Vegetative rescue and *ex vitro* plants production system for *Ginkgo biloba* by cuttings and mini-cuttings

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ABSTRACT: This study aimed to evaluate the rooting potential of cuttings from year and epicormic shoots of *Ginkgo biloba* and evaluate the efficiency of the mini-cuttings technique for the species. Therefore, two experiments were implemented: I - Cuttings from current year shoots and epicormic shoots were prepared with 8 ± 1 cm, planted in 55 cm^3 plastic tubes and placed in a greenhouse for 60 days. II - Mini-stumps grown in 2 L pots were subjected to successive sprouts collections during 600 days. From the produced shoots, 5 ± 1 cm mini-cuttings were prepared, planted in 55 cm^3 plastic tubes and placed in a greenhouse for 45 days. Propagules from epicormic shoots presented rooting (82.5%) and root vigor higher than those from current year shoots. Despite the high survival of mini-stumps (100%), the morphological characteristics and reduced plant vigor of the species did not favor mini-cuttings productivity in the first trial year. On the other hand, in the second year, high rooting (92.5%) and root vigor proved the efficiency of the mini-cuttings technique for this species.

Keywords: rhizogenesis, juvenility, clonal mini-garden, vegetative propagation, reinvigoration/rejuvenation.

RESUMO: Resgate vegetativo e sistema *ex vitro* de produção de mudas de *Ginkgo biloba* por estaquia e miniestaquia. Objetivou-se no presente trabalho avaliar o potencial de enraizamento de estacas provenientes de brotações do ano e brotações epicórmicas de *Ginkgo biloba* e avaliar a eficiência da técnica de miniestaquia para espécie. Para tanto, foram implantados dois experimentos: I - Estacas provenientes de brotações do ano e epicórmicas foram preparadas com 8 ± 1 cm, plantadas em tubetes de 55 cm^3 e acondicionadas em casa de vegetação por 60 dias. II - Minicepas cultivadas em vasos de 2 L foram submetidas a coletas sucessivas durante 600 dias. A partir das brotações produzidas foram preparadas miniestacas com 5 ± 1 cm, plantadas em tubetes de 55 cm^3 e acondicionadas em casa de vegetação por 45 dias. Propágulos provenientes de brotações epicórmicas apresentaram enraizamento (82,5%) e vigor radicial superiores aqueles oriundos de brotações do ano. Apesar da elevada sobrevivência de minicepas (100%), as características morfológicas e o reduzido vigor vegetativo da espécie não favoreceram a produtividade de miniestacas no primeiro ano de avaliação. Por outro lado, no segundo, o elevado enraizamento (92,5%) e vigor radicial comprovaram a eficiência da técnica de miniestaquia para a espécie.

Palavras chave: rizogênese, juvenilidade, minijardim clonal, propagação vegetativa, revigoramento/rejuvenescimento.

INTRODUÇÃO

Adventitious rooting has played a key role in the survival and evolution of human beings. It has been the main method for plant propagation of clonal forestry (Martins *et al*., 2011) and vegetables over the past decades, representing significant gains to industry and society (Steffens & Rasmussen, 2016).

Ginkgo biloba L. (Ginkgoaceae) is a deciduous species, originally from Asia whose leaves extracts and fruits have been used in the treatment of mental illness for over 2000 years (Zhang *et al*., 2011). It is among the medicinal plant that is

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most studied and popular in the market, presenting flavone glycosides in its extract (≥24%) and terpene lactones (≥6%), both with proven effect on the human cardiovascular system, particularly on brain vascular activity (Lin *et al*., 2008; Van Beek & Montoro, 2009; Song *et al*., 2010; El-Ghazaly *et al*., 2015). It also has a high ornamental potential (Tommasi & Scaramuzzi, 2004; Bitencourt *et al*., 2010).

However, the diocey of the species (Singh *et al*., 2008), the necessity of plant growth regulators application and low vigor of plants produced through asexual propagation (Acharya *et al*., 2001; Valmorbida & Lessa, 2008; Bittencourt *et al*., 2010), generate the need to develop and evaluate vegetative rescue and reinvigoration techniques for optimization of their vegetative propagation by cuttings protocols. The attainment of juvenile propagules can be easily reached by shallow cutting or drastic pruning in adult trees for epicormic shoot induction (Stuepp *et al*., 2014; Rickli *et al*., 2015).

Mini-cuttings technique, however, presents some advantages over conventional cuttings propagation, such as reduction in the size of the propagules and increased productivity in shoots per area, in addition to exploitation of juvenile characteristics of propagules (Ferriani *et al*., 2011; Dias *et al*., 2012; Stuepp *et al*., 2015a). Its use in the propagation of ornamental plants is still unknown, especially because of the lack of technologies adaptation for small producers of this segment.

Thus, given the importance of developing an efficient production system of *Ginkgo biloba* plants, we aimed to evaluate the rooting potential of cuttings from current year shoots and epicormic shoots, the efficiency of the mini-cuttings technique and, the management system of clonal mini-garden on mini-cuttings productivity and rooting.

Experiment I - Current year shoots and epicormic shoots of the canopy

The experiment was conducted between November/2013 and February/2014 in the city of Curitiba (PR), Brazil (25°44’ S and 49°23’ W, 920 m). The collection of plant material occurred from 12-mother trees, all about 10 years old. Two types of cuttings were prepared: current year shoots collected from the top of the canopy and epicormic shoots pruned near the base of the canopy in May/2013.

Both cuttings were prepared with semi-hardwood shoots, 8 ± 1 cm long, with the remaining two leaves reduced to 1/3 of their surface. The disinfection was carried out with sodium hypochlorite at 0.5% for 10 min, followed by washing in running water for 10 min. Planting was carried out in tubes of 55 cm^3, filled with fine-grained vermiculite and carbonized rice husk (1:1), placed in greenhouse with intermittent mist (temperature of 24 ± 2 °C and air relative humidity higher than at 80%).

Sixty days after installation, the following variables were evaluated: percentage of rooting; number of roots/cutting; length of the three largest roots/cuttings; survival percentage (live cuttings without formed roots or calluses); percentage of cuttings with callus (live cuttings without roots, presenting mass of undifferentiated cells at the base); mortality percentage and maintenance of original leaves. The experimental design was completely randomized, with two types of cuttings and four replications containing 20 cuttings per experimental unit.

Experiment II – Clonal mini-garden management and mini-cuttings technique

The *Ginkgo biloba* clonal mini-garden, formed from plants produced by cuttings (Experiment I), was conducted in conditions of full sunlight, in 2 L pots containing commercial coir and peat-based substrates, with 20 x 20 cm spacing, periodic irrigation (three daily irrigations, lasting 5 minutes and flow of 144 L $hour^{-1}$) and monthly fertigation (25 ml nutrient solution consisting of 4 g L^{-1} ammonium sulphate, triple superphosphate and potassium chloride and 1 g L^{-1} FTE BR-12), installed in February/2014.

By being a deciduous species, with production of sprouts only in Spring and Summer, only two collections were made in the first year of assessment, the first at 210 days after installation (September/2014) and the second at 330 days after installation (January/2015). After the winter period, there was a new collection at 600 days after installation (October/2015). For cuttings preparation, only shoots with more than 10 cm long and more than three pairs of leaves were collected.

The mini-cuttings were prepared with 5 ± 1 cm length and a mean diameter of 0.4 ± 0.1 cm, keeping two leaves reduced to 1/3 of their surface. Planting was carried out in tubes of 55 cm^3, filled with fine-grained vermiculite and carbonized rice husk (1:1), placed in a greenhouse with intermittent mist. After 45 days, the same variables described in Experiment I were evaluated.

The evaluations of the clonal mini-garden occurred between February/2014 and October/2015. The following parameters were evaluated: survival percentage of mini-stumps and production of mini-cuttings m^{-2} $month^{-1}$. The experimental design was completely randomized, with five replications of five mini-stumps per experimental unit.

Due to the low productivity of mini-stumps in the first year, only results for rooting of the installation with propagules collected at 600 days after installation in a single installation are presented. The experimental design was completely randomized with four replications of 20 cuttings per experimental unit.

All data (Experiments I and II) were evaluated for homogeneity using the Bartlett´s test and the means were compared by the Tukey´s test at 5% probability.

Data analysis showed significant differences between current year shoots and epicormic shoots for all variables, except for the production of shoots, in which both showed 0%. Epicormic shoots were significantly superior to current year shoots for rooting (82.5%), number of roots (3.75), average root length of the three largest roots (5.04 cm) and leaf maintenance (82.5%) (Figure 1 and Table 1).

The reduction in rooting capacity between the two types of cuttings may be due to differences in the endogenous levels of hormones. The cuttings taken from epicormic shoots at the base of plants are characterized by a greater juvenility and vigor (Stuepp *et al*., 2014; Wendling *et al*., 2014; Rickly *et al*., 2015) compared to the current year shoots (Stuepp *et al*., 2015b).

The use of epicormic shoots for rooting has been successfully registered for several species, particularly forest species (Dias *et al*., 2012; Stuepp *et al*., 2014; Rickly *et al*., 2015; Stuepp *et al*., 2015c; Wendling *et al*., 2013). In addition to the excellent levels of verified rooting, most juvenility of propagules brings the possibility of producing plants with great vigor without the need to use plant growth regulators, as found in this study.

It is possible to verify a close relationship between adventitious roots formation and survival and leaf maintenance for the two sources of propagules (Figure 1). The importance of leaf maintenance for these two variables has been highlighted in the literature, especially in relation to the presence of certain compounds in the leaves, such as auxin and cofactors and can be translocated via phloem to the base of the cuttings, thereby stimulating root formation (Bona; Biasi, 2010; Fragoso *et al*., 2015).

In addition, the reduced callus formation in cuttings from epicormic shoots (27.5%) is an excellent indicator of the increase in the vigor attributed to these propagules, unlike those from current year shoots. Similarly, the survival and reduced mortality of cuttings from epicormic shoots are reflective of the high percentage of rooting (Table 1).

TABLE 1. Means for rooting percentage, number of roots per cutting (n) and average length of three major roots per cutting (cm) in *Ginkgo biloba cuttings.*

	Epicormic shoots	Current year shoots
Rooting (%)	82,5 a	47,5 b
Number of roots	3,75 a	2,26 b
Length of roots (cm)	5,04 a	2,51 b

Means followed by the same letter in each variable do not differ by Tukey´s test at 5% probability.

The high survival of mini-stumps (100%) over the 600 days evaluation reflects the adaptability of these to environmental and nutritional conditions provided in full sunlight. Moreover, the low productivity measured in the first assessment year (63 mini-cuttings year^{-1}) from only two collections is attributed

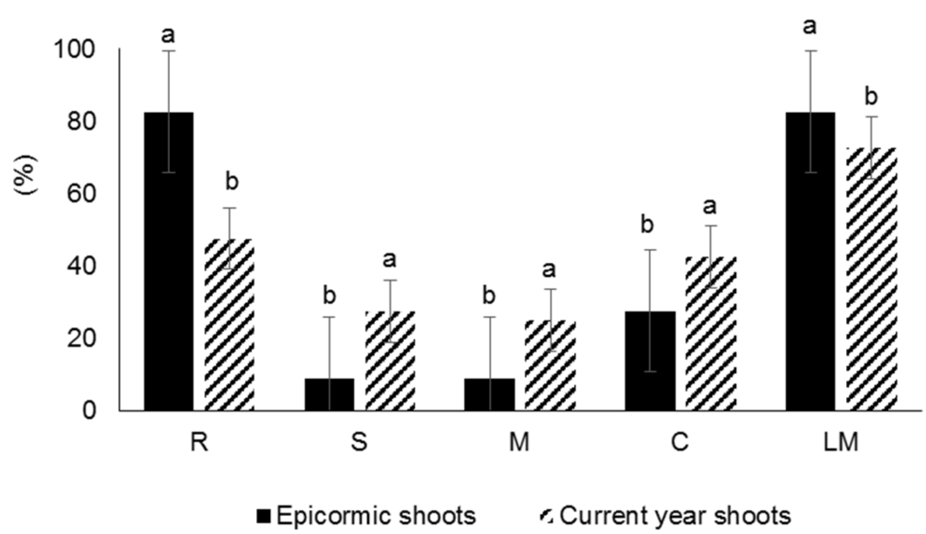

FIGURE 1. General means for rooting percentage (R), survival (S), mortality (M), callus formation (C) and leaves maintenance percentage (LM) in *Ginkgo biloba* cuttings from current year shoots and epicormic shoots. Means followed by the same letter in each variable do not differ by Tukey´s test at 5% probability. The bars indicate the standard error of mean (n = 20).

to the deciduous characteristic of the species, indicating the need for management adjustments for the application of the technique in the first year.

Despite not having been carried out the assessment of propagules productivity in the second year of the experiment, it was possible to make an installation with material produced at 600 days after installation. The results for rooting mini-cuttings were excellent, reaching 92.5% of rooting and root vigor (NR: 5.25 and CMR: 7.13 cm) higher than those obtained for epicormic and current year shoots (Table 1). Likewise, that material showed low percentage of live (5%) and dead (2.5%) mini-cuttings and calli emission (10%) (data not shown). Added to high leaves maintenance (92.5%) and the reduction of time that propagules remain in greenhouse, these results indicate the efficiency of the technique in maintaining the youthful vigor of propagules 600 days after installation (data not shown). This fact highlights the efficiency of the continuous pruning of mini-stumps in maintaining the juvenility of propagules (Aimers-Halliday *et al*., 2003; Wendling *et al*., 2015).

Based on the obtained results, we conclude that the potential of *Ginkgo biloba* rooting is higher in cuttings from epicormic shoots. The mini-cuttings technique for *Ginkgo biloba* plants production is feasible from the second year of the clonal mini-garden installation.

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