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BIOLOGY

Grafting technique and rootstock species for the propagation of *Plinia cauliflora*

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ABSTRACT: *One of the biggest challenges for expansion of jabuticaba fruit tree cultivation is the high cost of seedlings due to to difficulties with vegetative propagation. Here, we aimed to evaluate graft survival in combinations of **Plinia cauliflora** and rootstocks of other species from the **Myrtaceae** family. The study was carried out at the Universidade Tecnológica Federal do Paraná - Campus Dois Vizinhos, Paraná State, Brazil. **Eugenia uniflora** L., **E. involucrata** DC, and **P. cauliflora** were used as rootstocks and were obtained as seeds. The 'whip-and- tongue' and cleft grafting techniques were tested for vegetative propagation. Grafted plants were cultivated in a greenhouse. The experimental design was a randomized complete block, in factorial 3×2 (rootstock species× graft type), with four replicates of 10 grafts by plot. Compatibility between rootstock species and scion was evaluated by diameter growth below and above the grafting point and by vegetative budburst and survival percentages. The use of **P. cauliflora** grafts with the same species was required for propagation. **E. involucrata** and **E. uniflora** rootstocks were not capable of propagation with **P. cauliflora**. Therefore, it is necessary to test other species from the **Myrtaceae** family.*
Key words: *Jabuticaba Açú tree, incompatibility, **Myrtaceae**.*

Enxertia e espécie de porta-enxerto na propagação de *Plinia cauliflora*

RESUMO: *Um dos maiores problemas enfrentados para a expansão do cultivo de jabuticabeira é o alto custo das mudas, principalmente, pela dificuldade de multiplicá-las vegetativamente. O objetivo deste trabalho foi analisar a sobrevivência da combinação da enxertia entre jabuticabeira Açú (**Plinia cauliflora**) sobre portas-enxerto de outras espécies da família **Myrtaceae**. O trabalho foi realizado na UTFPR – Campus Dois Vizinhos (PR). Foi usada a pitangueira (**Eugenia uniflora** L.), a cerejeira-do-mato (**Eugenia involucrata** DC.) e a própria jabuticabeira Açú (**P. cauliflora**) como porta-enxertos, sendo estes obtidos por meio de sementes. Foram testados dois tipos de enxertia, um a fenda cheia e outro inglês com entalhe. As plantas enxertadas foram mantidas em casa-de-vegetação. O delineamento experimental foi em blocos completamente casualizados, em fatorial 3x2 (espécie de porta-enxerto x tipo de enxertia), com quatro repetições, considerando-se o uso de dez enxertos por parcela. A compatibilidade entre as espécies foi avaliada com base na aferição do crescimento entre os diâmetros do porta-enxerto e enxerto, abaixo e acima do ponto de enxertia, da brotação e a sobrevivência dos enxertos. As enxertias de jabuticabeira Açú com uso de porta-enxerto de mesma espécie foi indicado para sua propagação. Os portas-enxerto de pitangueira e cerejeira da mata não foram indicados, devendo testar outras espécies da família **Myrtaceae**.*
Palavras-chave: *Jabuticabeira Açú, incompatibilidade, **Myrtaceae**.*

INTRODUCTION

Although the jabuticabeira fruit tree is well appreciated by consumers, domestication of this tree is still in its early stages. The main traits required for domestication of the jabuticabeira are early maturity, productivity, and fruit quality. However, one of the main problems facing the implementation of commercial cultivation of jabuticabeira is the high cost of the seedlings produced, mainly due to difficulties in vegetative propagation; this compromises the propagation of genotypes selected as naturally superior (SASSO et al., 2010) and leads to long periods of juvenility.

Cutting, layering, and grafting techniques have been examined for asexual propagation of the jabuticabeira. According to SASSO et al. (2010), the grafting method is the most appropriate method for propagation of this fruit tree because this method has been shown to be more successful than the other two techniques. However, results from preliminary studies have suggested that grafts between different species of jabuticabeira may be incompatible after the second year in the field, making not possible the complete union between two genetic materials, which can be by genetic or physiological factors.

Species selection is pivotal in plant grafting because grafting failure is often the result

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of incompatibility between the rootstock and the scion, characterized by absence of normal tissue development at the graft, with the occurrence of vascular vessels that are not completely lignified. This interrupts the vascular and cambial continuity with consequent physical problems at the junction (CANIZARES et al., 2003). Three hypotheses may explain the cause of such incompatibility: 1) differences in growth characteristics between the rootstock and the scion (vigor and starting and ending seasons of the vegetative cycle); 2) physiological and biochemical differences between rootstock and scion and 3) production of a substance by parts of the graft union that is toxic to the other part of the plant (HARTMANN et al., 2010).

Incompatibility studies performed between scion and rootstock have sometimes shown poor vascular connection, vascular discontinuity, and phloem degeneration, which can be identified early on or after weeks following the establishment of the graft (PINA & ERREA, 2005; PINA et al., 2009). This may be due to water and nutrient unbalance, which will inhibit initial cell development at the graft region, resulting in incompatibility (PINA et al., 2009). Vascular connections; are therefore. interrupted due to lack of differentiation.

In some cases, this incompatibility is only observed years after the grafting is performed, resulting in differences in growth and often breaking the connectivity of the host and graft at the graft point. However, depending on the specific conditions used, incompatibility may be detected early, as was the case in apricott grafting, in which the accumulation of phenolic compounds was indicative of incompatibility during the first week after grafting (ERREA et al., 2001).

Rootstock selection is a critical determinant of grafting success and directly influences the cultivation process. As such, traits such as uniformity in plant vigor, homogeneous production, and increased cultivation can be modified, and plants can develop the ability to better adapt to the adverse conditions (biotic and abiotic soil factors) and climatic characteristics of each region.

Rootstocks from other species of the same family can be tested for the jabuticabeira; compatibility must be the first characteristic observed, followed by development in the field (i.e., plant vigor). According to the findings of HARTMANN et al. (2010) and FACHINELLO et al. (2005), host/graft interactions with higher botanical affinity will lead to better chances of graft survival. As such, grafting combinations involving species from the same family (apple, plum, peach, and pear trees) and from different genera (citrus) have been successfully performed for fruit production. FRANZON (2008), SUGUINO et al. (2003), and LATTUADA et al. (2012) have studied the Myrtaceae family. However, for the jabuticabeira, combinations with other genera involving species of native fruit trees of the same family (***Myrtaceae***) have yet to be tested; such studies could result in advantages for the cultivation of jabuticabeira.

Therefore, the aim of this research was to analyze the survival of grafting combinations between jabuticabeira Acú (***Plinia cauliflora***) and rootstocks from other species of the ***Myrtaceae*** family.

MATERIALS AND METHODS

The research was performed in the Universidade Tecnológica Federal do Paraná - Campus Dois Vizinhos. Rootstocks of Surinam cherry (***Eugenia uniflora*** L.), Cherry of the Rio Grande (***Eugenia involucrata*** DC), and ***P. cauliflora*** were tested for the grafting of jabuticabeira Açu (***P. cauliflora***), which were obtained from seeds.

The rootstocks used were between 2 and 3 years old (diameter: ≈ 0.6cm) and were kept in 2-L plastic growing bags. Two grafting techniques, i.e., whip-and-tongue and cleft grafting, were performed. The experimental design was a 3×2 factorial design (rootstock species × type of graft) with a randomized complete block, with four repetitions and 10 grafted plants per plot.

Scions used as top cultivars were collected in the morning period and carried to the place of grafting in a container with water in order to avoid dehydration and oxidization of the material. Top cultivar scions were approximately 5cm long, and the leaves had been removed during their preparation.

The leaves below the grafting point were kept in order to maintain the photosynthetic activity of the plants until the graft had taken, like the methodology performed by SASSO et al. (2010). Grafting was performed at a height of 12–15cm from the root of the rootstock. Following grafting, any new rootstock growth below the graft was pruned.

After the grafting, the cambium between the rootstock and the scion was connected using an appropriate grafting tape (*Buddy Tape*), and the grafts

were covered with clear plastic bags (18×30cm) in order to form a high humidity chamber and avoid tissue dehydration. The grafted plants were kept in a greenhouse, and the plastic bag was kept in place until the graft's first leaves developed.

At 150 days after grafting, the final percentages of primary growth and graft survival were evaluated, and the numbers of primary growths and leaves were determined. Only the grafts that survived this period were considered in the survival evaluation.

After the grafts took, cuts were performed to observe graft union. A sliding microtome was used for this purpose, with cuts performed at the grafting point in order to observe the junction of the tissues from the rootstock and scion. This procedure was performed for all the types of grafting and for all plants tested, even when there was no growth observed. The cuts were visualized using a 10× magnifying glass.

The diameter of the rootstock (cm) was also measured monthly during the 150 days following grafting at 5cm below and above the graft point using a digital caliper. Each month corresponded to one treatment. The data were analyzed using the Lilliefors normality test, followed by analysis of variance and Duncan test (P=0.05) using SANEST software. The normality test did not reveal the need for data transformation.

RESULTS AND DISCUSSION

Significant differences were observed between the rootstock species for all the variables analyzed. However, no significant differences were observed for any of the variables regarding the type of grafting performed and the interaction between rootstock species and type of grafting (Table 1). This result confirmed that the type of species influenced the propagation of jabuticabeira Açú.

In the present research, the grafted plants started to sprout at around 30 days after grafting only when a rootstock from the same species (***P. cauliflora***) was used. OLIVEIRA et al. (2012) reported that in their experiment, jabuticabeira grafts started to sprout at approximately 50 days after grafting for combinations between jabuticabeira Paulista and Sabará, Paulista and Olho de Boi, Sabará and Hybrid, and Hybrid and Olho de Boi and at 71 days for the combination between Sabará and Hybrid. Comparing the results from the present study with those obtained by OLIVEIRA et al. (2012) suggested that the reduced sprouting start time could be explained by the use of the plastic bag with a humidity chamber, which promoted the accumulation of heat. This may lead to a greenhouse effect, allowing rapid cell differentiation and subsequent sprouting.

No new buds were observed on the grafted jabuticabeira using Surinam cherry (***E. uniflora*** L.) or Cherry of the Rio Grande (***E. involucrata***) as rootstock, which resulted in death of the scion (Table 2). This suggested the existence of incompatibility between these combinations, as established by the anatomical longitudinal sections examined in both tissues (Figure 1). This observation could be explained by physiological and anatomical aspects, preventing union between the two tissues.

Grafting incompatibility was also observed for Camu camu (***Myrciaria dubia***) grafting with guava and Surinam cherry in a study by SUGUINO et al. (2003),as demonstrated by anatomical analyses in which the existence of physiological and biochemical differences between the grafted parts was suggested as a hypothesis to explain the incompatibility. A similar result was obtained by LATTUADA (2012), with grafting combinations involving Cherry of the Rio Grande and Surinam cherry, both as rootstocks and scions.

Table 1 - Square mean of number of primary buds, length of primary buds, number of leaves, and percentage of survival of specie and grafting type factors and the interaction of these factors.

Variation Causes	GL	Square mean Number of primary buds	Square mean Length of primary buds (cm)	Square mean Number of leaves	Square mean Survival (%)
Block	3				
Specie (S)	2	1.9319923*	11.3040504*	27.179168*	1812.7135*
Grafting type (G)	1	0.0047852 ns	0.0006687 ns	0.0007468 ns	3.0850997ns
S x G	2	0.0052149 ns	0.0005207 ns	0.0009221ns	2.5956498ns
Residue	15	00396346	0.0881351	0.1301562	21.8161687

*Significant by test's F by 5% of probability. ns. no significant by test's F.

Table 2 - Number of primary buds, length of primary buds, number of leaves, and percentage of survival of jabuticabeira Açu scions grafted onto the same species, Surinam cherry,or Cherry of the Rio Grande.

Rootstock	Number of primary buds	Lengthof primary buds (cm)	Number of leaves	Survival (%)
Jabuticabeira Açú	2.46 a*	8.41 a	16.65 a	20.38 a
Surinam cherry	0.00 b	0.00 b	0.00 b	0.00 b
Cherry of the Rio Grande	0.00 b	0.00 b	0.00 b	0.00 b
CV (%)	15.37	17.50	17.40	49.38

Mean values followed by different letters in the column differ significantly from one another according to the Duncan test (P=0.05).

One of the factors that may have influenced the results of the present study was the presence of substances that inhibited the formation of calluses, such as phenolic compounds. Myrtaceaes generally exude a large amount of these toxic substances, according to FACHINELLO et al. (2005).

In the present study, 20.38% of grafts survived when jabuticabeira Açú was grafted onto rootstocks of the same species (Table 2 and Figure 2). This percentage was relatively low given the potential success of this technique. We hypothesized that the low percentage of survival for this combination could be explained by the lower than ideal C/N because the grafting coincided with the period following the jabuticabeira harvest in February.

In a study by SASSO et al.(2010), a low budding percentage (15.6%) was observed following grafting between ***P. jaboticaba*** species. This observation could be attributed to the time of collection of the scions, which also coincided with the period of fructification of the original cultivar.

Because the jabuticabeira Açu scions did not survive on Cherry of the Rio Grande and Surinam cherry rootstocks, the use of the same species as rootstock showed superiority for multiple variables, including the number of buds, bud length, and number of leaves (Table 2).

An average of 2.46 primary buds (Table 2) was obtained for the jabuticabeira Açu scion on rootstocks of the same species. Similar results were obtained by SASSO et al. (2010), who reported 2.2 and 2.3 buds for grafts onto the same species during two separate grafting seasons (May and August, respectively). Additionally, 2.06 buds per grafted plant were obtained by MALAGI et al.(2012), who used the same jabuticabeira species as scion and rootstock.

In addition to the number of primary buds, it is important that the length is sufficient to allow rapid plant development. An average length of 8.41cm was obtained for primary buds in the union between jabuticabeiras (Table 2), surpassing the values obtained by OLIVEIRA et al.(2012) for jabuticabeira Olho de Boi and Sabará (scions) grafted

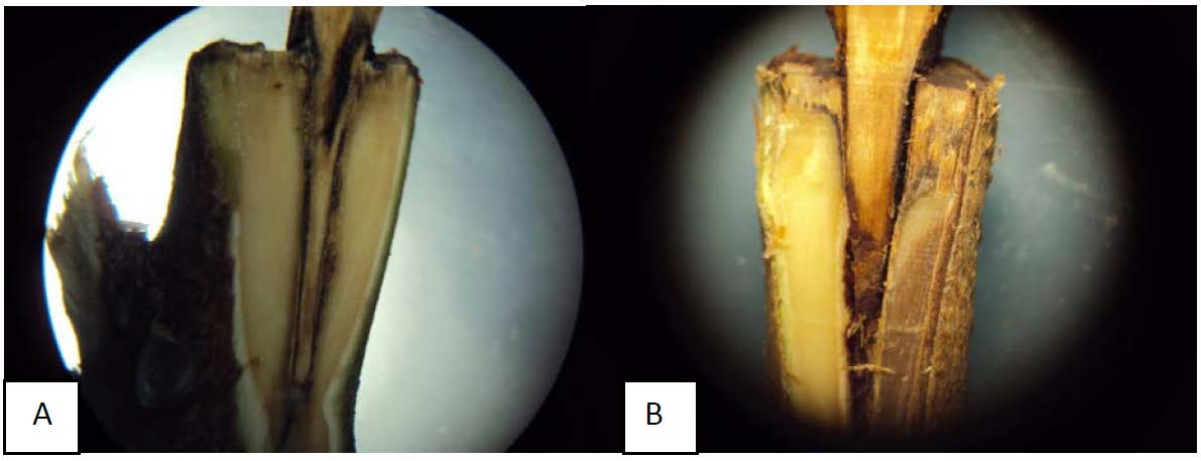

Figure 1 - Transverse section of the grafted area showing incompatibility between the combinations of jabuticabeira Açu and rootstocks of Surinam cherry (A) and Cherry of the Rio Grande (B) at 30 days after grafting. UTFPR, Dois Vizinhos - PR, 2013.

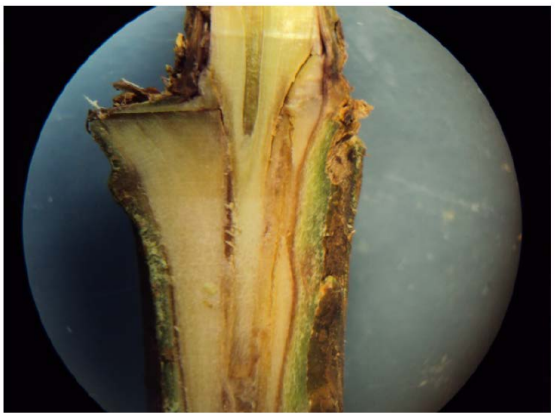

Figure 2 - Transverse section of the grafted area showing compatibility between the combinations of jabuticabeira Açu at 30 days after grafting. UTFPR, Dois Vizinhos - PR, 2013.

onto Hybrid jabuticabeira (rootstock) (5.47 and 0.28cm, respectively), in which both combinations were grafted using the cleft grafting technique.

MALAGI et al.(2012) obtained a mean bud length of 11.1cm for combinations between species of jabuticabeira Açu. The increased length obtained for the same species combination, when compared with the results obtained in the present study, could be related to the lower number of buds obtained in the previous study, which may have increased the amount of resources available per bud and consequently their increased growth.

An average of 17 leaves per grafted plant was obtained (Table 2). The number of leaves is important for subsequent development of the plant, resulting from the perfect union between scion and rootstock, because the scion initially depends completely on the resources from the xylem, and if those resources are exhausted, the plant will become dependent on the products of photosynthesis.

The whip-and-tongue and cleft grafting techniques used in the present study were statistically similar; therefore, either one can be used. However, when FRANCO et al. (2010) compared the whip-and-tongue and cleft grafting methods, in August, they observed lower graft survival for jabuticabeira Sabará when cleft grafting was performed. Thus, the season of grafting may affect the results obtained by different methods.

In the 150 days following grafting, the diameter of the surviving plants increased with time in all points assessed, indicating graft survival during this period (Table 3). However, a comparison of the data obtained in the first and last analyses at the points assessed revealed that there was a greater increase above the graft point (0.53cm), when compared with that at the graft point (0.30cm) and the point below the graft point (0.26cm). This effect may have been related to the growth behavior between the scion and rootstock, as increasing growth averages were observed above the grafting point as early as at day 60 and below the grafting point at day 120. This may have contributed to the fact that growth at the grafting point was observed only in the last month (150 days; Table 3). This could be indicative of future survival or failure between these combinations after grafting.

In the present study, survival of the grafted plants after grafting was low (20.38%) even when the same species was used as rootstock and scion. As such, future studies are needed to examine the effects of different seasons and grafting techniques on the ability to obtain higher survival percentages and to observe grafted plants in the field if will have cases in which late incompatibility occurs.

CONCLUSION

The whip-and-tongue and cleft grafting techniques were the most promising methods for the vegetative propagation of jabuticabeira Açu, in combination with plants of the same species. Rootstocks

Table 3 - Diameter (cm) of the jabuticabeira Açu plants grafted onto rootstock of the same species, measured above, below, and at the grafting point, in the months of June, July, August, September, and October.

Point analyzed	----------Mont----------					CV (%)
	June	July	August	September	October	
Above the grafting point	0.42 b*	0.50 ab	0.53 ab	0.69 ab	0.95 a	13.77
At the grafting point	0.44 c	0.47 c	0.53 bc	0.61 b	0.74 a	3.12
Below the grafting point	0.45 c	0.49 c	0.54 bc	0.62 ab	0.71 a	3.44

Mean values followed by different letters in the line differ significantly from one another according to the Duncan test (P=0.05).

of Surinam cherry and cherry of the Rio Grande were not indicated for jabuticabeira Açu grafting.

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REFERENCES

CANIZARES, K.A.L. et al. **Enxertia em hortaliças**. São Paulo: UNESP, 2003. V.1. p.11, 21-23, 25-27.

ERREA, P. et al. Early detection of graft incompatibility in apricot (***Prunus armeniaca***) using in vitro techniques. **Physiologia Plantarum**,v.112, p.135-141, 2001.

FACHINELLO, J. C. et al. **Propagação de plantas frutíferas**. Brasília, DF: Embrapa Informação Tecnológica; Bento Gonçalves: Embrapa Uva e Vinho, 2005.V.1, p.221.

FRANCO, L.R.L. et al. Pegamento e crescimento inicial de mudas de jabuticabeiras 'Açu' e 'Sabará' submetidas a dois tipos de enxertia. **Revista Ceres**, v.57, p.535-538, 2010.

FRANZON, R.C. **Propagação vegetativa e modo de reprodução da pitangueira(*Eugenia uniflora* L.)**. 2008. 100f. Dissertação (Mestrado em Agronomia). Universidade Federal de Pelotas, RS.

HARTMANN, H.T. et al. **Plant propagation**: principles and practices. New Jersey: Prentice Hall, 2010. 915p.

LATTUADA, D.S. et al. Estaquia herbácea em myrtaceae nativas no Rio Grande do Sul. In: CONGRESSO BRASILEIRO DE FRUTICULTURA,22., 2012, Bento Gonçalves, RS. **Anais...** Bento Gonçalves: Sociedade Brasileira de Fruticultura, p. 1285-1288.2012.

MALAGI, G.et al. Enxertia interespecífica de jabuticabeira: influência do tipo de garfo. **Ciência Rural**, v.42, p.221-224, 2012.

OLIVEIRA, M.S.S. et al. Influência do tipo de enxertia em combinações copa/porta-enxerto na produção de mudas de jabuticabeiras. In: CONGRESSO BRASILEIRO DE FRUTICULTURA, 22., 2012, Bento Gonçalves, RS. **Anais...** Bento Gonçalves: Sociedade Brasileira de Fruticultura, 2012.

PINA, A. et al. A review of new advances in mechanism of graft compatibility – incompatibility. **Scientia Horticulturae**, v.106. p.1-11, 2005.

PINA, A. et al. Cell-to-cell transport through plasmodesmata in tree callus cultures. **Tree Physiology**, v.29, p.809-818, 2009.

SASSO, S.A. et al. Propagação de Jabuticabeira por enxertia e alporquia. **Revista Brasileira de Fruticultura Jaboticabal**, v.32, p.571-576, 2010.

SUGUINO, E. et al. Propagação vegetativa de camu-camu por meio de enxertia intergenérica na família Myrtaceae. **Pesquisa Agropecuária Brasileira**, v.38, p.1477-1482, 2003.