

Vegetative propagation of rootstocks and budding of 'Iratí' Japanese plum in recently rooted softwood cuttings

Propagação vegetativa de porta-enxertos e enxertia da ameixeira 'Iratí' em estacas herbáceas recém-enraizadas

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ABSTRACT

In Brazil, nursery plum trees are traditionally produced by interspecific budding, where the rootstock is propagated by peach seeds often obtained from the waste of peach industrialization, which promotes heterogeneity among rootstocks. In addition, the conventional system of nursery plum tree production under field conditions requires approximately 18 months, from taking seeds to selling grafted trees. This research has aimed to assess the technical feasibility of adventitious root formation in the softwood cuttings of six cultivars of *Prunus* spp., as well as the budding of 'Iratí' plum in the recently rooted softwood cuttings developed in a greenhouse, in order to reduce the time required to produce nursery plum trees with cloned rootstocks. Two tests were carried out in a greenhouse involving the rootstock propagation step (1) and the 'Iratí' plum budding on the recently rooted cuttings (2). Under the experimental conditions adopted, we have concluded that it is technically feasible to propagate the cultivars of *Prunus* spp. under an intermittent mist system with 22 cm-long softwood cuttings. The cultivars 'Genovesa', 'Marianna 2624', and 'Myrobalan 29C' showed good propagation ability with high percentage of live rooted cuttings (>90%) and low mortality in acclimation ($\leq 5.0\%$). On the other hand, the budding of the 'Iratí' plum by the chip budding method made in April in the original rootstock cutting showed low percentages of success (between 17.1% and 31.4%) and the beginning of scion growth was only observed at the end of winter. Considering the time necessary for the softwood cutting to root and the satisfactory growth of the scion for planting the nursery trees at the appropriate time (winter), we could not produce budded nursery trees of 'Iratí' plum in less than 12 months, counting from the cuttings made.

KEYWORDS: nursery tree production; adventitious rooting; intermittent mist system; *Prunus salicina*.

RESUMO

No Brasil, as mudas de ameixeira são tradicionalmente produzidas por enxertia interespecífica, sendo o porta-enxerto propagado a partir de sementes de pessegueiro, muitas vezes obtidas do resíduo da industrialização do pêssego, o que promove heterogeneidade entre os porta-enxertos. Além disso, o sistema convencional de produção de mudas de ameixeira em condições de campo demanda em torno de 18 meses, desde a obtenção dos caroços à comercialização das mudas enxertadas. O objetivo do presente trabalho foi avaliar a viabilidade técnica do enraizamento adventício de estacas herbáceas de seis cultivares de *Prunus* spp., bem como a realização da enxertia da ameixeira 'Iratí' nas estacas recém-enraizadas em casa de vegetação, visando reduzir o tempo necessário para produzir mudas com porta-enxertos clonados. Dois experimentos foram conduzidos em casa de vegetação, envolvendo a fase de propagação do porta-enxerto (1) e a fase da enxertia da ameixeira 'Iratí' nas estacas recém-enraizadas (2). Nas condições experimentais adotadas, conclui-se que é tecnicamente viável a propagação vegetativa de cultivares de *Prunus* spp. sob nebulização intermitente, utilizando-se estacas herbáceas com 22 cm de comprimento. As cultivares Genovesa, Marianna 2624 e Myrobalan 29C apresentam boa capacidade de propagação, com alta porcentagem de estacas enraizadas vivas (>90%) e baixa mortalidade na aclimação ($\leq 5,0\%$). A enxertia de "borbulhia de escudo com lenho" da ameixeira 'Iratí', realizada em abril na estaca original do porta-enxerto, apresenta baixas porcentagens de pegamento (entre 17,1% e 31,4%) e o início do crescimento dos enxertos só é observado no final do inverno. Considerando os períodos necessários ao enraizamento da estaca herbácea e ao crescimento satisfatório do enxerto, para o plantio da muda na época adequada (inverno), não é possível produzir mudas enxertadas da ameixeira 'Iratí' em tempo inferior a 12 meses, contado a partir da estaquia.

PALAVRAS-CHAVE: produção de mudas; enraizamento adventício; câmara de nebulização intermitente; *Prunus salicina*.

INTRODUCTION

On the world stage, China had the highest production of Japanese plums in 2020 (6,465,219 tons), while in South America, Chile stood out (416,215 tons). In 2020, Brazil imported 12,222 tons of the fruit (FAO 2022), while Ceagesp-SP sold 19,615 tons, which shows the country's dependence on the product's imports, as well as the opportunity to increase production to meet the domestic demand (AGRIANUAL 2022). Because of the high chilling requirement, the European plum (*Prunus domestica* L.) does not have a significant cultivated area in the country, and the Japanese plum (*Prunus salicina* L.) stands out, which had 3,834 hectares in 2017, of which more than 60% were located in the states of Rio Grande do Sul and Santa Catarina (IBGE 2017). In the state of Santa Catarina, Japanese plum cultivars 'Fortune', 'Letícia', 'SCS438 Zafira', 'SCS428 Oeste', 'Reubennel', 'Harry Pickstone', 'Gulf Blaze', 'Santa Rosa', and 'Poli Rosa' are the ones that best adapt to the climatic conditions of the state (DALBÓ & BRUNA 2021).

Although the Japanese plum culture has economic importance and expansion potential in the South and Southeast of the country, the nursery trees are traditionally produced through interspecific grafting using the peach tree (*Prunus persica*) as a rootstock. Unfortunately, many nurseries in southern Brazil still do not have their own mother plant for rootstocks, so they use the waste from the peach canning industries to obtain the seeds, which promotes non-uniformity among the plants and lack of genetic identity for the rootstock (MAYER et al. 2017). In addition, to justify interspecific grafting its technical effectiveness must be proven in relation to monospecific grafting, and a rootstock tolerant to soil adversities must also be used, which have not been properly proven for the combinations of Japanese plum on peach tree in Brazilian growing conditions.

In Europe, rootstocks for stone fruits have been selected aiming at resistance to root asphyxia and alkaline soils, which are the plants' main adversities (MORENO et al. 1995). In addition to abiotic factors, rootstocks tolerant to root-knot (*Meloidogyne* spp.) and lesion nematode genera (*Pratylenchus vulnus*) have been selected (PINOCHET et al. 1996); there are also studies that demonstrate the effect of rootstock on the severity of leaf scorch (*Xylella fastidiosa*) on the scion (KRUGNER & LEDBETTER 2016), this being a disease of great importance for the plum crop in southern Brazil (DUCROQUET & DALBÓ 2007). In view of the graft compatibility (REIG et al. 2019) and the possibility of hybridization between some species of the *Prunus* genus (GRADZIEL 2003), there are already several rootstocks involving other species, such as 'Adesoto 101' (*P. insititia*), 'Myrobalan 29C' (*P. cerasifera*), in addition to interspecific hybrids, such as 'Julior' (*P. insititia* x *P. domestica*), 'Marianna 2624' (*P. cerasifera* x *P. munsoniana*), and 'Ishtara' [(*P. cerasifera* x *P. salicina*) x (*P. cerasifera* x *P. persica*)], being them commercially used for plum in several countries (MORENO et al. 1995, SOTTILE et al. 2012). However, in several countries, including Brazil, Japanese plum grafting is still used on peach trees, mainly because of the availability of seeds, ease of germination, and grafting compatibility.

The rootstock has an effect on fruit quality (RADOVIC et al. 2020, BENDER et al. 2021), productivity, vigor, leaf nutrient contents (REIG et al. 2018a, MAYER et al. 2018), soil waterlogging tolerance (GUERRA et al. 1992, RUBIO-CABETAS et al. 2018), adaptation to heavy soils (REIG et al. 2018b), and nematode resistance (PINOCHET et al. 1999, PAULA et al. 2011). Considering the importance of preserving the genetic identity of the rootstock, which is ensured by an efficient method of vegetative propagation, and that the rooting capacity varies according to the species and cultivar (STANICA 2007), it is essential to use a rootstock that is easy to propagate to enable the production of uniform nursery trees on a large scale (OLIVEIRA et al. 2018).

Therefore, the ease of adventitious rooting (rooting percentage and root quality) is one of the characteristics desired in a good rootstock, in addition to its adaptation to soil and climate conditions, grafting compatibility with the scion cultivar, improvement of scion performance, fruit quality, and increased production (REIG et al. 2019). The formation of adventitious roots is the result of the interaction between endogenous factors that act as inhibitors or inducers (TSAFOUROS et al. 2019). The dose of auxin applied changes the hormonal balance and influences the number of roots grown (RIBAS et al. 2007, JOHNSON et al. 2020); however, its effect on tissue can be inactivated by oxidation or conjugation, which can be caused by peroxidase enzymes and amino acids, respectively, and which are present in different concentrations according to the genotype and age of the tissue (GASPAR et al. 1992, RODRIGUES et al. 2002, OSTERC et al. 2016).

In the several studies carried out in southern Brazil, the best results for adventitious rooting of *Prunus* spp. have been obtained with softwood cuttings under intermittent misting (DUTRA et al. 2002, RADMANN et al. 2014, ROSA et al. 2017, MAYER et al. 2020, MAYER et al. 2021, LACKMAN et al. 2022). However, the time required to complete the formation of nursery trees budded onto clonal rootstocks has been

practically the same (18 months) as the time required in the conventional system (propagation of rootstocks by seeds in a nursery in the field), as the stem of the clonal rootstock needs to reach an adequate diameter (8 mm or more) to enable budding on this stem (REIS et al. 2010). Thus, as an alternative to reduce the production time of a grafted nursery tree, it is necessary to implement a budding method on the original cutting of the rootstock.

Some grafting or budding methods have been successfully used in nursery tree production systems to reduce the time in the nursery stage, such as cleft grafting for grape vines (*Vitis* spp.) (REGINA 2002, MAROLI et al. 2014) and simultaneous grafting with softwood cuttings and hypocotyl grafting in passion fruit (CHAVES et al. 2004, CAVICHIOLI et al. 2009). In peach, cleft grafting, together with hardwood cuttings, is limited by the low rooting percentages (MIRANDA et al. 2004), while in omega-type grafting and cleft grafting together with softwood cuttings under a mist system, there was no effect of the type of graft when protecting the graft with a transparent plastic bag (SILVA et al. 2014). Although the protection of the graft with plastic bags promotes better grafting success (budding or cleft) in newly rooted softwood cuttings (NACHTIGAL 1999), the percentages of grafting success are still low.

The objective of this research has been to assess the technical feasibility of adventitious rooting of softwood cuttings of six cultivars of *Prunus* spp., as well as the budding of 'Irtai' Japanese plum on newly rooted cuttings, in order to reduce the time required to produce nursery trees with cloned rootstocks.

MATERIAL AND METHODS

This research consisted of two experiments, as described below:

Adventitious rooting of softwood cuttings of *Prunus* spp. under intermittent mist system

For this experiment, six cultivars of *Prunus* spp. were used, as described in Table 1. The 12-year-old mother plants kept in the "Prunus Rootstock Collection", from Embrapa Clima Temperado (Pelotas/RS/Brazil), were managed with drastic pruning. To this end, at the beginning of August 2020, all the structural branches of the plants were pruned with a hand saw or battery pruning shears at approximately 1 to 1.2 m above the ground, aiming at the growth of vigorous softwood shoots with hormonal balance favorable to adventitious rooting (MAYER et al. 2020).

Table 1. Cultivars of *Prunus* spp. tested in the vegetative propagation experiment by softwood cutting. Embrapa Clima Temperado, Pelotas/RS/Brazil, 2022.

| Rootstock | Species |
|---------------|---|
| Genovesa | <i>P. salicina</i> |
| Marianna 2624 | <i>P. cerasifera</i> x <i>P. munsoniana</i> |
| Myrobalan 29C | <i>P. cerasifera</i> |
| Santa Rosa | <i>P. salicina</i> |
| Okinawa | <i>P. persica</i> |
| Tsukuba-2 | <i>P. persica</i> |

On January 21, 2021, softwood shoots were collected from the mother plants (Figure 1A) and were immediately taken to a greenhouse equipped with an intermittent mist system to keep them hydrated. Cuttings measuring 22 cm in length were prepared (Figure 1B) by performing a transversal and bevel basal cut at the apical end, in addition to removing the leaves located at the two basal nodes. The remaining leaves, located in the distal $\frac{3}{4}$ of the cuttings, were cut in half to reduce transpiration and facilitate storage in the propagation bed.

The base of the cuttings (3 cm) was treated with a hydroalcoholic solution of indolebutyric acid at 3,000 mg L⁻¹ for five seconds (Figure 1C), and they were placed in perforated plastic boxes (46 x 30 x 10 cm) containing vermiculite medium as rooting substrate. The cuttings were kept under intermittent misting (Figure 1D), which was programmed to be activated for 15 seconds every five minutes, between 7 AM and 8 PM, being turned off between 8 PM and 7 AM.

Five days before the assessments, intermittent misting was turned off in order to promote the hardening of the cuttings and facilitate the assessments. Fifty-five days after cutting, the cuttings were carefully removed from the vermiculite, immersed in water for cleaning, and the following variables were determined: 1) percentage of dead cuttings, which did not grow any roots; 2) percentage of dead rooted cuttings, being those that grew at least one root, but died; 3) percentage of live rooted cuttings, which had one or more roots; 4) number of roots per cutting, determined by manual counting on live rooted cuttings; 5) root length, measured with a ruler in the three largest roots of each live rooted cutting, expressed in cm.

Subsequently, the cuttings classified as live rooted, from each treatment and repetition, were classified as: 6) percentage of rooted cuttings suitable for transplanting, being considered as those with at least four roots satisfactorily distributed around the base of the cutting, which were transplanted into JKS[®] citrus pots (plastic pots, 15 cm (width) x 15 cm (length) at the top, with a capacity of 3.78 L) for establishment of the experiment 2; 7) rooted cuttings unsuitable for transplantation, considered as those with three or fewer roots and/or with inadequate distribution around the base of the cuttings, which were discarded. Prior to the removal of the cuttings from the vermiculite, the following were also assessed: 8) percentage of cuttings with an original leaf, classified as those that kept at least one original leaf; 9) percentage of sprouted cuttings, in which those cuttings with at least one visible sprout were counted.

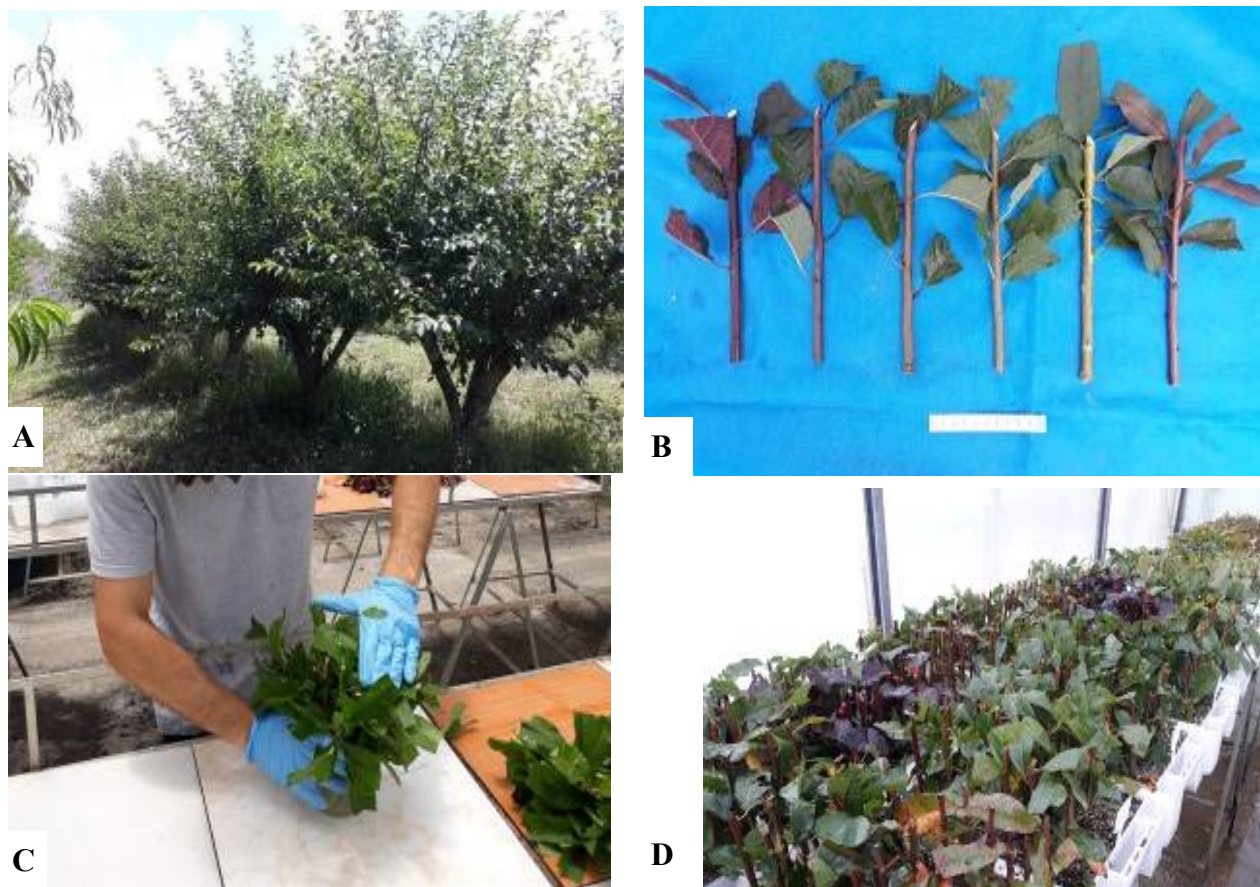


Figure 1. A) Mother plants of the rootstock cultivar 'Marianna 2624', managed with drastic pruning in winter, ready to supply softwood shoots; B) Prepared 22 cm-long softwood cuttings (from left to right: 'Genovesa', 'Marianna 2624', 'Myrobalan 29C', 'Santa Rosa', 'Okinawa', and 'Tsukuba-2'); C) Treatment of the cutting base with indolebutyric acid for five seconds; D) Experiment carried out with softwood cuttings under intermittent mist system for 55 days. Photos: Newton Alex Mayer.

After rooting assessment, cuttings classified as suitable were transplanted to the plastic pots with commercial substrate Turfa Fértil[®] (SSP Hortalíça CA). The substrate, according to the manufacturer's specifications, is composed of peat (70% v/v) and carbonized rice hull (30% v/v), with the addition of N (0.04%), P₂O₅ (0.04%), K₂O (0.05%), and calcitic limestone (1.5%). It has the following characteristics: electrical conductivity: 0.7 mS/cm ± 0.3; dry basis density: 260 kg/m³; pH: 5.8 ± 0.5; relative humidity: 55%; water holding capacity: 60%. Prior to filling the pots, the substrate was enriched with fertilizer at a dose of 4 g L⁻¹ of the following mixture (1:1:1 v/v): Osmocote[®] (N= 15%; P₂O₅= 9%; K₂O= 12%; Mg= 1%; S= 3%; B= 0.02%; Cu= 0.05%; Fe= 1%; Mn= 0,1%; Mo= 0,001%; Zn= 0.05%), Topmix[®] (N= 4%; P₂O₅= 14%; K₂O= 8%; Ca= 16%; S= 14%; B= 0.05%; Cu= 0.05%; Mn= 0.1%; Zn= 0.1%), and Calcinit[®] (N= 15.5% and Ca= 19%). The pots containing the newly transplanted cuttings were kept for 27 days in the acclimatization chamber (Figure 3A), on galvanized iron benches (1 m high), under an Aluminet[®] reflective screen to reduce the incidence of direct radiation on the plants, inside an agricultural greenhouse with no temperature and relative humidity control, with a polyethylene roof and sides with anti-aphid screen. The acclimatization chamber is equipped with an aerial irrigation system programmed to be activated for two minutes every four hours. After

the acclimatization phase was completed, the pots with the rootstocks were transferred to another agricultural greenhouse (growth stage), also on galvanized iron benches (1 m high) but equipped with a localized drip fertigation system. The growth fertilization, applied via fertigation, consisted of the addition, once a week, in a 500 L box for irrigation, of the following fertilizers: 1) 50 L of NPK nutrient solution [0.81 kg of potassium phosphate monobasic (KH_2PO_4) + 1.52 kg of potassium nitrate (KNO_3) + Ca (4.5 kg of Calcinit®)]; 2) 1 L of the solution containing 265 g of magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$); 3) 100 ml of solution with YARA Rexolin® micronutrients at 10%; 4) 50 mL of 1% Copper (Cu) and Zinc (Zn) nutrient solution.

After 38 days of transplanting the cuttings to the pots (17/Mar/2021), the mortality of the cuttings in the acclimatization stage was evaluated, expressed as a percentage (%). The variables expressed in percentage were transformed to arcsine $\sqrt{x/100}$, where x is the value of the variable. The other variables did not undergo transformation.

The experimental design used was completely randomized with six treatments (cultivars) (Table 1) and four replications of 15 cuttings. Analysis of variance was performed using the F-test (1 and 5% probability) and the comparison of means was performed using Tukey test (1 and 5% probability) using the RStudio software.

Budding of 'Iratí' Japanese plum on newly rooted cuttings

After 38 days of transplanting the rooted cuttings to the pots, 'Iratí' Japanese plum was budded on the original cuttings of the rootstocks, on 23/Apr/2021. Bud shoots were collected in a semi-hardwood stage of seven-year-old 'Iratí' plants, kept in the field without irrigation. Manual budding was performed by a single trained grafter, using the chip budding method (HARTMANN et al. 2002, PEREIRA et al. 2002), being it made between 3 to 5 cm below the distal end of the original cutting of the rootstock (Figure 3B).

After the grafting, any shoots of the rootstock located above the grafting point were manually broken to force budding. When the scions reached a length greater than 10 cm, a bevel cut of the rootstock was performed immediately above the growth of the scion, in this way removing the remaining aerial part of the rootstock. Fertigation was performed twice a day (2 minutes each) using an automatic drip system. Considering the plant losses that occurred during the acclimatization stage of the rooted cuttings, this experiment consisted of only three treatments (rootstocks 'Genovesa', 'Marianna 2624', and 'Myrobalan 29C') arranged in a completely randomized design with seven replications of five plants per plot.

After 199 days of grafting (on 08/Nov/2021), the following variables were evaluated: 1) percentage of successful grafts; 2) scion length, expressed in cm. The percentage data were transformed to arcsine $\sqrt{x/100}$, where x is the value of the variable. Analysis of variance was performed using the F-test (1 and 5% probability) and the comparison of means was performed using Tukey test (1 and 5% probability) using the RStudio software.

RESULTS AND DISCUSSION

Adventitious rooting of softwood cuttings of *Prunus* spp. under intermittent mist system

Treatments 'Genovesa', 'Marianna 2624', and 'Myrobalan 29C' (Figures 2A, 2B, and 2C, respectively) showed greater retention of the original leaves, as well as greater sprouting in the cuttings, in this way providing a greater source of energy for the formation of the root system (HARTMANN et al. 2002) and producing a large number of roots with good distribution around the cuttings. The higher mortality of rootless cuttings in treatment 'Tsukuba-2' (Figure 2F) is caused by the lower growth of adventitious rooting because of the lower dedifferentiation capacity in the tissues of this cultivar, considering that the optimal dose of IAA varies according to the cultivar (TSIPOURIDIS et al. 2006, TIMM et al. 2015).

The percentage of dead cuttings was significantly lower in the treatments 'Genovesa', 'Marianna 2624', and 'Myrobalan 29C' compared to 'Tsukuba-2' (Table 2). The viability of cuttings depends on the formation of adventitious roots (JOHNSON et al. 2020, MAYER et al. 2020), in addition to the presence of leaves on the cutting. In view of the high percentage (40%) of dead cuttings in the cultivar Tsukuba-2, this cultivar proved to be more recalcitrant to tissue dedifferentiation than the other cultivars, being thus less responsive to the exogenous application of auxin (OSTERC & STAMPAR 2015).

The percentage of dead rooted cuttings was low for all cultivars, ranging from 1.7 to 10% (Table 2), which indicates that the substrate used has adequate drainage, in this way avoiding pore saturation and supplying the roots with oxygen (PIMENTEL et al. 2014). Water deficit also causes plant death (JIMÉNEZ et al. 2013, GU et al. 2021); however, medium grade vermiculite presents satisfactory physical characteristics for the rooting of cuttings (CARDOSO et al. 2011, YAMAMOTO et al. 2013). Another factor that reduces the mortality of already rooted cuttings is the high relative humidity, as this reduces transpiration and keeps the leaves photosynthetically active (STEFANCIC et al. 2007, TETSUMURA et al. 2017).

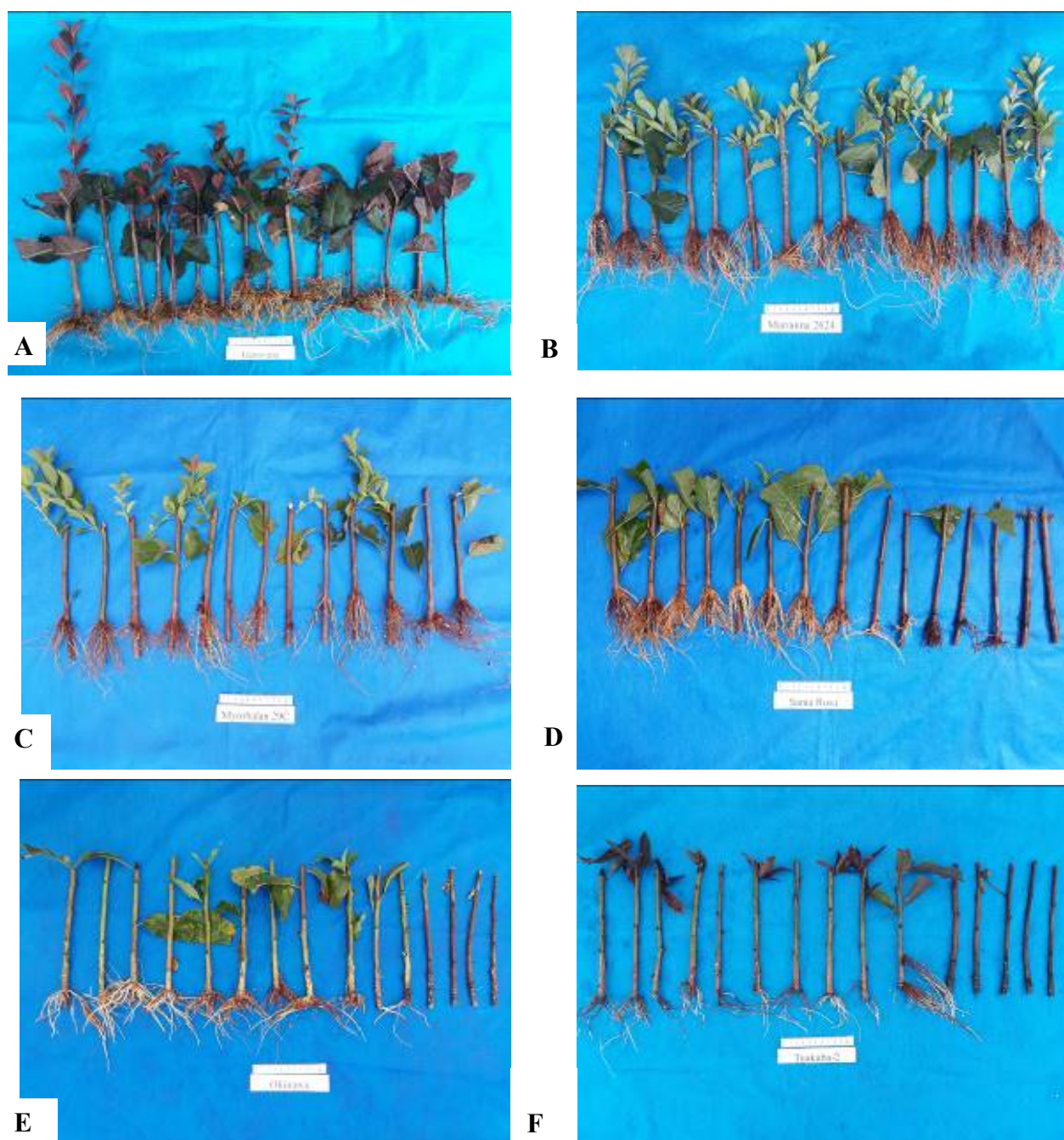


Figure 2. Softwood cuttings after a 55-day rooting period in medium grade vermiculite under intermittent misting, illustrating the result obtained in some of the treatments: A) Genovesa; B) Marianna 2624; C) Myrobalan 29C; D) Santa Rosa; E) Okinawa, and F) Tsukuba-2. Photos: Newton Alex Mayer.

Table 2. Assessment of adventitious rooting of softwood cuttings of cultivars of *Prunus* spp. Percentages of dead unrooted cuttings, dead rooted cuttings, live rooted cuttings, suitable rooted cuttings, and unsuitable rooted cuttings for transplanting. Embrapa Clima Temperado, Pelotas/RS/Brazil, 2022.

| Rootstock | Dead cuttings (%) | Dead rooted cuttings (%) | Live rooted cuttings (%) | Suitable cuttings (%) | Unsuitable cuttings (%) |
|------------------------|-------------------|--------------------------|--------------------------|-----------------------|-------------------------|
| Genovesa | 5.0 b | 3.3 a | 91.7 a | 83.5 a | 16.5 a |
| Marianna 2624 | 0.0 b | 1.7 a | 98.3 a | 77.9 a | 22.1 a |
| Myrobalan 29C | 5.0 b | 1.7 a | 93.3 a | 75.6 a | 24.4 a |
| Santa Rosa | 16.7 b | 10.0 a | 73.3 ab | 75.9 a | 24.1 a |
| Okinawa | 16.7 b | 5.0 a | 78.3 a | 67.3 a | 32.7 a |
| Tsukuba-2 | 40.0 a | 10.0 a | 50.0 b | 56.3 a | 43.7 a |
| F _{rootstock} | 11.50** | 1.59 ^{ns} | 8.15** | 1.71 ^{ns} | 1.71 ^{ns} |
| CV (%) | 66.10 | 133.28 | 15.45 | 18.93 | 53.6 |

Means followed by distinct letters in the column differ from each other by Tukey test. * significant at 95% confidence; ** significant at 99% confidence; ^{ns} not significant.

The percentage of live rooted cuttings (Table 2) was influenced by the treatments, and 'Genovesa', 'Marianna 2624', and 'Myrobalan 29C' showed values greater than 90%, while for 'Tsukuba-2' this was only 50%. Thus, despite forming roots, the cultivar Tsukuba-2 showed high leaf abscission, thus leading to higher mortality than the other cultivars. As observed by MAYER et al. (2020), maintenance of the original leaves is essential for the adventitious rooting of cuttings under intermittent misting.

The cultivars studied did not show significant differences for the percentages of suitable and unsuitable cuttings for transplanting. The aptitude for transplanting reflects the quality of the root system, being visually classified by the distribution and number of roots in the cutting, which are influenced by the fertilization adopted in the mother plants (SZABÓ et al. 2014), adequate shoots collected at the appropriate time, and an adequate environment for adventitious rooting.

The percentage of cuttings with original leaves (Table 3) was influenced by the cultivars tested. The low percentage of original leaves (Table 3) in the cultivar Tsukuba-2 (5%) may have led to low rooting, since according to MINDELLO NETO & BALBINOT JUNIOR (2004) the maintenance of leaves in softwood cuttings of the peach cultivar Jubileu is essential for rooting. Leaf retention, an energy source organ, is essential for the viability of cuttings (TCHOUNDJEU et al. 2002, DICK & LEAKEY 2006); however, this retention depends on the maintenance of cell turgor (HAIDER et al. 2018), which resumes only after the rooting of the cuttings has begun (SMALLEY et al. 1991).

The percentage of sprouted cuttings was statistically influenced by the treatments (Table 3), and the cultivars Marianna 2624 and Myrobalan 29C showed higher values compared to 'Santa Rosa', 'Okinawa', and 'Tsukuba-2'. New shoots are initially energy drains; however, they transform into tissues that provide carbohydrates (sugars, mainly sorbitol), which are translocated to the base of the cuttings for use in the maintenance and growth of the root system, as well as for generating energy for the absorption of nutrients to other parts of the plant (TORO et al. 2018). The higher percentage of sprouting in cultivars Marianna 2624 and Myrobalan 29C is a genotype effect, as observed by ROSA et al. (2017), in which work 96.6% of the cuttings of the cultivar Myrobalan 29C had shoots, while 'Julior' had only 13.3% (ROSA et al. 2017, MAYER et al. 2021).

Table 3. Assessments carried out in the adventitious rooting experiment of softwood cuttings of cultivars of *Prunus* spp. considering the percentage of cuttings with original leaf, sprouted cuttings, mortality during acclimatization, and number and length of roots per cutting. Embrapa Clima Temperado, Pelotas/RS/Brazil, 2022.

| Rootstock | Cuttings with original leaf (%) | Sprouted cuttings (%) | Number of roots per cutting | Root length (cm) | Mortality on acclimatization (%) |
|------------------------|---------------------------------|-----------------------|-----------------------------|------------------|----------------------------------|
| Genovesa | 91.7 a | 63.3 ab | 45.4 a | 12.9 ab | 5.0 b |
| Marianna 2624 | 66.7 ab | 96.7 a | 26.3 b | 13.4 a | 1.7 b |
| Myrobalan 29C | 50.0 b | 96.7 a | 24.8 b | 12.9 ab | 0.0 b |
| Santa Rosa | 61.7 ab | 48.3 b | 27.1 b | 10.0 c | 16.7 ab |
| Okinawa | 41.5 bc | 41.7 b | 15.5 b | 8.4 c | 16.7 ab |
| Tsukuba-2 | 5.0 c | 46.7 b | 15.8 b | 10.4 bc | 40.0 a |
| F _{rootstock} | 16.81** | 16.98** | 16.72** | 15.43** | 11.50** |
| CV (%) | 26.81 | 18.62 | 20.67 | 9.00 | 66.10 |

Means followed by distinct letters in the column differ from each other by Tukey test. * significant at 95% confidence; ** significant at 99% confidence; ^{ns} not significant.

The number of roots per cutting was significantly higher in the cultivar Genovesa, with 45.4 roots per cutting, while in the other cultivars the number of roots varied from 15.5 to 26.3 with no significant difference (Table 3). Despite the effect of the cultivar on the growth of roots, all genotypes showed a high capacity for dedifferentiation and subsequent formation of adventitious roots. The number of roots/cutting obtained by ROSA et al. (2017) in the cultivar Myrobalan 29C was only 11.08 roots/cutting without the exogenous application of auxin. The growth of adventitious roots also varies between *Prunus* species (TWORKOSKI & TAKEDA 2007), a characteristic that may be a result of the favorable anatomy of the cultivar and/or species (HARTMANN et al. 2002). The largest number of roots is desired to increase the area of contact with the substrate and to increase the water and nutrient absorption capacity of the new growing plant (SOLARI et al. 2006).

Root length was significantly higher in the cultivar Marianna 2624 when compared to the 'Okinawa'

cuttings (Table 3). The growth of adventitious roots depends on the carbohydrate and nutrient reserves of the mother plant; however, we cannot state that the differences occurred because of differences in the carbohydrate and nutrient reserves between the mother plants of the cultivars (TSAFOUROS et al. 2019), but because of the ability of morphogenetic differentiation of the tissues of this cultivar.

The percentage of mortality during acclimatization varied between cultivars (Table 3). The change from nebulization to acclimatization requires physiological adaptations such as better regulation of stomatal closure to avoid excessive water loss and maintain the carbohydrate synthesis necessary for the maintenance and growth of root and shoot tissues (MOING & GAUDILLÈRE 1992, JIMÉNEZ et al. 2013, TORO et al. 2018, GOMEZ et al. 2020). The growth of shoots and roots after transplanting varies according to the rootstock cultivar in *Prunus* spp., and this growth influences both water and nutrient absorption, as well as the survival of the plants (MONDRAGÓN-VALERO et al. 2017, OPAZO et al. 2020).

Therefore, in our study, we could clearly see the effect of the genotype on the capacity for rhizogenesis and stomatal control in the acclimatization stage, conditions that directly influence the success and quality of the rooted material, and several studies have already highlighted the greater capacity for rhizogenesis of plum cuttings, in relation to the peach tree, as well as their survival rate (KERSTEN et al. 1994, DUTRA & KERSTEN 1996, SCHWENGBER et al. 2002, TONIETTO et al. 2005).

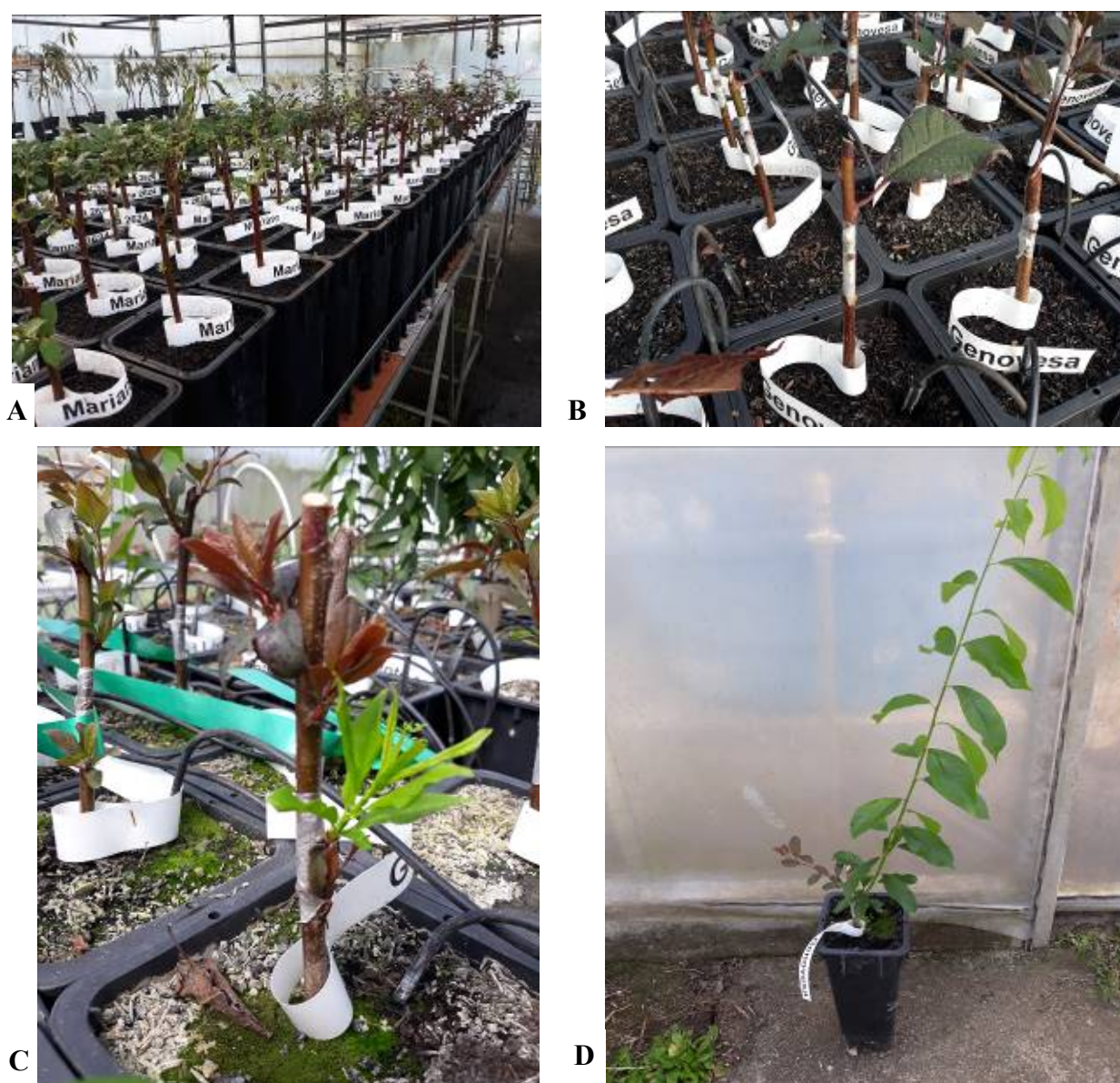


Figure 3. Process of budding of 'Irtati' Japanese plum on the original cutting of newly rooted *Prunus* spp. A) Rooted rootstocks recently transplanted to citrus pots in an acclimatization environment with aerial irrigation; B) 'Irtati' plum budded directly onto newly rooted cuttings of 'Genovesa'; C) Beginning of scion growth at 146 days after budding; D) Scion growth (cultivar 'Irtati') on 'Genovesa' rootstock in citrus pots at 199 days after budding. Photos: Newton Alex Mayer.

Budding of 'Irati' Japanese plum on newly rooted cuttings

After 199 days of the budding performed on the original cutting (Figure 3D), we found that bud taking success ranged from 17.1 to 31.4% with no significant difference between the three rootstocks analyzed. Regarding the growth of the scions, averages between 10.7 and 19.8 cm were observed with no significant effect of the rootstocks analyzed (Table 4). The data suggest that, although the percentages of budding success are low and well below the percentages commonly observed in nurseries (>80%), the budding technique on the newly rooted cutting may become a viable alternative, if it is improved. With the assessment of the length of the scions, carried out on 08/Nov/2021 (Table 4), it appears that the nursery trees showed little growth, that is, the nursery trees would not be suitable for planting at this time of year, considered as late.

Table 4. Percentage of budding success and scion growth of 'Irati' Japanese plum budded by the chip budding method on newly rooted cuttings of rootstocks. Embrapa Clima Temperado, Pelotas/RS/Brazil, 2022.

| Rootstock | Budding success (%) | Scion length (cm) |
|------------------------|---------------------|--------------------|
| Genovesa | 17.1 a | 19.7 a |
| Marianna 2624 | 31.4 a | 19.8 a |
| Myrobalan 29C | 25.0 a | 10.7 a |
| F _{rootstock} | 3.04 ^{ns} | 0.57 ^{ns} |
| CV (%) | 83.47 | 109.59 |

Means followed by distinct letters in the column differ from each other by Tukey test. * significant at 95% confidence; ** significant at 99% confidence; ^{ns} not significant.

The percentage of maximum success of the simultaneous grafting with the softwood cutting observed in *P. persica* by SILVA et al. (2014) was 24.16%, but with no significant difference between omega and full cleft grafting, while TOMAZ et al. (2014) obtained 72% in November and 39% in May with budding on rooted cuttings. According to NACHTIGAL (1999), the type of grafting and the protection of the scion help in the faster production of peach nursery trees, and up to 99% of success could be obtained in the cleft grafting performed on softwood cuttings after 30 days of rooting using protection of the scion region, carried out at the time of rootstock transplantation in November in the state of São Paulo, Brazil.

The difference in grafting success obtained by NACHTIGAL (1999), SILVA et al. (2014), and TOMAZ et al. (2014) may be related to environmental conditions, such as temperature and photoperiod, which are responsible for inducing dormancy in *Prunus* (HEIDE 2008). The decrease in temperatures and photoperiod induces physiological changes, such as an increase in ABA synthesis and a decrease in endogenous gibberellins (LANG et al. 1987, ZHANG et al. 2018). Tissue age also influences grafting success (TOMAZ et al. 2014), which depends on callus formation. Callogenesis in mature tissues of *Prunus avium* showed less proliferation than in softwood tissues, with a higher level of endogenous ABA, a growth-inhibiting hormone (OLEIRA & BROWNING 1993).

According to CARVALHO et al. (2010), Japanese plum shoots of the cultivar Poli Rosa collected in mid-May showed only 12.5% of bud sprouting rate, which indicates the establishment of dormancy, while from July onwards the sprouting rate reached 90%. In this way, it is possible that the buds of the cultivar Irati collected in April were already in a dormant stage that would lead to a low budding rate. The use of the dormancy index proposed by CARVALHO & BIASI (2012) should be used to identify the period in which dormancy starts for buds of the cultivar Irati for the collection of shoots with levels of endogenous hormones favorable to cell division and growth.

Considering that the cultivar Irati requires between 200 and 350 hours of cold to overcome dormancy, the storage of shoots in a cold chamber is an alternative to meet the chilling requirement in order to stimulate the sprouting of the scion inside the greenhouse during the winter (WREGG et al. 2005). Therefore, for future studies involving budding on cuttings, we propose that cuttings should be brought forward to November, also aiming to anticipate budding by two months, so that scion growth can occur in a period with still favorable photoperiod and temperature. Therefore, it is essential to properly carry out the treatments of the mother plants of the rootstocks (especially pruning, fertilization, irrigation, and phytosanitary control) to enable the production of softwood shoots with a minimum diameter of 10 mm in most of their extension (cutting production yield), in a period between 90 and 110 days after drastic pruning.

CONCLUSION

The vegetative propagation of cultivars of *Prunus* spp. is technically feasible under intermittent misting

using 22 cm long softwood cuttings.

The cultivars Genovesa, Marianna 2624, and Myrobalan 29C have good propagation ability with a high percentage of live rooted cuttings (>90%) and low mortality on acclimatization ($\leq 5.0\%$).

The budding of 'Iratí' Japanese plum by the chip budding method, made in April in the original cutting of the newly rooted rootstock, presents low percentages of success and the beginning of scion growth is only observed at the end of winter.

Considering the time necessary for the softwood cutting to root and the satisfactory growth of the scion for planting the nursery trees at the appropriate time (winter), we could not produce budded nursery trees of 'Iratí' Japanese plum in less than 12 months, counting from the cuttings made.

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