

Maintenance of leaves and indolebutyric acid in rooting of juvenile Japanese Flowering Cherry cuttings

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ABSTRACT

The Japanese Flowering Cherry (*Prunus serrulata* Lindl.), also known as Hill Cherry, is an exotic species native to the East Asia, widely used in landscaping, with an excellent ornamental potential due to its flowering and vegetative characteristics such as bright bark and showy foliage. Despite its commercial importance, there is no information in literature about the vegetative propagation of this species, a method that could facilitate the production of seedlings with selected characteristics and flowering anticipation. Thus, this study aimed to evaluate the effect of different concentrations of indolebutyric acid (IBA) to promote rooting of *Prunus serrulata* cuttings, with and without leaves, obtained from current year shoots, with the intent to subsidize the development of a propagation protocol for the species. The IBA concentrations used in both types of cuttings were 0, 1000, 2000 and 4000 mg L⁻¹. After 60 days in the greenhouse it was observed that the maintenance of leaves is essential to the viability of the technique for *Prunus serrulata*, as well as the application of IBA, to induce rooting, with a recommended concentration of 2000 mg L⁻¹.

Key words: current year shoots, ornamental potential, *Prunus serrulata*, stem cuttings, synthetic auxin, vegetative propagation

Manutenção das folhas e ácido indol butírico no enraizamento de estacas juvenis de cerejeira-do-Japão

RESUMO

A Cerejeira-do-Japão (*Prunus serrulata* Lindl.), também conhecida como cerejeira ornamental, é uma espécie exótica de origem no leste da Ásia, largamente utilizada no paisagismo, apresentando excelente potencial ornamental, por sua floração e características vegetativas, como casca brilhante e colorida e folhagem vistosa. Apesar de sua importância comercial não há, na literatura, informações sobre a propagação vegetativa desta espécie, o que poderia facilitar a obtenção de mudas com caracteres selecionados e antecipação do florescimento. Desta forma, objetivou-se avaliar o efeito de diferentes concentrações da auxina sintética ácido indol butírico (IBA), na promoção do enraizamento de estacas caulinares com e sem folhas, de brotações do ano de *Prunus serrulata*, visando subsidiar a elaboração de um protocolo de propagação da espécie. As concentrações de IBA utilizadas nos dois tipos de estaca, foram: 0, 1000, 2000 e 4000 mg L⁻¹. Após 60 dias em casa de vegetação verificou-se que a manutenção de folhas é essencial à viabilidade da técnica para *Prunus serrulata*, tal como a aplicação de IBA para promoção do enraizamento, sendo recomendada a concentração de 2000 mg L⁻¹.

Palavras-chave: brotação do ano, potencial ornamental, *Prunus serrulata*, estaquia caulinar, auxina sintética, propagação vegetativa

Introduction

The Japanese Flowering Cherry (*Prunus serrulata* Lindl. – Rosaceae family), also known as Hill Cherry, is an exotic species native to the East Asia, considered a temperate climate species. It is an arboreal plant, generally from 4 to 6 meters tall but sometimes reaching 15 meters in natural conditions. The trunk is cylindrical, shows simple alternate leaves with serrate margin (Lorenzi, 2003) and two petiole glands close to the leaf base, has white or pink flowers on short racemes (Mondin et al., 2010). This species grows at temperate climate, in cold and highland regions, like south and south eastern Brazil, presenting abundant flowering from July to September (Paiva et al., 2004; Mondin et al., 2010).

The commercial importance of this species is mainly due to its ornamental potential, it is widely used in landscaping for its flowerings and vegetative characteristics, like colored and shining bark and showy foliage in autumn (Seth, 2003). Despite all the described qualities, there is no information in literature about the vegetative propagation of this tree, a procedure that could facilitate the production of seedlings and flowering anticipation.

Macropropagation techniques are considered the most common, including cuttings and grafting methods. Grafting is the most used method for fruit plants of the Rosaceae family, however it presents technical difficulties being a delicate and time spending process (Hartmann et al., 2011). Therefore, cuttings method can be a good option, once the segments of the stock plants used are able to develop a complete new plant when submitted to favorable environmental conditions (Brondani et al., 2009; Beyl & Sharma, 2014).

However, one of the biggest problems related to cuttings is providing a viable propagation material, with good rooting performances and able to develop a new plant in field. Factors to be considered for the success of this technique are different for each species (Ferriani et al., 2010). For example, the maintenance of leaves can have relation with rooting process due to translocation of the metabolites produced by the stock plant towards the rooting region. Meanwhile, information about this factor is still very scarce for several species (Ruedell et al., 2013).

Likewise, the maturity of the plant, mainly in woody species, interferes on quality and speed of the roots formation, on growth rate, growth forms and on morphology of leaves (Wendling et al., 2014b). Some species also present a low rooting level due to low endogenous auxins concentrations, requiring the application of exogenous synthetic auxins to increase the induction of rooting (Pizzatto et al., 2011).

Thus, we aimed to assess the effect of the synthetic auxin indolebutyric acid (IBA), in different concentrations, applied to induce rooting in stem cuttings, with and without leaves, obtained from current-year shoots of *Prunus serrulata*, with the intention to support the elaboration of a propagation protocol for this species.

Material and Methods

Collection of the propagation material from *Prunus serrulata* stock plants was performed in the end of winter in the

municipality of Curitiba-PR, where the climate is temperate (cfb) according to Köppen classification, with an average temperature around 17 °C, being 23 °C the maximum and 13 °C the minimum, maintaining reduced temperatures until mid-spring. Relative humidity of the air averages 82% and rainfall is generally higher than 1300 mm.

The experiment was performed in a greenhouse with a controlled temperature of 24 °C ± 2 °C and 85% of relative humidity. Non-ligneous stem cuttings, obtained from current-year shoots of juvenile material, were prepared with 10 to 12 cm length and 3.48 mm average diameter. Two types of cuttings were prepared: the first with a bevel cut on the base and a straight cut above the last apical bud, maintaining two leaves reduced to half of the original size in the upper third of the cutting, and the second type with same characteristics but without leaves. Cuttings were submitted to phytosanitary treatment by immersion for 10 min in 0.5% sodium hypochlorite solution and then rinsed for another 10 min in running water.

Immediately afterward, the treatment with different concentrations of indolebutyric acid (IBA) was performed on the base of the cuttings. Treatments consisted in immersion of the bases for 10 s into a hydroalcoholic solution (50% v/v) with 0, 1000, 2000 and 4000 mg L⁻¹ IBA concentrations. Cuttings belonging to the control group (0 mg L⁻¹) did not receive the plant growth regulator treatment, they were simply immersed into distilled water and alcohol solution (50% v/v). Cuttings were then installed into 53 cm³ polypropylene pots with fine vermiculite and carbonized rice hull (1:1 em v/v).

After 60 days in the greenhouse, the following variables were evaluated: percentage of rooted cuttings (alive cuttings that presented roots at least 2 mm long); number of roots per cutting; length of the three longest roots per cutting; percentage of cuttings with callus (alive cuttings, without roots, with undifferentiated cells formation on the base); percentage of survival (alive cuttings, without roots or callus formation); percentage of mortality (cuttings with dead tissues); percentage of original leaves maintenance and percentage of new shoots.

Data were analyzed according to a completely randomized design, with a factorial arrangement 2 x 4 being 2 types of cuttings (with and without leaves) and 4 IBA concentrations, with 4 replications of 20 cuttings per each experimental unit, for a total of 640 cuttings. Data homogeneity was evaluated through Bartlett test and the averages were compared through the Tukey range test at 5% level of significance ($p > 0.05$).

Results and Discussion

The evaluations performed showed different reactions between cuttings with leaves and cuttings without leaves, for all the considered variables. Significant variations were observed between the results of cuttings with leaves for the different IBA concentrations applied.

Cuttings without leaves presented 100% mortality under all the IBA treatments applied, with a 17% presenting new shoots beginning to form. On the other hand, cuttings prepared with a pair of halved leaves presented only 1.87% mortality. Since mortality in cuttings without leaves occurred independently

from the IBA concentrations, the absence of leaves is almost certainly the decisive factor causing the high mortality observed.

Permanence of leaves in stem cuttings has been assessed in many works to represent an important factor determining both survival and rooting (Ferriani et al., 2008; Ferreira et al., 2014). Leaves produce carbohydrates and natural plant growth regulators, like endogenous auxins and rooting co-factors that are transferred to roots through the polar transport, increasing their concentrations on the base of the cutting and allowing better physiological conditions for roots induction (Hartmann et al., 2011).

As a result, in cuttings without leaves of *Prunus serrulata*, the rooting process may not have been fast enough, thus demanding metabolites that would have been provided by leaves, if present, and so leading to death of the cuttings. It is then possible to assume that not only the presence of leaves is important, but also the surface of the remaining leaves must be ideal to obtain a better rooting, and this is related to what seems to be equilibrium between photosynthesis and transpiration.

Bigger leaves present an increased water loss, reducing the photosynthesis rate and carbohydrates production, while very little leaves do not produce the necessary metabolite quantity to provide a good rooting. Definition of the most efficient surface of maintained leaves is necessary for each species (Beyl & Sharma, 2014).

The shoots observed in some of the cuttings without leaves, in the same way and when there is no rooting observed after a certain period of time, can become a worsening factor due to the stocked energy consumption for their formation, deviating this energy from the formation of roots where it would be required (Hartmann et al., 2011).

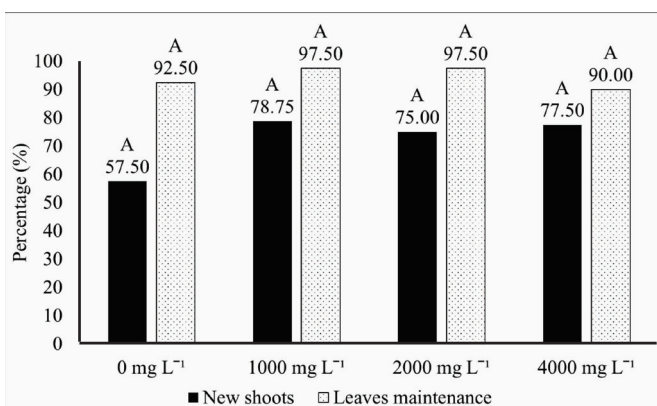
Together with the change of relation between source and drain, the new formed leaves could have contributed to water loss through transpiration, drying the tissues and leading to death of the cuttings, a process also worsened by the absence of roots.

About cutting with leaves, 72% of them presented new shoots, without statistical difference between the IBA concentrations, though the lower results were observed in the control group. Maintenance of leaves was also independent from treatments, reaching 94% of the results (Figure 1).

Despite the importance of leaves maintenance for root induction, results also demonstrated significant differences between the concentrations of IBA applied on cuttings with leaves, being the higher rooting percentages observed with treatments of 2000 and 4000 mg L⁻¹, with respectively 86.25 and 81.25% (Figure 2).

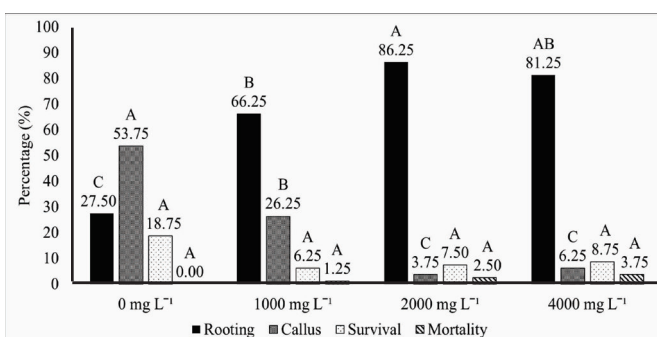
This slight reduction of the values observed with the 4000 mg L⁻¹ concentration, the higher applied, may have occurred due to the fact that the rooting induction, caused by the increase in concentration of the exogenous auxin, could have been close to its maximum, starting from which the inhibitory effects take place (Pop et al., 2011).

The highest rooting percentage observed, in turn, was significantly bigger than the one obtained from cuttings without IBA application, that reached a mere 27.5%, thus demonstrating the importance of the exogenous auxin application to produce clonal seedlings of *P. serrulata*.



Averages followed by the same letter in each variable do not differ between them according to Tukey range test at 5% level of significance

Figure 1. Averages of new shoots percentage and leaves maintenance percentage, in cuttings obtained from current-year shoots of *Prunus serrulata* juvenile material, prepared with leaves and submitted to treatments with different indolebutyric acid concentrations



Averages followed by the same letter in each variable do not differ between them according to Tukey range test at 5% level of significance

Figure 2. Averages of rooting, callus formation, survival and mortality percentages of cuttings obtained from current-year shoots of *Prunus serrulata* juvenile material, prepared with leaves and submitted to treatments with different indolebutyric acid concentrations

Since the concentrations of endogenous auxins are not always big enough to induce rooting, being still possibly degraded by IAA-oxidase enzymes, the application of synthetic auxins like IBA becomes important to help out this process, as was observed in this study, because they are less sensible to biological degradation (Lopes et al., 2011).

Meanwhile, the response of material to roots induction depends also on the physiological conditions of the stock plant that, in turn, can change depending on the season of the year (Ferreira et al., 2014; Wendling et al., 2014a). Thus, in case of new experiments taking in consideration the season of collection, rooting results of *P. serrulata* could be different.

Considering the number of roots per cutting, there was significant difference between IBA concentrations, with the higher number observed for the 4000 mg L⁻¹ concentration, with an average of 6.95 roots per cutting (Table 1), differing from the other concentrations. This variable is related to the adventitious roots emission potential in a certain period of time that is enhanced by the application of IBA, which is involved with cell division, promoting the anticipation of rootlets formation in treated cuttings (Hartmann et al., 2011) and increases the physiological quality of seedlings.

Table 1. Averages of the rooting percentage, number of roots per cutting and length of the three longest roots per cutting obtained from current-year shoots of *P. serrulata*, prepared with leaves and submitted to different indolebutyric acid concentrations

IBA (mg L ⁻¹)	Rooting (%)	Root number (cutting ⁻¹)	Length of roots (cm)
0	27.50 c	2.61 c	3.52 b
1000	66.25 b	4.31 b	6.39 a
2000	86.25 a	5.01 b	7.01 a
4000	81.25 ab	6.95 a	6.24 a

Averages followed by the same letter in column do not differ between them according to Tukey range test at 5% level of significance

The highest number of roots in the 4000 mg L⁻¹ concentration was frequently followed by formation of thinner roots than the ones formed with the 1000 and 2000 mg L⁻¹ concentrations. This behavior may be interesting for the plant once taken to field, improving its initial performance, because an increase of thin roots could mean a better absorption of water and nutrients from soil, making the plant more capable to withstand harsh environment conditions (Freitas et al., 2005; Severino et al., 2011).

Furthermore, longer roots may go deeper into soil, revealing a better ability to exploit the available resources, and providing a better fixation in the soil (Taiz & Zeiger, 2009; Hartmann et al., 2011). This last variable was similar for the three IBA concentrations, statistically differing from the control group, being 7.01 cm the highest value (2000 mg L⁻¹) and 3.52 cm the lowest value (0 mg L⁻¹).

Considering the formation of callus in cuttings (alive cuttings, without roots, with formation of undifferentiated mass of cells in the base), it was observed that the control group presented the highest percentage, followed by the 1000 mg L⁻¹ treatment. Treatments with 2000 and 4000 mg L⁻¹ presented the lowest callus percentages.

However, considering the percentage of rooted cuttings and at the same time with the presence of callus (data that were not statistically analyzed), it was observed that varied from 20% (corresponding to almost 70% of the rooted cuttings in the control group) to 41.25 and 46.25% (corresponding to almost 50% of rooted cuttings in 4000 and 2000 mg L⁻¹ IBA treatments), thus demonstrating that in the treatments with the best rooting, almost half of the cuttings presented also formation of callus (Figure 3).

The relationship between callus and roots was object of investigation in many studies, from which it is possible to understand that this relationship may change depending on species, with roots that can appear out of callus or directly from the stem tissue (Mayer et al., 2008; Stuepp et al., 2013). That way, the highest percentage of callus observed in cuttings with lowest rooting percentages may mean that permanence of cuttings in the greenhouse for a longer period could result in an increase of roots formation, because callus formation, in this case, is an indicator of favorable environmental conditions (Oliveira & Ribeiro, 2013).

Considering survival and mortality percentages, there was no statistical difference between the various IBA concentrations applied on cuttings with leaves, being both very small for all the concentrations. The low percentage of alive and dead cuttings, in all the treatments on cuttings with leaves, is related to the

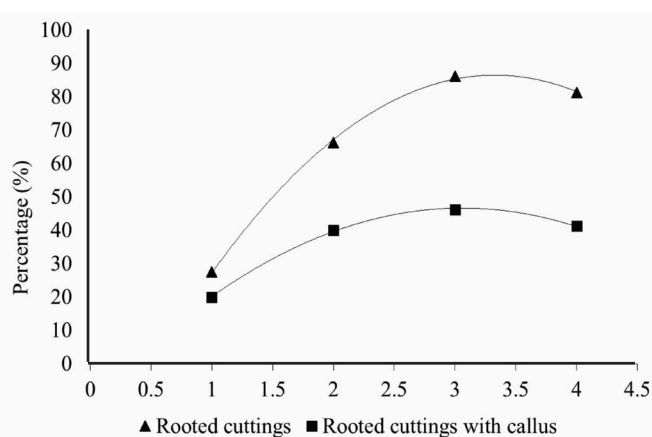


Figure 3. Trend lines of the averages of rooted cuttings and rooted cuttings with formation of callus, obtained from current-year shoots of *Prunus serrulata* juvenile material, prepared with leaves

high percentages of rooted cuttings and callus formation (when in the control group), thus demonstrating the good adaptation of this material to greenhouse and its high potential in roots formation.

Conclusion

Maintenance of leaves is fundamental for the viability of the stem cuttings propagation technique applied to current-year shoots of *Prunus serrulata*, given the conditions of the described work. Application of IBA is also necessary to induce rooting, being recommended in the concentration of 2000 mg L⁻¹.

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