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EX VITRO SYSTEM FOR *Acer palmatum* PLANTS PROPAGATION BY MINI-CUTTINGS TECHNIQUE

Keywords:
Adapted minicutting technique
Length of mini-cuttings
Mini-stumps shading
Ornamental plants cloning
Vegetative propagation

ABSTRACT: This study presents an efficient system with high productivity and quality for *Acer palmatum* propagation. We evaluated the efficiency of mini-cuttings technique, management of mini-stumps, production of shoots, rooting and root vigor of mini-cuttings on two seasons. The mini-stumps were planted in pots of two liters, put up in two environments (full sunlight area and shade house) and submitted to weekly fertigation. Over six collections, we evaluated the survival and productivity of clonal mini-garden (Experiment I). From these, we performed two experiments with mini-cuttings: Experiment II - mini-cuttings originated from two environments, with 8±1 cm, put to root in two seasons (Spring and Summer); Experiment III - mini-cuttings originated from full sunlight area, with 4±1, 6±1, 8±1, 10±1 cm, put to root in Summer. The mini-cuttings planting was made in plastic tubes of 55 cm³, filled with fine vermiculite and carbonized rice hulls (1:1 v/v), in a greenhouse with intermittent irrigation system. The high rooting (95.0%), mini-stumps survival (100%) and mini-cuttings production (217.5 mini-cuttings m^{-2} $month^{-1}$) in clonal mini-garden conducted in full sunlight area shown the technique viability for the species. Mini-cuttings of 8±1 cm favored rooting (96.3%). The mini-cuttings technique is viable for *Acer palmatum* propagation.

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SISTEMA EX VITRO DE PRODUÇÃO DE MUDAS DE *Acer palmatum* POR MINIESTAQUIA

Palavras chave:
Clonagem de plantas ornamentais
Comprimento de miniestacas
Miniestaquia adaptada
Propagação vegetativa
Sombreamento de minicepas

RESUMO: Um sistema de produção de mudas eficiente, com elevada produtividade e qualidade é apresentado neste estudo para *Acer palmatum*. Avaliou-se a eficiência da miniestaquia, manejo das minicepas e produção de brotos em duas estações no enraizamento e vigor radicial de miniestacas. As minicepas foram plantadas em vasos de dois litros, acondicionadas em dois ambientes (pleno sol e casa de sombra) e submetidas a fertirrigação semanal. Ao longo de seis coletas avaliou-se a sobrevivência e produtividade do minijardim clonal (Experimento I). A partir destes, realizou-se dois experimentos de miniestaquia: Experimento II- miniestacas de dois ambientes, com 8±1 cm, instaladas em duas estações do ano (primavera e verão); Experimento III- miniestacas de pleno sol, com 4±1, 6±1, 8±1, 10±1 cm, instaladas no verão. O plantio das miniestacas foi realizado em tubetes de 55 cm³, preenchidos com vermiculita de granulometria fina e casca de arroz carbonizada (1:1 v/v), acondicionados em casa de vegetação com sistema de irrigação intermitente. O elevado enraizamento (95,0%), sobrevivência de minicepas (100%) e produção de miniestacas (217,5 miniestacas m^{-2} $mês^{-1}$) em minijardim clonal conduzido a pleno sol mostram a viabilidade da técnica. Miniestacas de 8±1 cm (96,3%) favoreceram o enraizamento. A técnica de miniestaquia é viável para a espécie.

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INTRODUCTION

Acer palmatum Thunb. (Aceraceae), popularly known as japanese maple or simply as "acer", occurs naturally in Japan, Korea and China. Its commercial importance stems from its use as an ornamental tree, highlighting the characteristics of its reddish leaves (HUGHES et al., 2007), which vary according to genotype, cultural factors and environmental practices (JAKOPIC et al., 2007). It has wide use in subtropical regions of North America and Europe (COPINI et al., 2014), and lower expression in all temperate regions of the world (LI et al., 1992).

The long stratification periods needed for *Acer palmatum* seed germination (FARMER; CUNNINGHAM, 1981; TREMBLAY et al., 1996) justify the need for more detailed studies related to other forms of propagation. Thus, the development of a propagation system capable to produce a large number of standardized and classified plants is the major challenge for deciduous species (BRONDANI et al., 2010; STUEPP et al., 2015). These limitations have been discussed throughout the years, especially by the need to produce shoots on only a part of the year due to dormancy of these species.

Vegetative propagation is used worldwide in the propagation of tree species. This technique consists in asexually multiplying parts of plants, resulting in individuals genetically identical to the mother plant (HUSEN; PAL, 2007). Among the vegetative propagation techniques, the mini-cuttings technique has presented efficient results and has been relatively fast to obtain clones (MCMAHON et al., 2014).

The mini-cuttings technique is similar to micro-cuttings technique (BRONDANI et al., 2012) and, compared to cutting, has some advantages such as reduction in the size of the propagules, increase shoots productivity per area, better rooting, often without the need of plant regulators (WENDLING et al., 2010; STUEPP et al., 2015). It has been successfully used in the propagation of selected clones of different species (BRONDANI et al., 2010; WENDLING et al., 2010; STUEPP et al., 2016), applied with great frequency in the *Eucalyptus* propagation in Brazil (BRONDANI et al., 2012).

The use of mini-cuttings technique for ornamental plants propagation is less known, among other factors, due to the lower production area compared to the forest sector and the lack of technology adaptation to the conditions of small farmers included in this segment. No information is available in literature regarding the use of this technique in *Acer palmatum*, having a limitation in the plant production and consequently, the underutilization of this species in general. Therefore, we evaluate the efficiency of mini-cuttings technique and management of clonal mini-garden on the productivity of mini-cuttings, rooting and root vigor of *Acer palmatum*.

MATERIAL AND METHODS

The studies were conducted between September/2014 and March/2015 in the city of Curitiba (PR), Brazil (25°25'40 "S and 49°16'23" W). The region's clime is characterized as temperate (Cfb) by the Köppen classification, with relative humidity around 82% and rainfall generally higher than 1300 mm without a defined dry season. The average temperature during the experimental period varied between 15.8 °C (September) and 22.8 °C (January) and the cumulative rainfall of 59 mm (October) and 269 mm (September).

During the mini-cuttings collections, we evaluated the survival and productivity of a clonal mini-garden (Experiment I) and from these, we established two experiments: Experiment II - seasons and shading effect on mini-stumps; Experiment III - mini-cuttings length effect.

Experiment I - Clonal mini-garden survival and productivity

Acer palmatum plants produced by cuttings and kept in vases containing two liters of the commercial substrate coir and peat base (Figure 1a) formed the clonal mini-garden. Elapsed 90 days for

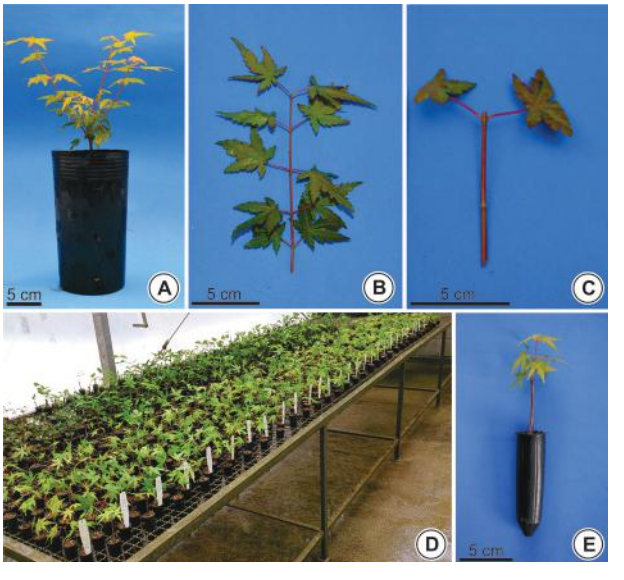

FIGURE 1 Schematic sequence for *Acer palmatum* mini-cuttings technique: general characterization of mini-stumps (a), collected sprout (b), prepared mini-cutting (c), mini-cutting planted in greenhouse (d) and general appearance of a plant formed by the technique (e).

plant adaptation, the sprouts apices were pruned (10 cm above the collaron) in order to homogenize the mini-stumps shoots.

The mini-stumps were arranged in 20 x 20 cm spacing (25 mini-stumps m^{-2}) in two environments. The first in shade house conditions, with 50% shade and irrigation by microsprinkler (three daily irrigations of 10 minutes and flow of 144 L $hour^{-1}$) and the second in full sunlight conditions, with irrigation by microsprinkler (three daily irrigations, 5 minutes duration and flow rate of 144 L $hour^{-1}$). In addition to irrigation, they received weekly fertigation with 25 mL of a nutrient solution composed by ammonium sulfate (4 $g \cdot L^{-1}$), triple superphosphate (4 $g \cdot L^{-1}$), potassium chloride (4 $g \cdot L^{-1}$) and FTE BR - 12 (1$g \cdot L^{-1}$).

The mini-cuttings were collected every 30 day, comprising three collections per season. Only shoots longer than 10 cm were used to prepare mini-cuttings. Mini-cuttings were put in polypropylene tubes with 55 cm^3 capacity, filled with fine-grained vermiculite and carbonized rice husk (1:1 v/v) in a heated greenhouse with intermittent mist (temperature of 24±2 °C and relative humidity of air greater than 80%).

The clonal mini garden was set up in a completely randomized design in split plot, with five replicates of five mini-stumps each.

Experiment II - Effect of the seasons and mini-stumps shading

Mini-cuttings were prepared with 8±1 cm and a mean diameter of 0.2±0.1 cm, leaving two leaves reduced to 50% of its original surface (Figure 1b). The experimental design was completely randomized with a 2x2 factorial arrangement [2 seasons (Spring and Summer) and 2 mini-cuttings sources (full sunlight and shade house)] and four repetitions of 20 mini-cuttings each.

Experiment III - Effect of mini-cuttings length

From shoots collected on mini-stumps established in full sunlight conditions, we prepared four types of mini-cuttings, with 4±1, 6±1, 8±1 and 10±1 cm in length and 0.2±0.1 cm in diameter, keeping two leaves reduced to 50% of its original surface. The experiment was installed in Spring/2014.

The experimental design was completely randomized, with four types of mini-cuttings (length) and four replicates of 20 mini-cuttings each.

Assessments

The clonal mini-garden was evaluated for six months (September/2014 to March/2015) comprising Spring (23/September to 21/December) and Summer (21/December to 21/March). The variables evaluated were: percentage of mini-stumps survival; mini-cuttings production mini-$stumps^{-1}$ $collection^{-1}$ (PPM) and production of mini-cuttings m^{-2} $month^{-1}$ (PPSM).

Regarding the mini-cuttings experiments, after 60 days in a greenhouse the following variables were evaluated: rooting percentage (live mini-cuttings that had roots of at least 2 mm in length); number of roots mini-$cutting^{-1}$; three largest length of roots mini-$cutting^{-1}$ (cm); percentage of mini-cuttings with callus (live mini-cuttings without roots, with a mass of undifferentiated cells at the base); survival percentage (live mini-cuttings that did not have root induction or callus formation); mortality rate (mini-cuttings that were with necrotic tissue); shoots emission (percentage of mini-cuttings with new shoots of at least 2 mm in length emission) and maintenance of mini-cuttings original leaves (percentage of mini-cuttings that kept the original leaves).

Data were analyzed by analysis of variance (ANOVA), the treatment variances were evaluated for homogeneity by Bartlett's test, and the variables with significant differences by the F test had their means compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

Unlike a traditional mini-garden (semi-hydroponic system in sand), the technique presented here is simplified. Fertigation has lower frequency and hence less salinization of the substrate, a common problem in the traditional systems (WENDLING et al., 2010; BRONDANI et al., 2012).

The survival of mini-stumps conducted in conditions of full sunlight (100%) indicates the technical efficiency and management of these, providing the production of quality sprouts for mini-cuttings for propagation of the species. On the other hand, the lower adaptation of mini-stumps under shadowing conditions is evident, based on the reduced survival recorded (76%) (Figure 2). The shading effects are also observed in the analysis of mini-cuttings productivity mini-$stump^{-1}$ $collection^{-1}$ (PPM), where the superiority of mini-stumps kept in full sunlight over all collections is verified.

The reduction in mini-stumps survival in shade house may be linked to the adaptive characteristics of the species. Plants naturally occurring in high irradiance environments (full sunlight) when placed under shade

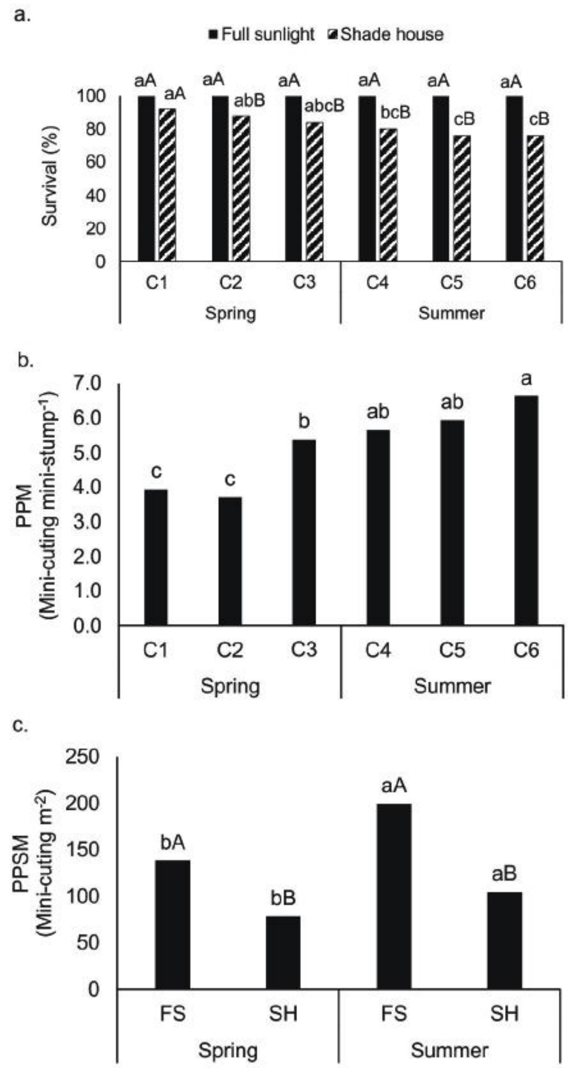

FIGURE 2 Means for (a) Survival of *Acer palmatum* mini-stumps in each environment; (b) average production of mini-cuttings mini-stump^{-1} collection^{-1} in both environments (PPM); (c) production of mini-cuttings m^{-2} month^{-1} (PPSM) in clonal mini-garden. Where: C1, C2, C3, C4, C5 and C6 are the collection 1, 2, 3, 4, 5 and 6, respectively; FS - full sunlight and SH - shade house. Means followed by the same lower case letter between seasons and capital between the environments do not differ by Tukey test at 5% probability.

conditions, tend to adapt (acclimatize) by morphological and anatomical changes such as thickening of the palisade parenchyma (CHENU; SCHOLES, 2015). This leaf modification increases energy expenditure to maintain their respiratory rate, making it a disadvantage for these plants in shade conditions (ROBSON et al., 2015).

Among the different collections, the 6th collection was superior to the 1th, 2th and 3th collections when evaluated the average of the two environments (Figure 2b). The increase in mini-cuttings production may be due to rising temperatures over this period. Another important factor may have been the longest period of mini-stumps permanence and consequent better adaptation to the substrate through the expansion of its root system and consequent increase in the absorption capacity of nutrients available via nutrient solution. These results demonstrate the skills in the management of mini-garden, showing a considered normal behavior in relation to the consumption of nutrients and reestablishment of shoots for subsequent collections (BRONDANI et al., 2010) (Figure 2b).

Good management of mini-stumps nutrition should reflect a homogeneous production over several collections, balance between income and exportation of nutrients (collected sprouts) and maintaining the nutritional condition for the restoration of the mini-stump. In contrast, improper handling can result in significant declines between collections, consequence of mini-stumps nutritional imbalance (WENDLING et al., 2010).

The volume of substrate available to the root system (two liters) was adequate, with a good production of mini-cuttings along the collections when compared to similar systems and species, such as *Cedrela fissilis* in 200 ml containers, which presented less productions (1.3) than the lowest value obtained in our study (3.7) (XAVIER et al., 2003). In semi-hydroponic system can be found even greater productivities depending directly of the propagated species, management and nutrition conditions of the mini-stumps, with results varying from 4.4 mini-cuttings mini-stump^{-1} month^{-1} in *Ilex paraguariensis* (WENDLING et al., 2007) to 32.5 mini-cuttings mini-stump^{-1} month^{-1} in *Eucalyptus grandis* x *E. urophylla* (WENDLING et al., 2010).

Mini-stumps grown in conditions of full sunlight showed superior productivity of mini-cuttings m^{-2} month^{-1} (PPSM) of 1.8 times in the Spring and 1.9 times in the Summer when compared to that conducted under shade. For the two seasons, productivity in the Summer was higher compared to Spring, about 1.4 times in conditions of full sunlight and 1.3 times in shading conditions (Figure 2c). One hypothesis is that carbohydrates availability in mini-cuttings has favored rooting and root vigor of *Acer palmatum*. Carbohydrates are an important energy source of vegetables (SIVACI, 2006; ASLMOSHTAGHI; SHAHSAVAR, 2010), and there is evidence of their participation in the root formation process, directly in the metabolism of auxin (HAISSIG, 1989). Similarly, the highest concentration of carbohydrates (fructose and glucose) in the Spring and Summer, coincided with the best results for rooting in *Olea europeia* cuttings (ASLMOSHTAGHI; SHAHSAVAR, 2010).

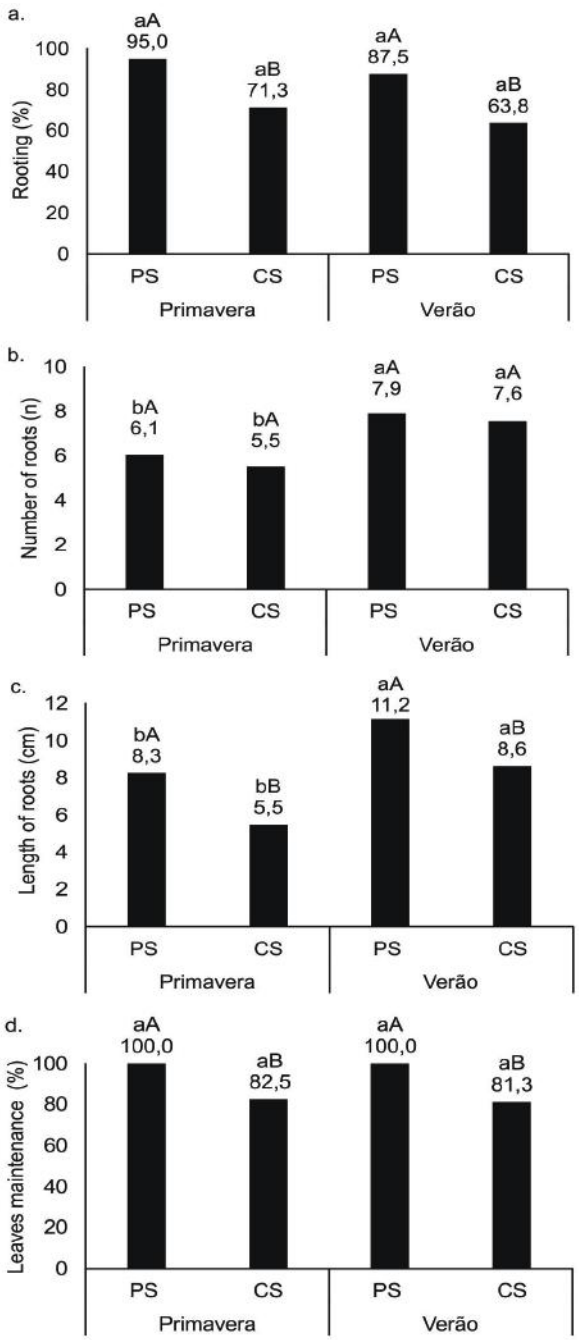

FIGURE 3 General means for (a) rooting percentage of *Acer palmatum*; (b) number of roots per mini-cutting; (c) average length of three major roots per mini-cutting and (d) leaves maintenance percentage in mini-cuttings, depending on the seasons and mini-stumps management. Where: FS - Full sunlight and SH - shade house. Means followed by the same lower case letter between seasons and capital between the environments do not differ by Tukey test at 5% probability.

Another effect of shading was the blanching and reducing of branches colaron diameter, greatly increasing the H/D ratio (height/diameter) of shoots. Similar results were observed for *Pterogyne nitens* (CESAR et al., 2014), where there was a reduction in stem self-support, leading to tortuous growth and tipping the branches.

The mini-stumps conducted grown in full sunlight (95.0% and 87.5%) favored rooting of mini-cuttings compared to thoseat grown conducted in shade house (71.3% and 63.8%) in the Spring and Summer, respectively (Figure 3a).

The best rooting obtained for full sunlight mini-cuttings can be explained initially by better adaptation of mini-stumps to this condition (Figure 2a). Photosynthesis is the energy generator in plants and has its efficiency based on three factors: CO_2, light and temperature. Thus, under stable conditions of temperature and CO_2, light has direct influence on the carbohydrate content in shoots (HAISSIG, 1989; SIVACI, 2006), favoring rooting.

The maintenance time of mini-cuttings in greenhouse (60 days) was adequate, since the results for rooting and root vigor (Figure 3a,b,c). Adequate control of environmental conditions in the greenhouse is highlighted in the literature as a key factor for rooting success, creating a good environment to maintain cell turgor and to improve plant survival after they are transferred to less controlled environments (WENDLING et al., 2010).

Adventitious roots emissions may be governed by several factors, including the sensitivity of cells to auxin signal (STEWART; NEMHAUSER, 2010), presence of inhibitors and carbohydrate levels (COSTA et al., 2013). The endogenous auxin level is considered a critical factor for rooting in many tree species (WENDLING et al., 2015). One of the mini-cuttings technique advantages is precisely the less necessity of auxin exogenous application due to the reinvigoration of the mother plants (STUEPP et al., 2015; STUEPP et al., 2016). Thus, the most consistent expression of maturation in woody species is the reduction in the regenerative capacity, directly reflected in the capacity and vigor of rooting (WENDLING et al., 2014). For this reason, the importance of using juvenile materials is highlighted for woody species propagation, especially when there is interest to propagate on a commercial scale (WENDLING et al., 2010; 2014).

The number and average root length per mini-cutting presented similar behavior to rooting. Summer was superior to Spring for both variables; however, there was no difference for the number of roots among studied environments (Figure 3b). Regarding the average length of roots mini-cuttings^{-1}, mini-cuttings obtained from full sunlight proved to be superior to the others (11.2 cm) (Figure 3c).

The number of roots per mini-cutting may vary between species, or even between clones of the same species (HUSEN; PAL, 2007). It is a factor highly dependent on environmental conditions, especially temperature, which has a direct effect on the metabolism of propagules, promoting cell division (PARENT et al., 2010). Most chemical reactions are accelerated in higher temperatures, thus favoring root vigor. These two variables are often correlated and, in general, are strongly controlled by genetic traits (PIJUT et al., 2011).

The leaves maintenance was superior on mini-cuttings from mini-stumps grown in conditions of full sunlight (100%) compared to that ones from shade house (82.5% and 81.3%) in the Spring and Summer, respectively (Figure 3d). Positive correlation was evident between the maintenance of the leaves and the rooting, which has been verified by several authors (STUEPP et al., 2014; FRAGOSO et al., 2015) and is directly related to the supply of carbohydrates and hormones present in the leaves (CORREA et al., 2005).

Similarly, mini-cuttings from full sunlight (22.5%) showed better sprouts emission levels compared to those from shade house (8.8%) in the Summer. The percentage of shoots emission was low, regardless of treatment, reflecting the high leaves maintenance, which showed a negative correlation with this variable, i.e., those who kept the leaves have not delivered new buds within 60 days. This fact was verified in *Olea europaea* cuttings and mini-cuttings of *Paulownia fortunei*, in which the leaves maintenance resulted in reduction of the percentage of shoots in axillary buds (PIO et al., 2005; STUEPP et al., 2015.).

Significant values were not found for callus formation on cuttings of *Acer palmatum*, indicating the direct root formation (PIJUT et al., 2011). This is a very desirable fact for plant propagation, because the presence of callus on vascular connection region is a limitation for rooting, because it is a region with high fragility, compromising the functionality of the roots (LI et al., 2009; BRONDANI et al., 2012). The reduced values for survival (17.5%) and mortality (18.8%) of mini-cuttings is a consequence of the rooting high levels, validating the effectiveness of mini-cuttings technique for the species.

The length of mini-cuttings from mini-stumps cultivated in full sunlight conditions significantly influenced rooting, root number, average length of three biggest

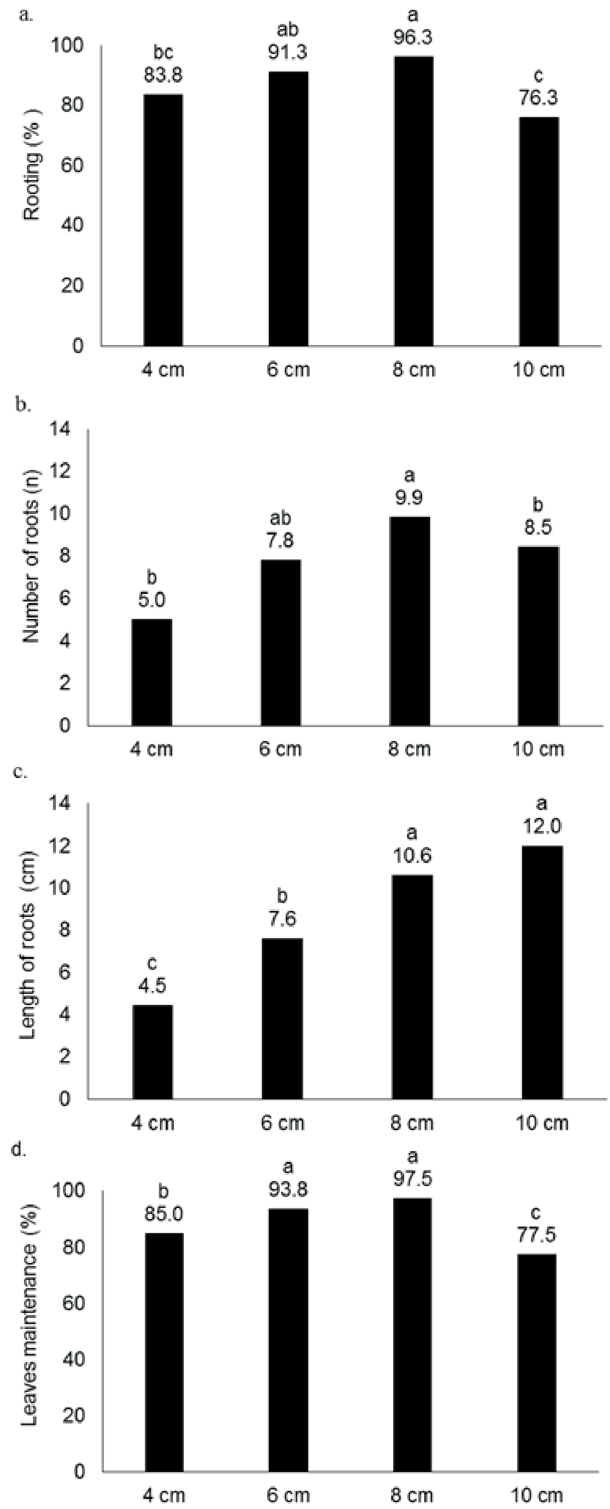

FIGURE 4 General means for (a) percentage of rooting; (b number of roots per mini-cutting; (c) average length of three biggest roots per mini-cutting and (d) leaves maintenance percentage in mini-cuttings, depending on the different mini-cuttings lengths. Means followed by the same letter do not differ by Tukey test at 5% probability.

roots and leaves maintenance in mini-cuttings of *Acer palmatum* ($p<0.05$). Mini-cuttings with 8±1 cm showed highest rooting percentage (96.3%), statistically similar to mini-cuttings with 6±1 cm (91.3%) and higher than the other lengths (Figure 4a). The good results recorded in all mini-cuttings lengths demonstrated the feasibility of the technique adapted for the propagation of *Acer palmatum*.

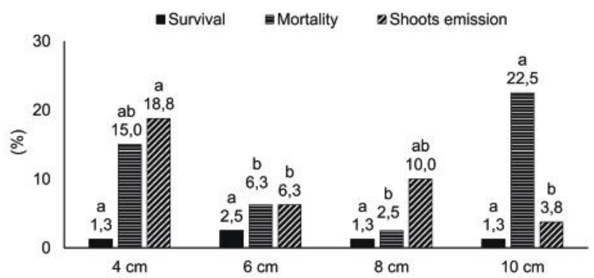

FIGURE 5 Means for survival percentage, mortality and shoots emission of *Acer palmatum* mini-cuttings, depending on the different mini-cuttings lengths. Means followed by the same letter do not differ by Tukey test at 5% of probability.

Rooting increased gradually until a length of 8±1 cm, from which fell sharply. This can be explained by oxidation and leaf fall in mini-cuttings of 10±1 cm. Shoots were collected selectively based on its length, where those higher than 10 cm were collected and others were kept for later collection. For the 10±1 cm mini-cuttings preparation we used the total length of the sprouts, showing thus herbaceous apex, which may have favored the reduction in rooting, leaves maintenance (Figure 4a,d) and, consequent increase in mortality (Figure 5).

Possibly, there is a relationship between the number of reserve and the major lengths of mini-cuttings interrupted on the 10±1 cm length depending on the observed oxidation. The carbohydrates function in the formation of adventitious roots has been widely discussed (SIVACI, 2006; COSTA et al., 2013), especially as carbon source for biosynthesis of nucleic acids and required proteins in the rooting process (HAISSIG, 1989; ASLMOSHTAGHI; SHAHSAVAR, 2010).

Mini-cuttings 8±1 cm also showed higher number of roots (9.9) (Figure 4b). For the average length of the three longest roots, the influence of mini-cuttings length is evident, showing a gradual increase, with apices in mini-cuttings of 10±1 cm (Figure 4c).

Leaves maintenance in mini-cuttings with 10±1 cm (77.5%) reinforces the hypothesis of mini-cuttings terminal portion oxidation, similar to the second experiment, indicating a positive correlation between these two variables (Figure 4 a,d). Similarly, the mortality in mini-cuttings of 10±1 cm (22.5%) (Figure 5) was above the results observed in both experiments for mini-cuttings from full sunlight, connoting the suggested oxidation effect.

Mini-cuttings survival (Figure 5) as well as calluses emission (data not shown) are consistent with results obtained in Experiment II, highlighting the origin of the mini-cuttings, coming just from clonal mini-garden established in full sunlight. The emission of shoots was superior in mini-cuttings of 4± 1cm, with a characteristic similar to each other, the presence of at least one remaining leave and a shoot, positioned in opposite axillary buds.

The results presented in this study show the importance of using appropriate plant propagules for rooting, particularly with respect to its lignification, and can lead to oxidation when using excessively herbaceous materials, even in high humidity conditions. Furthermore, it should be noted that mini-cuttings technique adapted to the condition of vases for *Acer palmatum* plants production is efficient. The obtained results serve as a basis for further studies in other species, which have limitations in terms of plants propagation.

CONCLUSIONS

The management system adopted on clonal mini-garden is suitable for the production of *Acer palmatum* mini-cuttings.

The highest percentages of productivity, rooting and root vigor of mini-cuttings were obtained in Summer.

Highest survival and productivity of sprouts per mini-stump, as well as the best rooting and root vigor of mini-cuttings are obtained from mini-stumps conducted in full sunlight.

Mini-cuttings of 8±1 cm are recommended for better rooting. The mini-cuttings technique is feasible for *Acer palmatum* propagation.

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