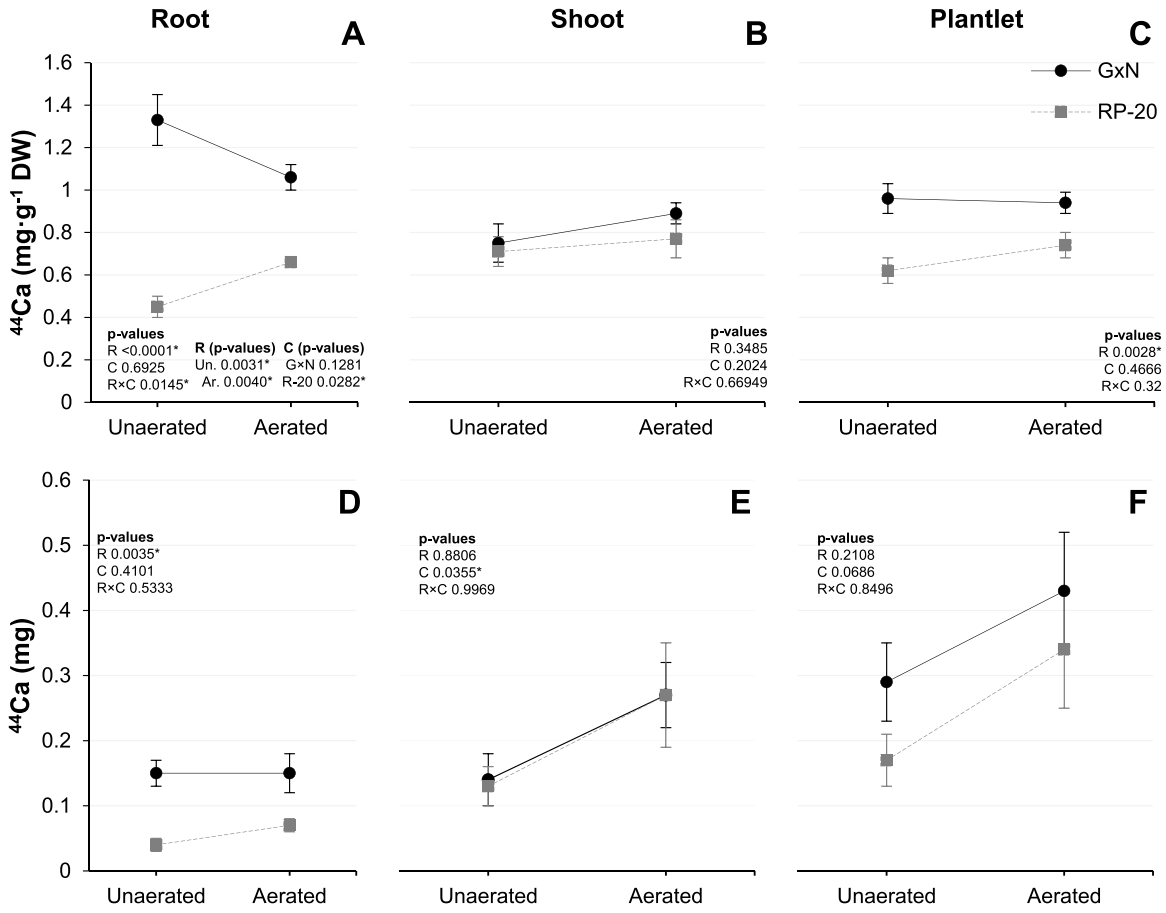

Fig. 5. Differential responses of rootstocks $G \times N$ (black circles and continuous lines) and RP-20 (gray squares and dashed lines), under aerated or unaerated conditions (abscissa axis), for ^{44}Ca (mg g^{-1} DW) (A, B and C) and ^{44}Ca (mg) (D, E and F) on roots (A and D), shoots (B and E) and the whole plantlet (C and F). A two-way ANOVA was used to test the main effects of conditions (C) and rootstocks (R). Statistical significance was assumed at $p < 0.05$*.

statistical differences between the conditions. However, no differences between aerated and unaerated conditions were found in total Ca concentration (Fig. 4A, B and C).

Concerning Ca (mg) content, as for Ca concentrations, $G \times N$ presented the highest values, but only statistically different in the roots. Even more, the $G \times N$ roots doubled the Ca content of RP-20 (0.56 and 0.26 mg respectively) independently from the conditions. Besides that, a higher Ca (mg) content, although not significant, was observed on aerated shoots and whole plantlets for both rootstocks when compared to unaerated (Fig. 4D, E and F).

3.3. ^{44}Ca-labelled concentration and content

In general terms, the results for ^{44}Ca (mg) and ^{44}Ca (mg g^{-1} DW) tended to present a similar pattern to those presented before for non-labelled Ca. $G \times N$ generally presented a higher ^{44}Ca concentration and content than RP-20. Concretely, ^{44}Ca (mg g^{-1} DW) concentration indicated that both rootstocks were differently influenced by the bioreactor aeration conditions. Meanwhile the $G \times N$ roots exhibited the highest ^{44}Ca (mg g^{-1} DW) under unaerated conditions, the RP-20 roots presented the highest ^{44}Ca concentration under aerated conditions, resulting in an opposite roots response to bioreactor conditions depending on the plant material (Fig. 5A, B and C).

The ^{44}Ca (mg) in roots was significantly higher in $G \times N$ than in RP-20 (0.15 and 0.06 mg respectively), although no statistical differences were found in roots regarding the conditions. Interestingly, aeration did not influence ^{44}Ca (mg) in the roots, however, it demonstrated a noticeable effect on the shoots. Under aeration conditions, the ^{44}Ca (mg) significantly increased in the shoots (0.13 mg for unaerated and 0.27 mg for aerated and). Shoot accumulation of ^{44}Ca was not different between rootstocks. In general terms, the total plantlet ^{44}Ca (mg) was not significantly influenced either by the rootstock or the bioreactor conditions. Although the aerated plant tended to present the highest ^{44}Ca (mg) contents (Fig. 5D, E and F).

For ^{44}Ca excess (%), the significant differences found in roots were only due to the rootstocks, where $G \times N$ presented the highest values (27.38 % for $G \times N$ and 23.08 % for RP-20). By contrast, in shoots there were significant differences for rootstocks and bioreactor conditions. In this part of the plantlet, RP-20 significantly presented more ^{44}Ca excess (%) than $G \times N$ (16.87 % for RP-20 and 14.23 % for $G \times N$). Moreover, aeration significantly increased ^{44}Ca excess (%) in the shoots compared to unaerated, for both plant materials (17.40 % for aerated and 13.7 % for unaerated). In the whole plantlet, no significant differences were found in rootstock and conditions. Cadff (%) followed the same patterns as ^{44}Ca excess (%) due to the fact that in every bioreactor the composition of the culture medium contained the same Ca and ^{44}Ca concentrations (Fig. 6).

4. Discussion

Peach rootstocks have been widely selected for their impact on fruit quality and productivity. However, compared to other fruit species such as apples and tomatoes (Ho et al., 1993; del Amor *et al.*, 2006), the nutrient uptake by peach rootstocks has not been as thoroughly characterized. Among the limited number of studies, peach rootstock evaluation has been focused on the fruit and leaf mineral content in productive trees (Reighard et al., 2012; Mestre et al., 2015). In this study, we conducted a comprehensive characterization of the plant

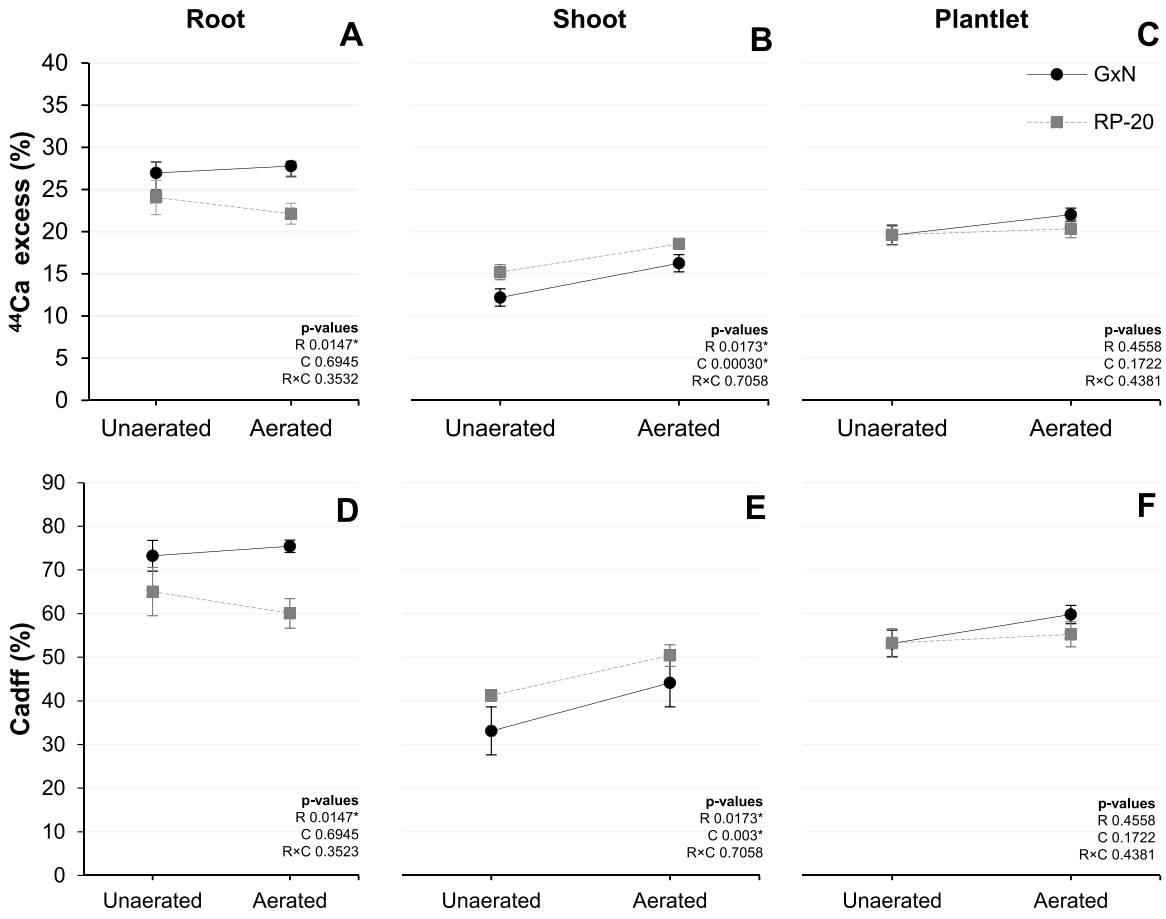

Fig. 6. Differential responses of rootstocks $G \times N$ (black circles and continuous lines) and RP-20 (gray squares and dashed lines), under aerated or unaerated conditions (abscissa axis), for ^{44}Ca excess (%) (A, B and C) and Cadff (%) (D, E and F) on roots (A and D), shoots (B and E) and the whole plantlet (C and F). A two-way ANOVA was used to test the main effects of conditions (C) and rootstocks (R). Statistical significance was assumed at $p < 0.05$*.

material using an *in vitro* approach within a short time frame. This approach enabled us to assess the root Ca uptake capacity independently of scion interactions in the GreenTray® system. Thus, the methodology presented herein represents a novel approach to clonal *in vitro* selection based on Ca uptake efficiency and could be extended to other nutrients.

The main factor influencing root Ca uptake was found to be the rootstock clone, rather than the aeration condition during culture in the bioreactor. It is worth mentioning that $G \times N$ exhibited higher levels of ^{44}Ca concentration and content in roots, suggesting a greater capacity for Ca uptake compared to RP-20. Additionally, the results showed that $G \times N$ presented a higher water content in roots compared to RP-20, what could be related to a greater water uptake capacity. These differences could be attributed to their differential vegetative growth. RP-20 is classified as a dwarfing rootstock (Pinochet *et al.*, 2010; Opazo et al., 2020).

On the other hand, $G \times N$ is a rootstock characterized by a higher yield production and trunk cross section in almond cultivars (Lordan et al., 2019), which may be associated with higher nutritional requirements. This implies that $G \times N$ rootstock would require a higher Ca fertilization compared to RP-20. Our results are in line with Aras et al. (2021), who evaluated the tolerance of the peach rootstocks $G \times N$ and GF-677 under Ca deficiency, concluding that $G \times N$ exhibited better tolerance to Ca deficiency.

The ability of $G \times N$ to uptake more Ca could be related to the higher capacity of transpiration in the aerial part under *in vitro* conditions. According to our results, there was a correlation between the total plantlet ^{44}Ca excess (%) and the FSS (mm^2) for both rootstocks under aerated conditions, ($G \times N$: $R^2 = 0.997$, $p = 0.0374$; RP-20: $R^2 = 1$, $p =$ 0.0096). Besides, we observed that $G \times N$ showed higher values of foliar area and FSS under aerated conditions. These results would be in line with those from Aras et al. (2021) who observed that the improved tolerance of $G \times N$ was attributed to an increase in stomatal conductance, which was achieved through physiological responses such as the increment in xylem thickness.

Accordingly, previous works under field conditions have reported that $G \times N$ presented a proper adaptation to drought and a high productivity compared to RP-20 and other eight almond rootstocks (Bellvert et al., 2021). On the other hand, even though RP-20 has been considered as a dwarfing rootstock, it has been reported that it induced a higher stomata density to grafted almond leaves compared to RP-40 (Opazo et al., 2020). This is consistent with our results where RP-20 leaves presented a higher stomata density than $G \times N$. However, this higher density implies a smaller stomata size, which together with a smaller leaf area result in a lower FSS (Fig. 3D).

It can be inferred based on the results presented herein that the aboveground part of the plant, particularly the leaves, may play an equally or even more significant role than the roots in the assimilation and transport of Ca. The size and density of stomata determine the leaf transpiration of the plant, meaning that larger stomatal area and higher stomatal density result in increased leaf transpiration (Roth-Nebelsick et al., 2009). Transpiration allows internal water movement within the plant, creating a column of water from the roots to the shoot (Brodribb and Holbrook, 2006). The results presented herein indicated that, for the examined rootstocks, stomatal size along the total surface leaf area could be a more significant influence on transpiration than stomatal density.

According to our findings, the Ca uptake in the roots was not

generally influenced by aeration conditions. Previous studies in apple trees reported that the amount of ^{44}Ca in roots and the whole plant was not influenced by the reduction of transpiration but did affect the proportion of ^{44}Ca remaining in roots and the ^{44}Ca partitioning to aboveground (Kalcsits et al., 2019). These results are consistent with the findings presented in this study, suggesting that a higher root Ca uptake is not only coupled with the water flow.

Interestingly, only under aerated conditions, there was a strong correlation observed between root FW (mg) (not the root DW) and ^{44}Ca excess (%) in shoots for both rootstocks ($G \times N$:$R^2 = 0.998$, $p = 0.0269$; RP-20: $R^2 = 1$, $p = 0.0127$). This suggests that the water content in the roots is closely associated with the Ca transport withing the plant, particularly when the VPD is sufficiently high, such as in aerated conditions. This association can be explained by the fact that an increment in root water content enhances both the apoplastic pathway and the xylem flux (Steudle, 2000), and therefore the Ca transport through this pathway.

The Ca transport withing the plant could also be stimulated by enhancing of plant growth. According to our results, under aerated conditions, the plantlets showed a tendency to increase their growth rate, particularly in the shoots, along with an increase in foliar area. Concretely, both plant materials responded equally under aeration conditions. This increase of plant growth rate could be related to an increase of transpiration and, consequently, photosynthesis rate. The forced air renewal (aeration) in the bioreactor could enhance plant transpiration, likely due to the increment in VPD by water vapor decrement. Consequently, the aerated plants could increase the photosynthesis rate and accumulate more photoassimilates, increasing the DW (Jones, 1998).

Simultaneously, the periodic removal of environmental water vapor seems to result in an increment of leaf surface what is correlated with the increment in foliar transpiration (Poorter et al., 2012; Pantin and Blatt, 2018). This positive effect of transpiration on photosynthetic capacity and plant growth is attributed to enhanced nutrient uptake and transport (Cramer et al., 2009). Based on the presented results, it can be inferred that plant growth, influenced by transpiration, is the primary driver for Ca accumulation in shoots under aerated conditions and plant growth appears to be the main factor promoting Ca movement withing the aboveground part of the plantlets. In contrast, under unaerated conditions with a low VPD, transpiration and Ca accumulation are decoupled.

5. Conclusion

In conclusion, the present study showed that the $G \times N$ rootstock exhibited a higher capability for Ca uptake compared to RP-20. The choice of rootstock had a significant impact on Ca uptake, highlighting the importance of rootstock selection for nutrient acquisition. On the other hand, the aeration conditions did not influence Ca uptake in the roots, but it did affect the mobilization of Ca within the aboveground part of the plantlet. The results indicated a strong correlation between root FW and Ca transport within the shoot. These findings emphasize the complex interplay between rootstock characteristics, transpiration, and Ca mobilization. Further research is needed to elucidate the underlying mechanisms and to explore the potential implications for improving nutrient uptake and plant performance in agricultural systems.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Francisca Carrasco-Cuello reports financial support was provided by Spain Ministry of Science and Innovation. Ramon Dolcet-Sanjuan has patent #ES2763637B1 issued to IRTA, Spain.

Data availability

Data will be made available on request.

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