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Selection of Prunus clonal rootstocks based on nutritional efficiency state.

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Abstract

Rootstock contributes to increase the absorption and nutrients translocation efficiency. We aimed to characterize rootstocks according to the nutritional physiological state of the scion cultivar. The experiment was carried out during three periods of leaf collect on the 4-year old peach cv. BRS Libra budded onto 21 rootstocks cultivated in a high density orchard. The following traits were evaluated: xylem water potential, dry leaf mass and chemical leaf nutritional analysis. The collected data were submitted to analysis of variance by the F test and some genetic parameters were estimated. When significant, the means were compared using the Scott-Knott test, at 5% significance. The principal component analysis was performed to verify the interrelationships between the rootstocks and the evaluated traits. The deviation from optimum percentage index was used to select the most nutritional stable rootstock. We observed that rootstock affects the dynamic absorption nutrient parameters in interactions with the scion. The highest foliar levels of P were found in the BRS Libra cultivar when budded onto the rootstocks 'Santa Rosa', 'Barrier', 'Tsukuba-1' and 'Rosaflor'. The leaf analysis revealed a high variation in the mineral nutritional status among the studied *Prunus* rootstocks. Most of the mineral macro-elements varied between medium and optimal levels. The rootstocks 'Rigitano' and 'Nemared' showed greater supply of nutrients to the canopy of 'BRS Libra'

Keywords: mineral elements, phenotyping, plant breeding, stone fruits

Introduction

In Brazil, peach orchards are grafted on rootstocks obtained by seeds, with no guarantee of genetic uniformity. Although the establishment of commercial peach orchards from vegetative propagated seedlings and own-rooted seedlings could ensure greater uniformity of the phenological stages, higher yield values as well as increase vigor control, this practice has not been verified in Brazil yet (Mayer et al., 2015; Santana et al., 2020). Modern trends in peach orchards have focused on high-density systems, such as the Y-shape and Central Leader systems, (Uberti et al., 2020), using different species of *Prunus* rootstocks vegetative propagated.

The viability of a peach orchard is linked to the adequate selection of scion and rootstock cultivars for a specific climate and soil conditions. In fact, the appropriate choice of rootstock is one of the most important decisions to maintain an orchard with great fruit production (Menegatti et al., 2019). The rootstocks directly influence the orchard's longevity, the plant vigor control and the fruit quality (Minas et al., 2018). The rootstocks also influence nutrient uptake from the soil and affect the mineral content in the leaves (Souza et al., 2019). An appropriate choice of rootstock contributes to increase the efficiency in the soil nutrients absorption and translocation.

Due to changing climate and the need for commercial production of fruits in low soil quality fields there is an urgent need for stress tolerant commercial lines of fruits such as peach. As the selection of an appropriate combination of scion / rootstock cultivar influences leaf gas exchange, plant size and soil nutrient uptake efficiency (Opazo et al., 2019; Menegatti et al., 2019), it is crucial to achieve high performance in *Prunus* orchards the right choice of scion/rootstock combination (Bielsa et al., 2018). Grafting commercial peach genotypes

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on more efficient in soil nutrient uptake rootstock may produce low nutrient tolerant commercial peach lines much more rapidly. Studies focusing on nutritional uptake efficiency in perennial crops such as peach trees, more specifically to evaluate the rootstock effect, are scarce and need more development. Some studies evaluating the leaf mineral content of peach cultivars have been carried out, however they have evaluated few numbers of rootstocks or just one period of leaf collect which causes bias on recommendation (Yahmed et al., 2020; Shahkoomahally & Chaparro, 2020). Therefore, to meet the increasing demand for food quality and grant the use of genotypes less stringent on soil fertility, we aimed to characterize rootstocks according to the nutritional physiological state of the scion cultivar BRS Libra budded onto 21 different rootstocks.

Material and Methods

Plant material and field trial

The trial was carried out on a 4-years old peach cv. BRS Libra budded onto 21 rootstocks at Experimental Station of Research and Education Unit from Federal University of Fronteira Sul, located in Chapecó, Brazil (27° 07' 30.15'' S, 52° 42' 20.14'' W, 605 m asl). The genotypes used as rootstocks are presented on **Table 1**. As control trees, own-rooted nursery trees (without rootstock) of cultivar BRS Libra were used, i.e., a total of 22 different genotype combinations. All rootstocks were cloned by cutting. Plants were grafted by chip-budding on BRS Libra buds.

Table 1. Rootstocks genotypes and their scientific names

Rootstock	Scientific name
Barrier	*Prunus persica* × *Prunus davidiana*
Cadaman	*Prunus persica* × *Prunus davidiana*
Capdeboscq	*Prunus persica*
Clone 15	*Prunus mume*
De Guia	*Prunus persica*
Flordaguard	*Prunus persica* × *Prunus davidiana*
G×N.9	*Prunus persica* × *Prunus dulcis*
GF 677	*Prunus persica* × *Prunus amygdalus*
I-67-52-4	*Prunus persica*
Ishtara	(*Prunus cerasifera* × *Prunus salicina*) × (*Prunus cerasifera* × *Prunus persica)*
México Fila 1	*Prunus persica*
Nemared	*Prunus persica*
Okinawa	*Prunus persica*
P. mandshurica	*Prunus mandshurica*
Rigitano	*Prunus mume*
Rosaflor	*Prunus persica*
Santa Rosa	*Prunus salicina*
Tardio-01	*Prunus persica*
Tsukuba-1	*Prunus persica*
Tsukuba-2	*Prunus persica*
Tsukuba-3	Prunus persica

According to the Köppen classification, the local climate is humid subtropical. The soil is classified as a dystrophic Red Latosol (Oxisol), with basic pH, that is, without liming. The trees are arranged in a Y-shape training system with 2 m of distance between trees and 5 m between rows, i.e., a total of 1,000 trees per hectare. Cultural treatments, such as management of diseases, insects and weeds, and pruning were carried out according to the techniques specified and required for the peach crop (Raseira et al., 2014). Fertilization was carried out according to the peach cultivation recommendations and soil chemical analysis (**Table 2**). No irrigation system was adopted. Data collection was performed for three periods during 2017/2018 season: i) immediately after harvest (0 days after harvest); ii) 35 days after harvest and iii) the beginning of senescence of the leaves (117 days after harvest). The experiment was conducted in a randomized block design with 22 treatments (21 rootstocks + BRS-Libra own-rooted) and four replications each one with one tree per plot.

Evaluated traits

The following traits were evaluated: xylem water potential, dry leaf mass and chemical leaf nutritional analysis. Yield data of all 22 combinations can be assed in Santana et al. (2020). The determination of the xylem water potential occurred with the aid of a Scholander pressure chamber (Soil Moisture), fed by N_2 at a pressurization speed of 0.2 Mpa every 30 sec. The leaves used for the measurement were protected with aluminum foil just after sunset the previous day and the measurements were taken before sunrise the next day. A fully expanded leaf was used for each tree, which is located in the middle third of the branch. The results obtained were expressed in Mega Pascal (Mpa). For the percentage of the dry leaf mass a sample of 10 middle age leaves per tree, located in the middle third of the plant, was used. The leaves were kept in a forced air circulation oven at 65 °C for 72 hours, and then weighed on a semi-analytical scale. Values were expressed as percentage (%).

Samples of fully expanded leaves, including blade and petiole, were collected from the middle portion of the branches located in the middle third of the plant. A total of 120 leaves were collected from BRS Libra budded or not onto each evaluated rootstock in three different periods between harvest and senescence as specified above.

Leaf samples were conditioned in properly identified Kraft paper bags and taken to dry in an oven with forced circulation of hot air at 65° ± 5° C. Subsequently, the dried leaves were milled in a Willye

Table 2. Soil chemical properties from the peach orchard conducted in Chapecó city, Santa Catarina State, Brazil, in the 2017/2018 season

Year	pH	SMP Index	P mg/dm³	K mg/dm³	% OM m/v	Al cmolc/dm³	Ca cmolc/dm³	Mg cmolc/dm³
2017	5.8	6.4	5.0	72.0	3.6	0.0	5.8	2.9
2018	6	6.5	3.8	64.0	3.4	0.0	3.3	2.3
Interpretation								
Class				N	P	K	Ca	Mg
Insufficient				< 2.00	< 0.05	< 0.50	< 0.65	< 0.20
Normal				3.30 – 4.50	0,15 – 0.30	1.40 – 2.00	1.70 – 2.60	0.50 – 0.80
Excessive				> 6.00	> 0.40	> 2.80	> 3.60	> 1.20

Source: Soil Laboratory of the Research Center for Family Agriculture, CEPAF/EPAGRI, Brazil. Methodology used: Sampled layer 0 – 20 cm; pH water (1:1) and SMP - potentiometer; P - Mehlich-1/ calorimetry; K - Mehlich-1/flame photometer; OM (organic matter) - spectroscopy; Al, Ca and Mg - KCl/ atomic absorption spectrophotometry; The other parameters were obtained by calculation.

knife mill, until passing through a 2-3 mm diameter mesh. The final sample was stored in plastic packaging, duly identified for adequate leaf diagnosis. The methodology adopted for chemical analyzes was based on Tedesco et. al (1995). This methodology allowed to determine five macronutrients Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) with a single digestion of 0.2g of plant material in hydrogen peroxide (H_2O_2) and sulfuric acid (H_2SO_4). Each element was evaluated in triplicates in each one of the four replicates, i.e., 12 chemical analyses for each genotype and each mineral element. The total contents were expressed as percentage (%).

Deviation from optimum percentage (DOP)

The ΣDOP index (deviation from optimum percentage) was estimated for the diagnosis of leaf mineral status of trees. The DOP index was calculated from the leaf analysis by the formula:

$$DOP = \frac{C \times 100}{C_{REF}}$$

where: C is the concentration of nutrients in the sample to be studied and Cref is the optimal nutrient concentration, both values based on the dry mass of the tissues. Cref was obtained from the optimal values proposed by SBCS (2016). For any nutrient, a negative DOP index indicates a deficiency, while a positive DOP index indicates an excess. The ΣDOP is obtained by adding the values of the DOP indices independently of the sign. The higher the ΣDOP, the greater the intensity of the imbalances between nutrients.

Statistical analysis

The data were submitted to the normality analysis of the residues, verified by the Shapiro-Wilk test, at the 5% level of significance. Once the assumption was attended, a joint analysis of variance (ANOVA) was performed based on data from genotypes and seasons, according to the following statistical model:

$$y_{ijkl} = \mu + G_i + E_l + GE_{il} + B_{k(jl)} + \epsilon_{ijkl}$$

where: y_{ijkl} is the observed value obtained from the i-th genotype evaluated at the l-th season, in the k-th block, within the j-th repetition; μ is a constant inherent in all observations; g_i is the random effect of the i-th genotype (i = 1, 2, ..., 23); E_l is the fixed effect of the l-th season (l = 1, 2, 3); GE_{il} is the random effect of the interaction of the i-th genotype with the l-th season; $B_{k(jl)}$ is the random effect of the k-th block within the j-th repetition at the l-th season; and ϵ_{ijkl} is the random error associated with the y_{ijkl} observation. The analyzes were performed using the software R, 'ExpDes' package (Ferreira et al., 2014).

When significant, the means were compared using the Scott-Knott test, at 5% significance. The principal component analysis was performed to verify the interrelationships between the rootstocks and the evaluated characters. The analyzes were performed using the software R.

Broad-sense heritability (h^2) was calculated as the proportion of genetic variance over the total phenotypic variance:

$$h^2 = \frac{\sigma_g^2}{\left[\sigma_g^2 + \left\{\frac{\sigma_E^2}{r}\right\}\right]}$$

where is σ_g^2 the genotypic variance, σ_E^2 is the error variance, and r the number of replications.

Results and Discussion

Analysis of variance and genetic parameters

The different rootstocks showed a significant difference for the content of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and dry leaf mass (**Table 3**). The nitrogen content and xylem flow did not show significant differences among the rootstocks which is why their averages are not shown in Table 3. For period effects, a significant effect was observed for potassium, calcium, magnesium and xylem flow. The heritability of the characteristics varied from 3.97×10^{-9}, for dry mass, to 0.732, for Mg.

Table 3. Mean square for the analysis of variance in the foliar contents of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), xylem flow (XF), dry mass (DM), sources of variation (SV) and degrees of freedom (DF) for 22 different peach clonal rootstocks

SV	DF	Mean Square						
		N	P	K	Ca	Mg	XF	DM
Genotype	21	0.10	0.029*	0.40*	2.19*	0.04*	2.34	22.5*
Period	2	5.93	0.084	1.14*	564.92*	0.29*	416.63*	14,333.9*
Block	3	0.45	0.0017	0.68*	0.03	0.009*	0.84	34.7
G×E	42	0.17	0.016	0.25*	2.03*	0.017*	1.86	32.9*
Residue	195	0.14	0.0035	0.16	0.14	0.003	1.89	18.7
σ^2_p		0.13	0.007	0.19	0.62	0.008	1.83	0.02
σ^2_g		0	0.001	0.012	0.01	0.002	0.03	1.86^{-7}
σ^2_{gxe}		0.006	0.003	0.023	0.47	0.003	0.02	2.65
h^2		-	0.55	0.235	0.28	0.732	0.07	3.97×10^{-9}
Accuracy		0.58	0.93	0.767	0.96	0.900	0.43	0.40
CV(%)		11.88	14.43	12.57	17.37	19.16	20.13	7.22

σ^2_p = Phenotypic variance; σ^2_g = genotypic variance; σ^2_{gxe} = variance of the genotypic × environment interaction; h^2 = heritability; CV = experimental coefficient of variation.

For dry leaf mass, the highest levels were found in the second period of collection (**Table 4**). The nitrogen content and the xylem flow values did not show significant influences from the different rootstocks, only differences between the periods. The nitrogen content was significantly higher in the first and second evaluation periods, with an average of 3.37 and 3.10%, respectively, in relation to the third period (2.85%). The xylem flow values were higher in the first and second period (8.19 and 7.95 Mpa) and differed significantly from the third evaluation (4.31 Mpa). The highest foliar levels of P were found in the BRS Libra cultivar when grafted onto the rootstocks ‘Santa Rosa’, ‘Barrier’, ‘Tsukuba-1’ and ‘Rosaflor’. The rootstocks ‘Capdeboscq’, ‘Flordaguard’, ‘I-67-52-4’, ‘México Fila 1’, ‘Nemared’, ‘Rigitano’, ‘Rosaflor’ and ‘‘Santa Rosa’ did not differ in terms of Mg contents.

Deviation from optimum percentage (DOP)

The leaf analysis revealed a high variation in the mineral nutritional status among the studied *Prunus* rootstocks (**Table 5**). Most of the mineral macro-elements varied between deficient and optimal levels. Based on DOP, the rootstocks tested induced deficient N content and some of them deficient in Ca and Mg in relation to the optimum levels. Excess mineral situation was observed for P and K levels. Better Ca values were observed in the rootstocks ‘De guia’ and ‘Rigitano’ whose values approximately adjusted to the optimal levels. According to ΣDOP average index, ‘Barrier’, ‘Capdeboscq’, ‘Rosaflor’, ‘Santa Rosa’, ‘Tardio-01’ and ‘Tsukuba-1’ showed the highest significant intensity of imbalances among nutrients with higher mean DOP values. The lowest ΣDOP average index was observed for ‘Rigitano’, ‘Nemared’ and ‘GF-677’, indicating their ability of stabilize the uptake soil nutrient according to plant requirements.

Principal components analysis – PCA

Data analysis revealed two principal components (PC) that explain 79.62% of the variability observed between the rootstocks studied (**Figure 1**). We noticed that leaf calcium levels are inversely correlated with nitrogen levels. The fresh mass of fruits has a high correlation with the magnesium content and the xylem water potential, depending due to the acute angles between the vectors and inversely correlated with the levels of phosphorus and potassium.

Three distinct groups of rootstocks can be viewed. The first consists of rootstocks that induced a higher leaf calcium and potassium content (‘De Guia’, ‘Flordaguard’, ‘Tsukuba-1’ and ‘I-67-52-4’). The second is formed by rootstocks with greater xylem water potential and leaf contents of magnesium and fresh leaf mass (‘Capdeboscq’, ‘Tardio 01’ and ‘Barrier’). The third group is related to the highest levels of nitrogen and dry mass. The rootstocks ‘Nemared’ and ‘Okinawa’ have the highest levels of N among the rootstocks. On the other hand, the rootstocks ‘G × N9’ and ‘Clone 15’ showed higher levels of phosphorus and potassium.

According to this study, rootstocks influence the absorption and transport of macronutrients P, K, Ca and Mg. Thus, it is possible to characterize rootstock genotypes that are more efficient in the absorption and translocation of mineral elements. Other studies have also verified the influence of rootstocks on the nutrient content in the leaves of the scion cultivar from different species (Jiménez et al., 2007; Mayer et al., 2015; Mestre et al., 2015; Reighard et al., 2013), however this work is the first one using different *Prunus* species cultivars and different

Table 4. Leaf nutrient content (%) and dry mass (%) in peach ‘BRs Libra’ budded onto different rootstocks and own-rooted

Rootstock	Phosphorus (%)			Potassium (%)			Calcium (%)			Magnesium (%)			Dry mass (%)		
	1°	2°	3°	1°	2°	3°	1°	2°	3°	1°	2°	3°	1°	2°	3°
Own-rooted	0.46aA	0.38bA	0.40cA	3.50aA	3.04aA	3.65aA	7.98aA	1.09aB	0.43aC	0.46aA	0.28bB	0.25cB	56.38*B	75.0A	47.55C
Barrier	0.43aA	0.33bB	0.42cA	2.93bB	2.69aB	3.43bA	4.98dA	1.03aB	0.53aB	0.49aA	0.38aB	0.29cC	56.04*B	72.35A	49.67C
Cadaman	0.29cB	0.36bA	0.40cA	2.95bA	3.15aA	3.10bA	4.38eA	0.84aB	0.45aB	0.44aA	0.26bB	0.32bB	55.64*B	79.47A	49.97*B
Capdeboscq	0.29cB	0.47aA	0.31cB	2.71bA	3.20aA	3.35bA	4.98dA	1.09aB	0.63aB	0.45aA	0.42aA	0.41aA	56.12*B	78.89A	51.63*B
Clone 15	0.43aB	0.34bC	0.61aA	3.19aA	3.26aA	3.12bA	7.69aA	1.07aB	0.54aB	0.29cA	0.20cB	0.19cB	55.71*B	67.97A	52.43*B
De Guia	0.37bA	0.41bA	0.39cA	3.15aA	3.07aA	3.26bA	4.30eA	0.74aB	0.79aB	0.33cA	0.15cB	0.33bA	56.36*B	79.59A	49.37C
Flordaguard	0.44aA	0.37bA	0.37cA	3.59aA	3.52aA	3.21bA	5.72cA	0.91aB	0.59aB	0.26cA	0.24bA	0.33bA	53.92*B	75.36A	50.46*B
G x N.9	0.47aB	0.51aB	0.61aA	3.45aA	3.15aA	3.76aA	4.36eA	0.72aB	0.58aB	0.26cA	0.16cB	0.28cA	57.97*B	78.34A	46.85C
GF 677	0.27cB	0.45aA	0.45bA	2.84bA	3.19aA	3.17bA	5.50cA	1.01aB	0.53aB	0.39bA	0.22bB	0.21cB	60.27*B	67.72A	50.02C
I-67-52-4	0.38bA	0.30bB	0.42bA	3.32aB	3.21aB	3.89bA	4.53eA	0.81aB	0.43aB	0.27cA	0.22cA	0.28cA	54.55*B	77.25A	46.89C
Ishtara	0.45aA	0.32bB	0.39cA	3.39aA	3.13aA	3.37bA	2.83gA	0.73aB	0.81aB	0.26cA	0.15cB	0.28cA	56.66*B	68.15A	49.23C
México Fila 1	0.35bB	0.30bB	0.41cA	2.91bA	2.84aA	2.79bA	5.46cA	1.13aB	0.42aC	0.44aA	0.42aA	0.35bA	58.29*B	73.30A	47.84C
Nemared	0.39bA	0.35bA	0.40cA	3.28aA	3.26aA	3.48bA	4.84dA	0.68aB	0.49aB	0.30cA	0.27bA	0.32bA	58.88*B	67.46A	49.24*B
Okinawa	0.43aA	0.30bB	0.45bA	2.69bA	3.00aA	2.73bA	4.84dA	0.65aB	0.49aB	0.52aA	0.23bB	0.21cB	57.68*B	76.34A	49.11C
P. mandshurica	0.54aA	0.50aA	0.57aA	3.17aA	3.07aA	3.23bA	3.34fA	0.64aB	0.73aB	0.40bA	0.20cB	0.28cB	58.43*B	73.06A	51.88C
Rigitano	0.45aA	0.36bB	0.53aA	3.04bA	3.46aA	3.17bA	4.39eA	0.74aB	0.58aB	0.23dA	0.20cA	0.27cA	53.50*B	73.54A	49.85*B
Rosaflor	0.49aA	0.31bB	0.44bA	3.37aA	3.09aA	3.17bA	3.67fA	0.73aB	0.50aB	0.20dA	0.23bA	0.33bA	52.01*B	73.64A	48.64*B
Santa Rosa	0.41aA	0.40bA	0.36cA	3.28aA	3.04aA	3.30bA	5.82cA	0.79aB	0.46aB	0.21dA	0.17cA	0.26cA	56.62*B	77.71A	49.07C
Tardio-01	0.49aA	0.39bB	0.42bB	3.65aA	2.99aA	3.23bA	5.31cA	0.87aB	0.77aB	0.39bA	0.26bB	0.41aA	55.68*B	72.94A	52.04*B
Tsukuba-1	0.37bA	0.45aA	0.46bA	3.30aA	3.10aA	3.45bA	6.76bA	0.83aB	0.39aB	0.46aA	0.21cB	0.24cB	55.45*B	74.16A	51.23*B
Tsukuba-2	0.39bA	0.47aA	0.47bA	3.57aA	2.98aA	3.19bA	5.04dA	0.75aB	0.45aB	0.36bA	0.19cB	0.26cB	55.48*B	69.89A	49.02C
Tsukuba-3	0.38bB	0.35bB	0.49bA	3.15aB	2.87aB	4.22aA	5.06dA	0.93aB	0.42aB	0.40bA	0.24bB	0.22cB	56.19*B	79.22A	47.21C

Means followed by the different lowercase letter differ among genotypes (lines) and capitals letter among sample periods (columns) at $P \leq 0.05$ according to the Scott-Knott test. *not significant

Table 5. DOP index (deviation from optimum percentage) determined from the leaf mineral concentration of the macro elements N, P, K, Ca and Mg

Treatment	N*	P	K	Ca	Mg	ΣDOP
Own-rooted	-24.06c	72.12b	87.65b	34.05b	-41.90b	259.77b
Barrier	-20.31b	109.60a	112.24a	55.65a	-54.28c	352.07a
Cadaman	-20.53b	68.69c	99.29a	52.13a	-48.29b	288.92b
Capdeboscq	-21.63b	83.47b	99.73a	47.39a	-50.70c	302.91a
Clone 15	-26.78c	70.63b	61.12c	8.98c	-37.78b	205.29c
De Guia	-20.84b	64.44c	78.59b	-0.30d	-44.53b	208.69c
Flordaguard	-14.47a	80.96b	92.82a	-4.47d	-38.48b	231.19c
G x N.9	-20.70b	73.49b	77.51c	1.34c	-40.14b	213.18c
GF 677	-13.27a	44.68d	66.94c	-12.69e	-40.31b	177.89d
I-67-52-4	-12.82a	73.12b	91.53a	-20.57f	-49.97c	248.01b
Ishtara	-27.01c	48.09d	82.47b	-2.65d	-53.74c	213.97c
México Fila 1	-17.70a	55.30d	80.31b	-11.88e	-47.75b	212.94c
Nemared	-19.21b	55.56d	60.47c	-9.30d	-43.69b	188.23d
Okinawa	-26.20c	56.75d	89.59b	8.68c	-24.31a	205.52c
P. Mandshurica	-17.73a	62.96c	94.12a	16.04b	-36.28b	227.13c
Rigitano	-21.05b	55.82d	81.39b	0.49d	-34.76a	193.50d
Rosaflor	-15.46a	98.87a	90.24b	49.04a	-62.05d	315.65a
Santa Rosa	-23.81b	123.65a	82.47b	57.21a	-62.40d	349.54a
Tardio-01	-29.98d	87.16b	90.24b	26.42b	-66.34d	300.13a
Tsukuba-1	-23.08b	103.30a	87.65b	44.22a	-66.83d	325.08a
Tsukuba-2	-32.03d	68.95c	64.35c	-15.14e	-58.98c	239.45c
Tsukuba-3	-24.80c	92.85a	90.88b	-4.08d	-55.53c	268.13b

*Means followed by the same letter in each column for each cultivar are not significantly different at *P*≤0.05 according to Scott-Knott test.

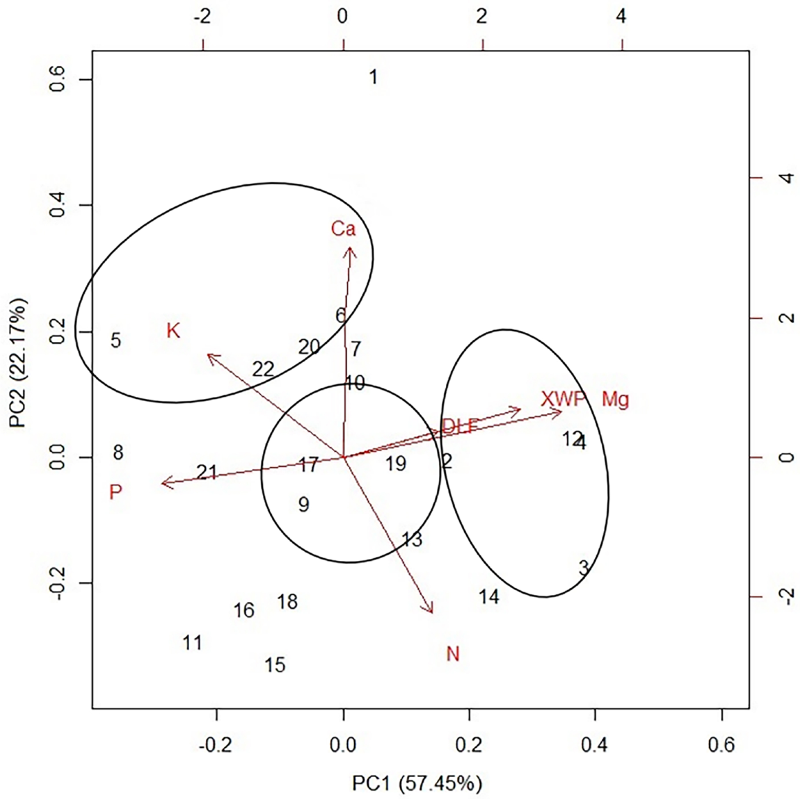

Figure 1. Principal component analysis for the 22 rootstocks evaluated. Abbreviations: own-rooted (1), Barrier (2), Cadaman (3), Capdeboscq (4), Clone 15 (5), De Guia (6), Flordaguard (7), G × N9 (8), GF 677 (9), I-67-52-4 (10), Ishtara (11), México Fila 1 (12), Nemared (13), Okinawa (14), *Prunus mandshurica* (15), Rigitano (16), Rosaflor (17), Santa Rosa (18), Tardio 01 (19), Tsukuba-1 (20), Tsukuba-2 (21) and Tsukuba-3 (22). DLM: dry leaf mass; XWP: xylem water potential.

periods of leaf collection which means more precise genotype recommendation for subtropical climate. In general, rootstocks differ in terms of root architecture, cation exchange capacity and root exudates which, together, can influence the concentration of nutrients in the leaf of the scion cultivar (Kucukyumuk & Erdal, 2011). Matínez-Ballesta et al. (2010) emphasized that, in addition to the rootstock effect, there is also the effect of the scion cultivar and environmental conditions on nutrient absorption and translocation. The differences on the leaf mineral content among the combinations evaluated in this work may be due to the water supply and the size variation of the xylem vessels of each genotype.

High magnitude heritability estimates, as seen for Mg (Table 3), provide greater reliability in the selection of superior genotypes, because the observed phenotypic variation is due to genetic causes and, therefore, greater genetic gains are obtained when applying selection based on the performance of the genotypes. Negative correlation between Mg and K was observed (Figure 1). Milosevic et al. (2013) mentions the antagonism that exists between Mg and other cations like K, Zn and Fe which can induce Mg leaf deficiency. As seen in figure 1, the leaf nitrogen content is negatively correlated with calcium. It is perfectly expected that mobile elements such as Nitrogen, Potassium and Phosphorus will be remobilized to other parts of the plant along the periods of leaf collect. For Magnesium the tendency would also be to reduce, but not as expressive as N, P and K. In our work we observed that the rootstocks didn't influence the N uptake significantly and the N level of plants was classified as normal (Table 2) which contribute to the orchard sanity. According to Saa et al. (2016), excess nitrogen results in excessive vegetative growth and increases the susceptibility of plants to diseases.

The Ca levels were higher in the first sample, reducing considerably in the second and third period of leaf collect. This behavior was observed in all rootstocks and may be explained by the fact that absorbed calcium tends to go to the forming buds for the next growing season (Xia et al., 2009). Ca is a constituent that exerts a profound influence on the integrity and firmness of the fruits and, during the reproductive period of the plants, the demand for this nutrient is greater, which justifies a higher rate of Ca absorption in this period (Manganaris et al., 2005). The higher Ca values observed in the rootstocks 'De guia' and 'Rigitano' may be explained by genetic causes. Genomic analyze, performed by using molecular markers, should be done to identify possible chromosomic that control this trait. In general, potassium was more stable in the plant, both among rootstocks and between sampling periods. This nutrient is considered to be the most abundant element in the fruit, providing an appropriate size, flavor balance and more intense color, while its excess is harmful to conservation (Rombolà et al., 2012).

The dry mass was higher in the second collection period, probably because this period coincides with the period where buds arise from meristem tissue in the plant. As there were no drains (fruits) in the plant, this period allowed a greater accumulation of dry mass. On the other hand, in the first sample, the trees had recently completed the reproductive period, of fruit harvest. In this reproductive phase, most of the carbohydrates are translocated to the fruits instead of being stored in other parts of the trees. (Borba et al., 2005). Finally, the third sample corresponded to the one with the lowest dry leaf mass content. This sample corresponds to the period of onset of vegetative senescence, which means translocation of leaf carbohydrates to the roots and/ orbuds.

According to Montañés et al. (1993), the index **ΣDOP** is an alternative to the interpretation of chemical analysis of plant tissues. According to the same authors, this index allows to identify if the nutritional limitation is due to the excess (positive indexes) or the deficiency (negative indexes) of mineral elements. Thus, the index **ΣDOP** is able to inform not only the quantity but the nutritional quality of the plant. Based on this, the rootstocks 'GF 677', 'Nemared' and 'Rigitano' showed higher nutritional balance expressed through lower indexes. However, according to Santana et al. (2020), the 'GF 677' rootstock was below the general yield average over three years. This shows how difficult it is to select genotypes that have multiple favorable traits of economic interest. The rootstock 'Santa Rosa' showed highest value for DOP index. This result confirm the high instability and low adaptability founded by Santana et al. (2020) for this rootstock, suggesting that 'Santa Rosa' is not the most suitable rootstock for our subtropical conditions. The use of more efficient cultivars for nutrient absorption and translocation is a sustainable alternative.

Conclusion

Based on the conditions in which the trial was carried out, with no nutritional limitation in the soil, there was a greater supply of nutrients to the canopy of 'BRS Libra' peach tree when grafted on the rootstocks 'Rigitano' and 'Nemared'. On the other hand, the rootstock Santa Rosa didn't show satisfactory supply of nutrients to the canopy 'BRS Libra'.

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