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ORIGINAL ARTICLE

Genetic diversity of the genus *Prunus* based on per se evaluation of peach clonal rootstocks

Diversidade genética do gênero Prunus *com base na avaliação per se de porta-enxertos clonais de pessegueiro*

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ABSTRACT: The multivariate analyses application and genetic divergence quantification can provide parameters that aid the selection of superior rootstocks. The objective of this study was to evaluate the technical feasibility of using the genetic diversity of the genus *Prunus* as a clonal rootstock of the peach cultivar 'BRS-Libra'. The experimental orchard used was part of a national network for evaluation of rootstocks for prunaceous trees, under the general coordination of *Embrapa Clima Temperado*. The design was randomized and data were collected during the 2016/2017 production cycle. Physical analyses (trunk section area, average fruit mass), chemical analysis (soluble solids), and quantification of estimated productivity of the plants were performed. The data were subjected to the Shapiro-Wilk normality test at a 5% probability level, after which the grouping of the rootstocks was performed using the Unweighted Pair Group Method Using an Arithmetic Average (UPGMA) hierarchical method. The grouping resulted in the formation of five groups. The cultivars of group I, Mirabolano 29C and Marianna 2624, showed graft incompatibility with the evaluated cultivar scion. Similarly, cultivars of group II showed characteristic symptoms of graft incompatibility, resulting in poor development. Group III and IV were composed of cultivars that showed low and medium vigor, with good characteristics for use in orchards with high density, while the cultivars of the group V stood out because they showed high vigor, recommended for orchards with low density. The use of various rootstocks influenced the behavior, vigor and fruit production of the BRS Libra cultivar, with genetic divergence.

KEYWORDS
Prunus persica
Stone fruits
Cluster analysis
Grafting

RESUMO: *A aplicação de análises multivariadas e quantificação da divergência genética fornecem parâmetros que favorecem a seleção de porta-enxertos superiores. O objetivo deste trabalho foi avaliar a viabilidade técnica do uso de parte da diversidade genética do gênero* Prunus *como porta-enxerto clonal do pessegueiro 'BRS-Libra'. O pomar experimental faz parte de uma rede nacional de avaliação de porta-enxertos para prunáceas, sob a coordenação geral da Embrapa Clima Temperado. O delineamento experimental foi em blocos ao acaso e a coleta de dados ocorreu no ciclo produtivo 2016/2017. Foram realizadas análises físicas (área de secção do tronco, massa média de frutos) e química (sólidos solúveis), além da quantificação da produtividade estimada das plantas. Os dados obtidos foram submetidos ao teste de normalidade de Shapiro-Wilk, ao nível 5% de probabilidade, sendo posteriormente realizado o agrupamento dos porta-enxertos através do método hierárquico UPGMA. A realização do agrupamento resultou na formação de cinco grupos. As cultivares do grupo I, Mirabolano 29C e Marianna 2624, apresentaram incompatibilidade de enxertia com a cultivar BRS-Libra. Semelhantemente, as cultivares do grupo II apresentaram sintomas característicos de incompatibilidade de enxertia, resultando em pouco desenvolvimento. Os grupos III e IV são compostos por cultivares que apresentaram baixo e médio vigor, com boas perspectivas de uso para formar pomares em alta densidade, enquanto as cultivares do grupo V destacaram-se por apresentar alto vigor, recomendadas para pomares de baixa densidade. O uso de diferentes porta-enxertos influencia no comportamento da cultivar BRS-Libra no vigor e produção de frutos, havendo divergência genética entre eles.*

PALAVRAS-CHAVE
Prunus persica
Frutas de caroço
Análise de agrupamento
Enxertia

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1 Introduction

Peach cultivation has been prominent during the last decade and is now considered the third largest temperate fruit crop in the world, after apple and pear (Roknulazam et al., 2019). However, in Brazil, production of the genus *Prunus* remains low. Rio Grande do Sul state is the largest producer of peaches in Brazil. However, in 2014, Rio Grande do Sul productivity achieved 9.78 t ha^{-1}, being below the world average (14.26 t ha^{-1}) (Mayer et al., 2019). In 2016, the Brazilian peach and nectarine production involved 17,283 hectares, with annual production of 191,855 tons. By contrast, the world's top leader, China, produced 838,768 hectares and 14,469,004 tons (64.35% greater than that of Brazil) (FOOD AND AGRICULTURE ORGANIZATION, 2018).

Among the aspects that contribute to the low Brazilian production are the use of low-quality nursery trees, with rootstock propagated by seeds, as well as the use of mixtures of industrial-grade pits that do not provide quality material for rootstock function.

Various factors directly or indirectly affect the success of peach cultivation. An important one is the selection of rootstocks that are more adapted to the conditions of environment. Each rootstock has a variable influence on the results depending to the vigor of the plant, as well as yield and physicochemical characteristics of the fruit (López-Ortega et al., 2016; Szymajda et al., 2019.).

The application of multivariate analyses and quantification of genetic divergence can provide parameters that aid the selection of superior rootstocks. Several techniques of multivariate statistics, including analysis of the main components, canonical variables and hierarchical grouping methods, have been used in studies of genetic divergence (Cruz et al., 2012). However few studies, such as Bakht et al. (2013) and Menegatti et al. (2019), have been developed to quantify the genetic dissimilarity among rootstocks of the genus *Prunus.* The objective of this work was to evaluate the technical feasibility of exploiting the genetic diversity of the genus *Prunus* as clonal rootstocks for the peach cultivar BRS-Libra.

2 Materials and Methods

The experiment was conducted in a peach orchard in the Experimental Area of the Fronteira Sul Federal University, Chapecó campus. The orchard is located at latitude 27°07'06"S, longitude 52°42'20" W, altitude 605 meters. The local climate, According to the classification of Köppen, the climate of the region is classified as C, subtype Cfa (Subtropical climate), with cold, humid winters and moderate, dry summers. The soil is classified as Rhodic Hapludox.

The orchard is part of a national network for evaluation of rootstocks of prunaceous trees, under the general coordination of *Embrapa Clima Temperado*. The planting occurred in 2014 with a spacing of 5 m between rows and 2 m between plants (5 m × 2 m), totaling 1,000 plants ha^{-1}. The cultivar scion was BRS-Libra, grafted on 24 different cultivars of clonal rootstocks (Table 1), propagated by herbaceous cuttings. As a control, we used own-rooted nursery trees (without rootstock) from cv. BRS-Libra. The nursery trees were originated from *Embrapa Clima Temperado* live germplasm bank, Rio Grande do Sul, Brazil.

Table 1. Identification and species of the 24 rootstocks used for peach cv. BRS-Libra with control (self-rooted plants)

Tabela 1. Identificação e espécies de 24 porta-enxertos sob a cultivar copa BRS-Libra, com testemunha (plantas autoenraizadas)

Treatments	Specie	Origin
Tsukuba-2	*Prunus persica*	Japan
Genovesa	*Prunus salicina*	Pelotas-RS, Brazil
Clone 15	*Prunus mume*	Jaboticabal-SP, Brazil
Nemared	*Prunus persica*	California, The United States
Tsukuba-1	*Prunus persica*	Japan
Barrier	*Prunus persica* × *Prunus davidiana*	Italy
Ishtara	*(Prunus cerasifera* × *Prunus salicina)* × *(Prunus cerasifera* × *Prunus persica)*	France
Cadaman	*Prunus persica* × *Prunus davidiana*	France
Capdeboscq	*Prunus persica*	Pelotas-RS, Brazil
BRS Libra Autoenraizado	*Prunus persica*	Pelotas-RS, Brazil
De Guia	*Prunus persica*	Pelotas-RS, Brazil
Rosaflor	*Prunus persica*	Pelotas-RS, Brazil
G × N.9	*Prunus persica* × *Prunus dulcis*	Unknown
Flordaguard	*Prunus persica* × *Prunus davidiana*	Florida, The United States
Rigitano	*Prunus mume*	Jaboticabal-SP, Brazil
Tardio-01	*Prunus persica*	Pelotas-RS, Brazil
P. *mandshurica*	*Prunus mandshurica*	Pelotas-RS, Brazil

Table 1. Continuation...

Tabela 1. Continuação...

Treatments	Specie	Origin
Mirabolano 29C	*Prunus cerasifera*	Unknown
Tsukuba-3	*Prunus persica*	Japan
Okinawa	*Prunus persica*	Okinawa, Japan
Santa Rosa	*Prunus salicina*	California, The United States
México Fila 1	*Prunus persica*	Mexico
Marianna 2624	*Prunus cerasifera* × *Prunus munsoniana*	California, The United States
I-67-52-4	*Prunus persica*	The United States
GF 677	*Prunus persica* × *Prunus amygdalus*	France

The experimental design was randomized with 25 treatments (24 rootstocks + own-rooted trees control) and four replicates, each plot consisting of one plant. The orchard was arranged in six rows, using the four central rows and the two on the edges considered as borders. The plants were managed in the form of a "Y", without irrigation.

Data collection occurred during the production year 2016/17. The analyzed variables were: a) trunk section area (cm^2), measured five centimeters above and five centimeters below the grafting point with the aid of a digital caliper; b) average mass of fruits (g), obtained by random harvesting of 20 fruits of each plant, weighed with a gram digital scale with accuracy of two decimal places; c) soluble solids (°Brix), determined with juice of 10 random fruits per plant, using a digital refractometer (Instrutherm®, RTD-95); d) estimated productivity per hectare (tons ha^{-1}).

The normality of the data was verified by the Shapiro-Wilk test, at a 5% probability level. When necessary, the Box-Cox transformation was used, represented by lambda (λ). Once the mathematical assumption was met, analysis of variance (ANOVA) was performed, and means were compared using the Scott-Knott test, at 5% probability.

The similarity between the cultivars was calculated using the Jaccard index with the Vegan package (Oksanen et al., 2017) based on data collection from analyzed variables. From the similarity matrix data, the rootstocks were grouped using the hierarchical method of UPGMA (Unweighted Pair Group Method Using an Arithmetic Average). The univariate and multivariate analyses were performed using program R.

3 Results and Discussion

The results for the Shapiro-Wilk normality test are presented in Table 2. We observed that the test was not significant for the analyzed variables, suggesting that the data were normally distributed.

As seen in Table 3, the Mexico Fila 1 and I-67-52-4 treatments stood out from the others, in that they were statistically superior for four of the five variables evaluated. By contrast, the treatments 'Mirabolano 29 C' and 'Marianna 2624' had low performance for the analyzed variables.

Table 2. Shapiro-Wilk test values for normality of the data obtained from the evaluated variables

Tabela 2. Valores do teste de Shapiro-Wilk para normalidade dos dados obtidos a partir das variáveis avaliadas

Variables	W	P
TSA above the grafting point (cm2)	0.99209	0.8279*
TSA below the grafting point (cm2)	0.99207	0.8264*
Average mass of fruits (g)	0.97506	0.0747*
Soluble solids (°Brix)	0.91914	0.4036*
Estimated productivity (tons ha-1)	0.97869	0.1051*

W values indicate normality, *P* values indicate confidence level with which one can reject the null hypothesis of normality, *TSA* trunk section area, *not significant at 5% probability.

The UPGMA grouping suggested the formation of five groups, based on cutoff of 0.2, (Figure 1). We observed that group V contained most of the treatments (11) followed by group III, with six treatments, group II and IV, both with three treatments each, and finally, group I with only two of the 25 treatments evaluated in the study.

Group I, comprising 'Mirabolano 29C' and 'Marianna 2624' treatments, consisted of dead plants. The cause of the mortality of these cultivars was the incompatibility with the peach tree, manifested five months after planting the nursery trees. Both rootstocks are recommended and used for plum cultivation (*Prunus domestica*). In the present study, the incompatibility of grafting, expressed through incomplete attachment at the point of grafting between the scion and the rootstock, was also observed in the initial phase of the cultivation of the plants (five months).

In an evaluation of grafting compatibility in genus *Prunus spp.*, Rodrigues et al. (2001) associated the incompatibility of grafting of cultivars Marianna 2624 and Mirabolano 29C with the highest activity of peroxidase and concentration of phenols in relation to the peach plants grafted on these rootstocks. That contributed to a difference in lignin content among species and led to a lack of vascular connections at the point of grafting. There was also incompatibility of translocated type grafting between these rootstocks and cultivar BRS-Kampai (Neves et al., 2017; Raseira et al., 2014).

Group II consisted of *P. mandshurica*, Genovesa and Santa Rosa. The plants grafted on these rootstocks had low vegetative and reproductive development throughout the evaluated cycle. The Genovesa cultivar produced only 30% of the estimated average yield of groups III, IV and V. In addition, at the end of the cycle, all the replicates of this cultivar died, demonstrating incompatibility of grafting from the third year of cultivation.

In similar fashion, the other cultivars of group II (*P. mandshurica* and Santa Rosa) showed characteristic symptoms of grafting incompatibility, similar to those observed for the Genovesa cultivar during the production cycle up to eight months after planting with leaf shrouding, necrosis and death of lateral branches and excessive thickening at the point of grafting.

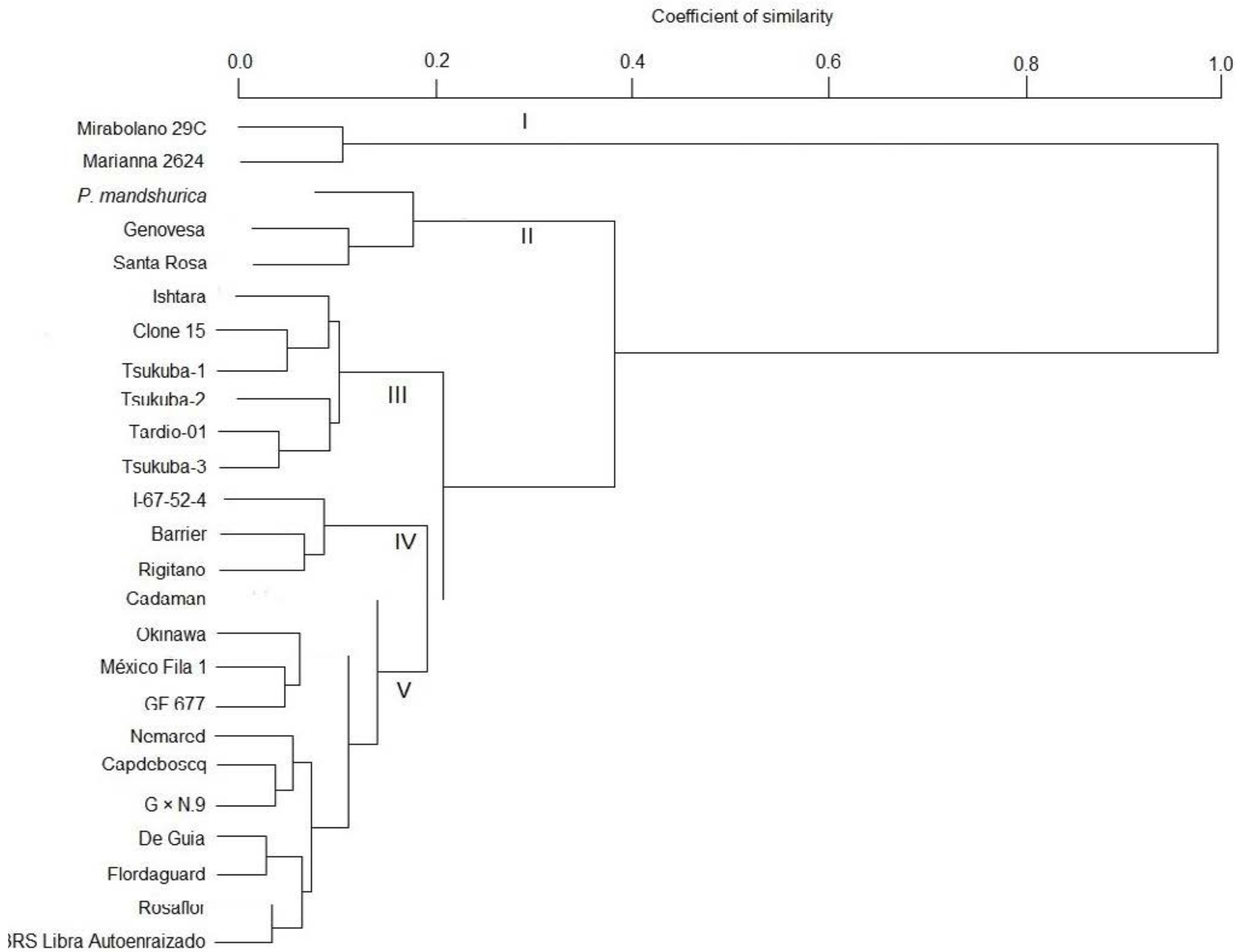
Table 3. Means and standard deviations for the variables evaluated in 'BRS-Libra' peach cultivar on different rootstocks and in own-rooted plants
Tabela 3. Médias e desvio padrão para as variáveis avaliadas na cultivar de pêssego 'BRS-Libra' sob diferentes porta-enxertos e plantas autoenraizadas

Treatments	EP (tons ha^{-1})	SS (°Brix)	FM (g)	SAA (cm^2)	SBA (cm^2)
Tsukuba-2	16.74±4.20b*	9.57±0.88a	94.03±3.88b	16.95±3.20d	17.47±2.38b
Genovesa	4.70±0.64c	8.04±0.57b	48.39±2.80c	20.03±3.98c	25.66±7.07a
Clone 15	14.48±5.23b	10.07±2.34a	113.02±26.48a	20.39±2.29c	22.49±1.87b
Nemared	18.01±6.48a	8.70±0.75b	109.24±21.93a	27.97±5.16b	35.85±5.90a
Tsukuba-1	14.45±2.7b	10.10±0.67a	99.44±15.30b	20.44±2.80c	20.56±1.76b
Barrier	13.53±4.90b	9.53±0.73a	130.24±7.54a	25.24±4.15b	28.97±8.07a
Ishtara	8.70±2.51c	9.16±1.22b	111.74±32.47a	14.02±1.10d	17.25±1.73b
Cadaman	18.09±2.63a	8.70±0.53b	80.11±7.34b	24.64±2.01b	29.65±6.03a
Capdeboscq	15.78±3.30b	10.66±0.50a	106.94±9.32a	25.28±4.46b	28.44±5.46a
Autoenraizado	24.45±5.35a	8.59±0.85b	112.87±25.53a	27.75±4.83b	31.21±5.43a
De Guia	20.45±1.64a	9.05±0.97b	101.45±7.19b	30.55±4.09a	35.72±4.95a
Rosaflor	20.24±6.40a	8.39±1.57b	108.56±13.37a	27.07±3.60b	32.55±6.93a
G × N.9	14.29±3.42b	9.44±1.24a	103.75±4.36b	25.66±5.36b	32.60±8.11a
Flordaguard	19.89±5.86a	8.04±1.95b	105.59±18.40b	32.86±5.09a	36.31±3.65a
Rigitano	11.94±2.32b	10.33±0.86a	124.99±10.75a	21.51±2.09c	23.23±3.21b
Tardio-01	14.85±2.27b	9.48±0.34a	112.28±4.28a	19.02±1.44c	20.33±1.79b
P. mandshurica	5.22±1.21c	9.47±0.64a	60.23±4.48c	13.22±1.87d	16.29±2.82b
Mirabolano 29C	0.00±0.00d	0.00±0.00c	0.00±0.00d	2.82±0.58e	7.23±0.64C
Tsukuba-3	17.37±3.42a	9.46±0.70a	103.68±18.30b	17.41±1.74d	19.86±5.04b
Okinawa	14.21±3.57b	9.96±0.70a	90.30±3.25b	27.69±4.05b	33.78±5.24a
Santa Rosa	4.39±0.39c	10.85±2.09a	50.28±15.03c	19.05±4.26c	24.45±4.93b
México Fila 1	19.28±2.83a	9.90±0.95a	99.51±10.47b	32.36±3.35a	37.64±8.07a
Marianna 2624	0.00±0.00d	0.00±0.00c	0.00±0.00d	6.05±0.71e	5.12±1.34C
I-67-52-4	20.31±5.52a	9.80±1.13a	124.96±20.17a	28.54±2.22b	34.40±3.77a
GF 677	12.34±4.93b	9.03±0.23b	95.86±9.61b	21.75±4.13c	36.63±5.67a
CV (%)	16.85	8.69	13.47	10.13	9.89

EP estimated productivity, *SS* soluble solids, *FM* mean fruit mass, *SAA* trunk section area above the grafting point, *SBA* trunk section area below the point of grafting
*Equal letters in the column do not differ statistically from one another by the Scott-Knott test, 5% probability.

Figure 1. Dendrogram of genetic similarity between rootstocks of the genus Prunus, engrafted with the cultivar BRS-Libra scion, generated by the UPGMA method from the Jaccard index. Cutoff = 0.2

Figura 1. Dendrograma de similaridade genética entre porta-enxertos do gênero Prunus, enxertados sob a cultivar copa BRS Libra, gerado pelo método UPGMA a partir do índice de Jaccard. Ponto de corte = 0,2



Good graft compatibility is a requirement for selecting appropriate rootstocks for agronomic use (Emmanouilidou & Kyriacou, 2017). In this regard, the cultivars of group II presented few factors that would favor their inclusion in cultivation systems.

On the other hand, as seen in Table 2, cv. Santa Rosa, also in group II, stood out in terms of soluble solids content, with an average value of 10.85 ± 2.09 °Brix. Soluble solids contribute to a variety of fruit quality characteristics, including flavor, texture and nutraceutical properties. According to Crisosto & Crisosto (2005), the degree of consumer acceptance was closely related to the content of soluble solids.

Group III included the cultivars Ishtara, Clone 15, Tsukuba-1, Tsukuba-2, Tardio-01 and Tsukuba-3. These cultivars showed productivity 15% lower than the average of groups IV and V.

Besides it had lower productivity, the group included plants that presented with less vigor, expressed as smaller trunk section area. De Rossi et al. (2004) also observed lower vigor in plants grafted on Tsukuba-1. Based on this observation, in addition to a lack of signs of incompatibility, cultivars of this group, due to their lower vigor, could be candidates for use in densely planted orchards, possibly requiring less intervention with pruning. Thus, the lower productivity observed in the plants of group III could be compensated for by the greater number of plants that they allow to be cultivated in the area, as well as by the lower labor requirement for the pruning management.

Group IV (I-67-52-4, Barrier and Rigitano) presented higher average fruit mass, 21% higher than the mean of groups III and V. This group showed lower productivity and vigor than did group V and showed higher productivity than did group III. The characteristics that highlight the importance of the cultivars of this group are the tolerance to nematodes of the genus *Meloidogyne*, conducive to the improvement of fruit quality, low vigor and ease of propagation by herbaceous cuttings (Couto et al., 2004; Pereira et al., 2007).

In contrast to the other groups, group V encompassed plants showing higher productivity and greater vigor. The cultivars of this group included Cadaman, Okinawa, Mexico Fila 1, GF 677, Nemared, Capdeboscq, G × N.9, De Guia, Flordaguard, Rosaflor and own-rooted BRS-Libra. Although these plants were more productive, they were more vigorous. Consequently, rootstocks of this group, as well as the own-rooted plants, required more manpower in terms of pruning and management, possibly increasing production costs.

Similar results with the Okinawa cultivar were reported by Mayer & Pereira (2006), who analyzed the vigor of peach clones propagated by herbaceous cuttings, confirming that the Okinawa cultivar presented larger trunk diameter and greater growth of the main branches, indicating high vigor and less possibility of success in the formation of orchards of high density. Varago et al. (2017) reported that cv. Cadaman initially showed high vigor, however with similar decreases from the fourth to the fifth productive cycle. The same authors reported that the cultivar Nemared provided vigorous plants with good productivity, as observed in the present study.

Based on the similarity coefficient, it can be seen that group IV and V were the most similar to each other (0.1), while group I was the most distinct (1.0) in relation to the others, with respect to graft incompatibility. Similarly, group II differed from group III (0.4) while the latter group was distinguished from group IV (0.2) with respect to the scale of genetic dissimilarity projected in the dendrogram (Figure 1).

Although the cultivars of group V were more productive, they showed more vigor. Consequently, rootstocks from this group required more manpower in terms of pruning and management, possibly increasing production costs. On the other hand, the cultivars of group III, although less productive, were less vigorous and required less expense in terms labor for their management. In this way, the choice of rootstock becomes fundamentally important for planning units of production.

4 Conclusion

We conclude that the use of various rootstocks influenced the behavior of the BRS – Libra cultivar in terms of vigor and fruit production. The cultivars of group V, showing greater vigor, gave good potential for use in low planting density orchards, while the cultivars of group III, composed of rootstocks with lower vigor, presented potential for use in orchards of medium-high density.

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