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Propagation

Advances in the propagation of Brazilian Cherry tree

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Abstratct - Brazilian Cherry tree is a native fruit tree belonging to the Myrtaceae family, with ample adaptation to the different edaphoclimatic conditions of Brazil, which makes this fruit widely known. However, there are still few commercial orchards, which may soon be reversed, since studies have shown the benefits of the consumption of this fruit for human health, as well as the potentialities for industrial processing. Thus, Brazilian Cherry tree will no longer be a backyard plant and will be grown in commercial orchards, which demands the production of seedlings with genetic quality. Sexual propagation with the use of seeds should be replaced by asexual propagation by means of cutting, grafting, spreading and tissue culture. The grafting method has proven to be efficient in more than 70% of cases and herbaceous cuttings have rooting greater than 77%. New studies on micropropagation and better control of herbaceous cutting processes should stimulate and economically increase the commercial production of this fruit for fresh consumption or processing. This review article included the results of other studies, addressing advances, limitations and protocols for propagation of Brazilian Cherry tree.

Index Terms: Brazilian Cherry, Myrtaceae, seeds, Surinan Cherry, propagation techniques.

Avanços na propagação da Pitangueira

Resumo - A Pitangueira é fruteira nativa da família Myrtaceae com ampla adaptação as diferentes condições edafoclimáticas do país, o que a torna muito conhecida. Todavia, ainda existem poucos pomares comerciais com a cultura, o que talvez seja em breve revertido uma vez que estudos vem demonstrando a importância do consumo de seu fruto para saúde humana, bem como, as potencialidades no processamento industrial e assim a pitangueira deixará de ser uma planta de fundo de quintal e passará a ser cultivada em pomares comerciais o que demanda produção de mudas com qualidade e genética. A propagação sexuada com uso de sementes deverá ser substituída pela assexuada através da estaquia, enxertia, alporquia e cultura de tecidos.O método de enxertia em garfagem têm-se mostrado eficiente em mais de 70% dos casos e a estaquia herbácea apresenta enraizamento superior a 77%. Novos estudos, como a micropropagação e melhor controle nos processos de estaquia herbácea, devem estimular e viabilizar economicamente a multiplicação desta frutífera para cultivo comercial, seja para produção de frutos frescos, seja para processamento. Neste artigo de revisão incluímos os resultados de trabalhos, abordando os avanços, limitações e protocolos para propagação da pitangueira.

Termos para indexação: Pitanga, Myrtaceae, sementes, técnicas de propagação.

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Introduction

Brazilian Cherry or Surinam Cherry (*Eugenia uniflora* L.) is a fruit tree native to Brazil which belongs to the Myrtaceae family. It is a perennial size tree, distributed throughout the country and due to its easy adaptability to different soils and climate conditions, Brazilian Cherry can also be found in different parts of the world. Myrtaceaes comprise one of the largest botanical families, with thousands of species distributed in approximately 140 genera (JOHNSON; BRIGGS, 1984; LANDROUM; KAWASAKI, 1997; WILSON et al., 2001). The genus *Eugenia* is one of the largest in the Myrtaceae family and it has more than 1,000 species distributed mainly in Central and South America (MERWE et al., 2004).

According to Costa (2004), Brazilian Cherry is a diploid species, having 11 chromosomes (n=11) and 2n=22. Almeida et al. (2012) reported that there is a morphologically similar species (*Eugenia pitanga*), but Costa (2004) reported that *E. pitanga* has n=22 or 2n=44. Thus, due to the morphological similarity, it is very difficult the correct identification of accessions, and the number of chromosomes an important parameter for the identification of the two species.

For Almeida et al. (2012), the knowledge of the reproductive mechanism of each species is important to ensure the perpetuation of their offspring, the colonization of new habitats, and it is the basis for the natural evolutionary process. According to these authors, both sexual and asexual propagation represent one of the main aspects to maintain an economically viable culture. In this context, aspects related to floral biology, pollination mechanisms and phenology are essential, both in relation to species ecology as for commercial production.

Flowers anatomy, morphology and biology

Brazilian Cherry has racemose inflorescences with the presence of inserted pedicellate flowers in the leaf axils (ANGELY, 1965; ROMAGNOLO; SOUZA, 2006; SILVA; PINHEIRO, 2007). Regarding to other species of the *Eugenia* genus, Brazilian Cherry has a distinct pattern, since more than two flowers are emitted from each leaf node (SILVA; PINHEIRO, 2007). According to these authors, the flowers of Brazilian Cherry have an average diameter of 16.1 mm, with the stigma and ovary measuring 0.24 and 1.2 mm in diameter, respectively. The measures of floral structure are ovale/ovary number of 20.8, style length of 6.08 mm, number of stamens per flower of 52.9 and flowers/ leaf axil of 3.3.

Brazilian Cherry flowers are hermaphrodite, with four curved white petals on the hypanthium and four sepals also bent on the structure, highlighting the androecium. This one with many stamens (52.9), with filiform fillets and globular anthers, and longitudinal dehiscence, and of yellowish color (SILVA; PINHEIRO, 2007). The stamens are distributed over the hypanthium around the style, and their high number provides a large quantity of pollen grains. They are floral attractive, since they constitute the only floral resources offered to the pollinators (FAEGRI, PIJL, 1979). The ovary is in the lower part, bilocular and pluriovulated (20.8) (ROMAGNOLO; SOUZA, 2006). The stigma is small, dry and in the shape of papillae.

During the anthesis, the stigma is receptive, presenting turgid and glossy papillae (SILVA; PINHEIRO, 2007). However, Almeida et al. (2012) reported that field observations showed that the Brazilian Cherry fruit usually has two to three seeds, which may suggest the existence of degenerative systems for the other ovules or restriction at fertilization. The style is erect, and the apical end being below the stigma, which is slightly curved, and this curvature becomes evident during the course of the day.

The anthesis begins at dawn and extends throughout the day, and this was characterized as being of the *Psidium* type, because the stamens' filaments and the style expand together with the blossoming of the petals during the floral opening. The flowers remain receptive until the afternoon of the second day, when the petals and the androecium fall, only persisting the chalice and style, which can be observed in the young fruit (SILVA; PINHEIRO, 2007).

The typical white appearance of Brazilian Cherry bloom is related to the great number of foliar nodes (4 to 7) at the peak of flowering period, and this aggregation of flowers is a strategy to attract pollinating agents (O'BRIEN, CALDER, 1993).

Regarding pollination, as in many species of Myrtaceae, Brazilian Cherry has generalist flowers, which are visited by many types of animals, including insects, bats, and birds (O'BRIEN, CALDER, 1993). In the study of Silva and Pinheiro (2007), it was observed that Brazilian Cherry flowers were visited by a total of 29 species of insects, but honey bees were the most common visitor (Figure 1). These authors reported that in the stigmas analysis of bagged flowers in bud stage, without the presence of pollinators, it was possible to observe a significant amount of pollen grains on stigmatic papillae, which may be indicative of automatic self-pollination. On the other hand, as the stigma usually stands above the stamens, this may represent a mechanism of control of self-fertilization (ROMAGNOLO; SOUZA, 2006; SILVA; PINHEIRO, 2007; FRANZON, 2008). According to Franzon (2008), self-pollination is possible, but effective fruiting is of only 6.4%. This author also reports that Brazilian Cherry is totally self-incompatible, but more information is needed to elucidate this process.

Figure 1 - Brazilian Cherry flowers visited by honey bee (*Apis mellifera*) and visual aspect of the Brazilian Cherry fruit. (Photos: Wagner Jr., A.)

Fruit anatomy, morphology and dispersion

The fruit, a result of fertilization of the ovary, is a globose ellipsoid berry, with seven to ten longitudinal grooves of 1 to 5 cm in diameter (ROMAGNOLO; SOUZA, 2007; SAMPAIO et al., 2005); with morphological variations in size and color of the epicarp, which starts from green, advancing to yellow, orange, red (Figure 2), which may reach purple or black coloration (Figure 1). The endocarp (pulp), 3 to 5 mm thick, has a pink to red color, sweet and acid flavor, and characteristic aroma. The embryos are of the eugenioid type, characteristic of the *Eugenia* genus, with globose, fleshy cotyledons, conferred with a line of separation between the cotyledons, with undeveloped hypocotyl-radicle axis (ROMAGNOLO; SOUZA, 2007).

The dispersion of the Brazilian Cherry fruit is zoocorical (CASTRO; GALETTI, 2004; BUDKE et al., 2005), but there are few reports on the dispersing agents. Teiús lizards (CASTRO;GALETTI, 2004) and birds (BUDKE et al., 2005) are cited as responsible for seed dispersal.

Phenology - According to Silva and Pinheiro (2007), Brazilian Cherry presents a pattern of annual mass blooming with defined peaks of floral emissions. Plants produce large quantities of flowers for a few weeks, one on average; however, there are episodes of production of some flowers throughout the year. Brazilian Cherry blooms in the dry season of the year (August to October), fruiting from September to November. These authors reported evident asynchrony among the plants within the populations, with individuals in the reproductive phase and others in the vegetative phase. This may increase cross-pollination rates by promoting greater range of foraging for pollinating agents.

SEXUAL PROPAGATION

According to Morton (1987), the use of seeds is the most used method for Brazilian Cherry propagation. Thus, most of the Brazilian Cherry orchards were formed from plants originated from sexual propagation, which results in great phenotypic heterogeneity (BEZERRA et al., 2004). According to Machado, Parente (1986) and Bezerra et al. (2000), the use of seeds (Figure 2) is still the most common mean to propagate this species. Thus, for the economic development of Brazilian Cherry crop, knowledge of seed germination is the first step to obtain seedlings with vigor and quality.

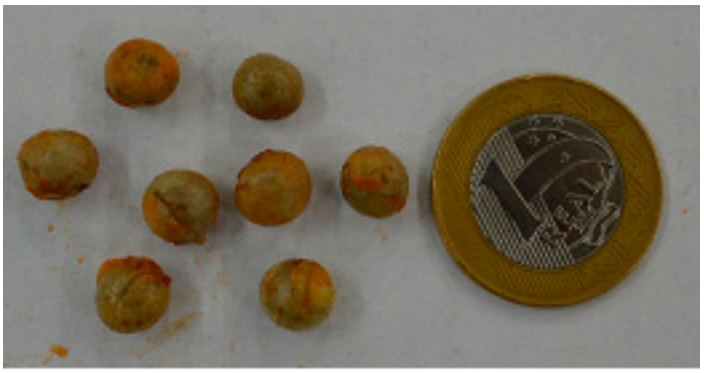

Figure 2 – Brazilian Cherry seeds newly separated from fruit pulp. (Photo: Wagner Jr., A.)

Brazilian Cherry seeds (Figure 3) were considered recalcitrant by Harrington (1972), that is, they are seeds of short longevity when lost humidity. These seeds do not undergo natural drying in the parent plant and they are released with high moisture content. If this moisture is reduced below a critical level during storage, the seeds will die (KING; ROBERTS, 1979). Kaiser et al. (2014) reported that the high moisture content of Brazilian Cherry seeds (40.6 - 43.4%) is characteristic of recalcitrant species, especially *Eugenia* genus, which moisture content varies from 40 to 70% at the moment of dispersion.

The germination of freshly harvested seeds with 43.4% moisture varied from 96% in vermiculite and sand (Figure 3) to 98% in paper after 35 days at 25°C, with a 12-hour photoperiod (KAISER et al., 2014). These results were superior to those found by Quintão Scalon et al. (2001), that is, values of 65.7%. The viability of the seeds is approximately 30 days, since Kaiser et al. (2014) stored Brazilian Cherry seeds in perforated polyethylene bags (5 holes) in a refrigerator, at 10 ± 3°C, for up to 45 days, and observed that after 30 days of storage the percentage of germination fell to 86% when compared to days 0 (98%), 15 (97%) and 30 (94%). Likewise, the percentage of viable seeds decreased to 85% in those stored for 45 days, compared to 96; 92 and 91%, for those stored for 0, 15 and 30 days, respectively.

Figure 3 – Brazilian Cherry seedlings emerged in sand. (Photo: Pirola, K.)

Regarding to the characteristics of the Brazilian Cherry seed, the weight of 1,000 seeds is 347.7 g, corresponding to 2,876 seeds Kg^{-1}, which is in accordance with the value proposed in the instructions for seed analysis of forest species (BRAZIL, 2013). According to Piña-Rodrigues and Aguiar (1993), seed size and weight are quite variable parameters, differing according to place of origin, year of production and among genotypes. One of the factors that may affect seed vigor is its size, and Brazilian Cherry presents a great variation in the number of seeds per fruit, which among other factors is the cause of seed size variation (LEITE et al. 2013). These authors classified Brazilian Cherry seeds as small, medium, large and extra-large and, they reported as values the ones described in Table 1.

Table 1- Mass, length and width of Brazilian Cherry (*Eugenia uniflora* L.) seeds classified according to their size*

Size	Mass (g)	Length (mm)	Width (mm)
Small	0.16	6.7	5.07
Medium	0.30	8.1	5.97
Large	0.40	9.1	6.97
Extra-large	0.56	12.2	7.40

*Average of 50 seeds (**Source:** Leite et al. (2013).

In addition to size classification, Leite et al. (2013) performed the percentage of emergence test and they determined the emergence speed index (ESI). These authors observed that the bigger the seed, the greater the percentage of emergence and ESI, that is, an emergence percentage of 28; 42; 62 and 82% was observed for small, medium, large and extra-large seeds, respectively. Likewise, ESI was 0.08; 0.12; 0.18 and 0.25 for the respective classes. Antunes et al. (2012) also observed that medium seeds with a diameter of 10.0 mm presented a higher percentage of emergence (80.5%) when compared to small seeds, with a diameter of 6.0 mm (27.7%). These results are due to the fact that larger seeds are generally better nourished, having well-formed embryos and a greater amount of reserve substances (CARVALHO; NAKAGAWA, 2000).

In addition to the percentage of emergence and ESI, the extra-large seeds resulted in seedlings with a greater number of leaves (18.04), larger stem size (14.79 cm) and root (20.17 cm), larger trunk diameter (3.63 mm) and higher dry matter (LEITE et al., 2013).

The seeds should be obtained from ripe and healthy fruit, as the maturity stage of the fruit can affect the later germination and the initial development of the seedlings (ANTUNES et al., 2012). The fruit should be pulped, and the seeds washed in running water and dried in the shade for 48 hours (LEITE et al., 2014).

Sowing should be carried out immediately after drying the seeds or in a maximum of 30 days when they are stored in perforated polyethylene bags (5 holes), kept in a refrigerator at 10 ± 3°C (KAISER et al., 2014).

It is recommended to plant two seeds per container, at a depth of 1 to 2 cm, with black plastic bags measuring 8 x 15 cm (FRAZON, 2008), 12 x 16 cm or 19 x 31 cm (LATTUADA, 2014). Sowing can also be performed in the seedbed, and the seedlings must be transplanted when they reach between 20 and 30 cm in height.

Concerning to the substrate, many authors used the mixture of soil and manure of cattle or poultry in different proportions, 6: 1, 4: 1 (LEITE et al., 2013) or 3: 1 (FRAZON, 2008), respectively. Other substrates were tested (Plantmax®, vermiculite and coconut fiber) for sowing Brazilian Cherry seeds, but with similar performance (ANTUNES et al., 2012). Abreu et al. (2005) recommended the use of organic compost + sand + soil substrates in the ratio of 1: 1: 3, and Plantmax® + sand + soil, as well as 1: 1: 3 (v/v), with the addition of simple superphosphate (5 kg m^{-3}) as being ideal for Brazilian Cherry. Plantmax HT® was also used by Peña et al. (2015) in mixture with expanded vermiculite and soil, in the ratio of 1: 1: 1, and the container being used the 240 cm^3 tube.

Lattuada (2008) also cites the use of residues of 'acacia' bark, São Jerônimo soil, carbonized rice husk and medium sand, in a ratio of 1: 1: 2: 2. Dalanhol et al. (2016) used pinus bark, vermicompost + carbonized rice bark in different proportions and inoculated arbuscular mycorrhizal fungi (AMF) on the substrates. However, the incorporation of AMF did not provide better development of the seedlings, which the most satisfactory results occurred when using vermicompost + carbonized rice husk in the proportion of 20/80.

In general, the emergence occurs around 22-50 days after sowing (LEITE et al., 2013). When the seedlings are 5 cm high, thinning should take place eliminating the less vigorous one. Up to three months after seedling emergence, they should be protected from direct sunlight, especially during the hottest hours of the day. After this period, it is best to leave the seedlings in full sun. Quintão and Scalon et al. (2001) verified that Brazilian Cherry seedlings showed higher height (52.9 cm), trunk diameter (7.35 mm) and dry mass (42.8 g) when kept in full sun, compared to those kept shaded at 50 and 70% light.

The seedlings should be taken to the field when they reach a height of approximately 25 cm, which usually occurs six months after sowing or seedlings can be grafted when they reach heights between 30 and 40 cm and diameter with averages of 25 mm at the point of grafting.

Asexual propagation

Asexual or vegetative propagation involves obtaining plants from vegetative tissues and this is possible because the vegetative organs have the capacity of regeneration (HARTMANN et al., 2002). In Brazilian Cherry culture, many techniques have already been tested with relative success (Table 2).

Table 2. Recommendations for Brazilian Cherry (*Eugenia uniflora* L.) asexual propagation

Method	Type	Age (months)	Dize	Genotype	Success (%)	Reference
Grafting	Top splice grafting	9-12		-	77.5	Bezerra et al., (1999)
Grafting	Top clef grafting	-	65 cm height 5 mm Ø	IPA-7.3, 2.2, 11.3, 4.3, 3.1, 14.3, 15.1 and 3.2	81.5-53.5	Bezerra et al., (2002)
Grafting	Top clef grafting	-	30 cm height / 3 mm Ø Semiwoody with leaves	-	19.1-79.7	Peña et al., (2015)
Grafting	Top clef grafting	-	4 mm Ø / Woody no leaves	-	0.0	Lattuada (2008)
Grafting	Top clef grafting	1 2	8-10 mm Ø Semiwoody with leaves	- -	20.5 80.0	Lattuada (2008)
Grafting	Top clef grafting	2	8-10 mm Ø Semiwoody with leaves	-	Nebulization – 52.4 Plastic bags – 0.0	Lattuada (2008)*
Grafting	Top whip and tongue grafting		30-40 cm height 2 mm Ø Semiwoody with leaves	Pit 15	60.0 44.2	Franzon (2008)**
Grafting	Top whip and tongue grafting		30-40 cm height 2.5 mm Ø Semiwoody with leaves	Pit 15, 61^{+}, 74, 75^{+}, 77, 137^{+}, 161	50-95	Franzon (2008)
Grafting	Top whip and tongue grafting		30-40 cm height 2 mm Ø Semiwoody with leaves	Rootstock: *E. uniflora* *E. pyriformis* *M. pungens*	28,.8 1.30 41.3	Franzon (2008)
Cutting	Semiwoody cuttings Softwood cuttings Minicutting	-	5.0 cm height 1-2 mm Ø	-	91 77 16.5-43.2	Lattuada (2008)
Cutting	Minicutting	-	4.0 cm height	-	19.1-79.7	Peña et al., (2015)
Cutting	Minicutting	-	5.0 cm height	-	69.0	Lattuada (2010)
Cutting	-	-	12 cm height 6 mm Ø	-	0.0	Hössel et al., (2012)
Cutting	Minicutting	-	6-8 cm	-	0.77-70%	Hössel (2016)
Cutting	-	-	12 cm height 6 mm Ø	-	0.0	Coutinho et al., (1991)
Micropropagation	*In vitro* multiplication	-	Apical segments with 2-3 mm: medium 1/2MS	-	Rooting: 25-85.7	Uematsu et al., (1999)
Micropropagation	*In vitro* germination	-	Seeds Mediums MS and WPM***	-	-	Silveira et al.(2015)
Micropropagation	*In vitro* germination *In vitro* multiplication	-	Medium germination (6.0g L^{-1} agar) Segments with 1.5 cm: mediums AS_{30} and 1/2MS	-	74.0 98.7	Silva et al., (2014)
Micropropagation	*In vitro* germination	-	Medium germination (8.0g.L^{-1} agar)	-	100 Rooting: 50.0	Griff (2006)
Micropropagation	*In vitro* multiplication	-	Segments with 1cm (one node): medium MS	-		Lattuada (2010)

* July/August (50%), ** September (77,5%), $^{+}$ better fixation, ***MS=Murashige; Skoog (1962) and WPM=Wood Plant Medium of Loyd; McCown (1980).

GRAFTING

Regarding grafting, most studies used the top clef grafting (Figure 5) (BEZERRA et al., 1999; BEZERRA et al., 2002; PEÑA et al., 2015; LATTUADA, 2008; FRANZON, 2008), splice grafting (BEZERRA et al., 1999) and whip and tongue grafting (FRANZON, 2008), and the best results were obtained with the use of the top clef grafting (Figure 4) with semiwoody material, whose survival reached between 77.5% (BEZERRA et al., 1999) and 81.5% (BEZERRA et al., 2002), Table 2.

Figure 4 – Clef grafting in Brazilian Cherry. (Photo: Franzon, R.C.)

In general, grafting should be performed when the plants reach two months of age (LATTUADA, 2008), with a height of 30 to 40 cm, and the trunk reaches about 2 mm in diameter. However, Bezerra et al. (1999) recommended the use of older plants, that is, 9 months of age and with 65 cm of height. The grafts should be obtained from semiwoody branches with leaves, as the use of woody scions did not result in graft survival (LATTUADA, 2008). Bezerra et al. (1999) recommended grafts with 10 cm in length, 5 mm in diameter and with approximately 5 buds, it has been collected from the median portion of annual lignified branches.

Regarding the protection of the grafting region, Lattuada (2008) tested the use of polyethylene bags and nebulization, without the use of these plastic bags. This authors observed superior survival rate in nebulization (52.4%) compared to the use of plastic bags (0.0%).

Most of the grafting studies used Brazilian Cherry plants as rootstocks, however Franzon (2008) tested other species besides *E. uniflora*, such as: *E. pyriformis* ('uvalheira') and *M. pungens* ('guabijuzeiro'). The survival percentage was higher when 'Pit 15' scions were grafted on *M. pungens* (41.3%) and *E. uniflora* (28.8%) compared to *E. pyriformis* (1.30%). However, according to personal information of the author, there was mortality of all material after evaluation, assuming there is late incompatibility.

Regarding the canopy variety, Bezzera et al. (2002) tested the genotypes developed by the Pernambucana Company of Agricultural Research (Empresa Pernambucana de Pesquisa Agropecuária - IPA), that is, IPA-7.3; IPA-2.2; IPA-11.3; IPA-4.3; IPA-3.1; IPA-14.3; IPA-15.1; IPA-3.2; IPA-1.3 and IPA-1.1, grafted onto IPA-2.2 rootstock. These authors verified that the genotypes IPA-7.3; IPA-2.2; IPA-11.3; IPA-4.3; IPA-3.1; IPA-14.3; IPA-15.1 and IPA-3.2 showed similar survival rates (53%), and the IPA-7.3 genotype showed results of 81.5%, similar to those reported by Bezzera et al. (1999). Franzon (2008) tested the genotypes maintained by the Brazilian Agricultural Research Company - Temperate Weather (Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA), denominated Pit 15; Pit 61; Pit 74; Pit 75; Pit 77; Pit 137 and Pit 161, grafted on rootstocks whose seeds were collected at random from the native fruit trees collection in southern Brazil. This author reported that the survival percentages ranged from 40 to 87.5%, where Pit 75; Pit 61 and Pit 137 genotypes presented results above 65%. Thus, there is variation in the success of grafting practice due to the interaction between rootstock and grafting.

CUTTING

With regard to cutting, variation was observed in the outcome of this technique depending on the material used (PEÑA et al., 2015; LATTUADA, 2008; LATTUADA, 2010; HÖSSEL et al., 2012; COUTINHO et al., 2001) and the season (PEÑA et al., 2015; LATTUADA, 2008; HOSSEL, 2016), Table 2.

Coutinho et al. (1991) reported that Brazilian Cherry softwood cuttings treated with indole butyric acid (IBA) did not root, even with the use of this plant hormone. Similarly, Lopes (2009), using intermittent mist did not verify rooting of Brazilian Cherry semiwoody cuttings treated with IBA at a maximum dose of 4,000 mg L^{-1}. The same was reported by Hössel et al. (2012), who concluded that concentrations of IBA up to 4,000 mg L^{-1} and up to 200 mg L^{-1} of benzylaminopurine (BAP) did not stimulate the formation of adventitious rhizogenesis in Brazilian Cherry cuttings.

On the other hand, Lattuada (2008), using softwood and semiwoody cuttings with a diameter of 1 to 2 mm and 5 cm long, with two opposing leaves, achieved a survival rate of 77% for softwood and 91% for semiwoody. This author also reported rooting percentage of 26% and 4% for softwood and semiwoody cuttings, respectively.

Regarding the time of year to carry out cuttings, Lattuada (2008) reported that the rooting percentages were higher in the months of June and August, with an average of 50%. Regarding to growth regulators, there was no effect of the application of IBA to 4000 mg L^{-1} at any time tested (June, April and August).

Lattuada (2008), Peña et al. (2015) and Lattuada (2010) reported the use of minicutting. These were obtained after the pruning of the mother plants and when the new branches had a length of 5 cm. These were placed to rooting in an intermittent nebulization chamber, and the rooting rate ranged from 16.5% to 43.2%, depending on the time of collection (LATTUADA, 2008). Peña et al. (2015) used a similar procedure, with the plants being pruned, leaving branches 5 cm in length with two pairs of leaves, and new shoots collected when they were 4 cm long, maintaining a pair of whole apical leaves. The minicuttings were treated with IBA and placed in an intermittent mist chamber. The percentage of survival minicuttings varied according to the collection season (3.8% to 79.7%), but the rooting percentage was very low (0.0% to 1.9%), even with the use of IBA, which did not provide improvement in these parameters.

Hössel (2016), testing two minicuttings lengths (6 and 8 cm) of young plants (2 years), applying three concentrations of IBA (0, 3,000 and 6,000 mg L^{-1}), collected in six months (February, April, June, August, October and December), obtained a mean of rhizogenesis varying from 0.77% to 70%, and in four interactions the results were superior to 50%, and the larger one, with 70.71%, was obtained in the minicuttings of 6 cm, applying 3,000 mg L^{-1} in June (Figure 5). This demonstrates the potential of the minicutting technique for production of Brazilian Cherry new plants.

What can be observed in both studies (LATTUADA et al., 2010; PEÑA et al., 2015; HÖSSEL, 2016) that the rhizogenesis process was formed by young materials, and the same result did not exist with adult material and, in the case of the last two, success with adoption of the minicuttings, which up to the present should be recommended for use.

Similar to grafting, the practice of cutting presents a great variation of results, which may be related to the genotypes, to the ontogeny of the material used and to the time of the year, since the use of IBA did not show improvement in survival results and the cuttings' rooting.

Figure 5 - Brazilian Cherry minicuttings in rhizogenesis process using commercial substrate (A) and rooted minicuttings. (Photo: Hössel, C)

MICROPROPAGATION

Some different techniques in micropropagation were reported with Brazilian Cherry propagation (Table 2).

In vitro germination of Brazilian Cherry seeds was studied by Griff (2006) and Silva et al. (2014). Griff (2006) placed seeds of Zill Dark cultivar to germinate on agar (8 g L^{-1}). The seeds remained in the dark for 3 weeks, and after this period they were transferred to light conditions, and between 7 and 14 days, 100% of the seeds germinated. After two weeks, 2 mL of ½ WPM medium were added to the tubes. This procedure was repeated every four weeks. This author reported that after 10 weeks, 50% of the seedlings sprouted shoots and produced adventitious roots. Silva et al. (2014) reported that *in vitro* germination and propagation of apical meristems and nodal segments with ½ Murashige and Skoog (1962) medium, supplemented with low concentrations of growth regulators, are an alternative for the mass production of healthy Brazilian Cherry materials.

Uematsu et al. (1999) reported that newly mature branches when used as a source of explants and placed in Murashige and Skoog (1962) culture medium, supplemented with 0.5 mg L^{-1} of BAP, were suitable for shoots regeneration and proliferation. Sprouting elongation, induction and root elongation were performed by transplanting to 1/2 MSHF medium, with a rooting percentage of 85.7%. Lattuada (2010) reported rooting percentages between 15% and 25% and sprouts from 24% to 67% when using bactericidal solutions in explants, establishing initially through McCown's Wood Plant Medium (WPM) with 0.2 mg L^{-1} naphthalene acetic acid (NAA) and subculturing them in medium supplemented with 0.2 mg L^{-1} BAP. According to this author, complete regeneration of the seedling can be obtained, and acclimatization can be performed in an intermittent nebulization chamber.

Thus, the micropropagation of Brazilian Cherry is possible, with an option for the production of healthy plants and in large quantities.

OTHER ASEXUAL PROPAGATION TECHNIQUES

There are reports in the literature of using other techniques of asexual propagation in Brazilian Cherry. Morton (1987) reported that air layering is used successfully in India. In the same way, the plunging and also the suckers from the mother plants can be used for the production of new plants.

Even with these reports, there is still a lack of information regarding the use of other propagation techniques of Brazilian Cherry, and therefore, more studies are needed on this subject so important for the establishment of this fruit species as a commercial alternative to national and international fruit growing.

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