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ROOTING OF JUVENILE CUTTINGS OF *Pinus patula* Schiede ex Schltdl. *et* Cham. HEDGES

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ABSTRACT

Pinus patula Schiede ex Schltdl. *et* Cham. is endemic to Mexico and is used in both reforestation programs and in the timber industry due to its fast growth, wood quality, and easy handling. With the advance in genetic breeding programs, there is a need to massively propagate new plants from cuttings. One of the main challenges for this purpose is to know the effect of the age of the mother plant (hedge) on the rooting of cuttings. The younger the plant, the greater the rooting is expected to be. Three ontological ages of *P. patula* mother plants were compared (7, 10, and 13 months). The experimental design was in complete random blocks, with four replications and 25 cuttings per experimental unit. The response variables were evaluated 20 weeks after the experiment was established. No significant differences were observed in the rooting of the three ages evaluated (67.3 to 81 %), nor in the quality of the root (number and length of primary roots, percentage of plants with secondary roots). The results point out an advantage for the propagation of *Pinus* cuttings since the mother plants at those ages are capable of generating juvenile cuttings that are adequate to obtain percentages of rooting of over 75 %.

Keywords: vegetative propagation, maturity, ontogeny, mother plants, juvenility.

INTRODUCTION

Pinus patula Schiede ex Schldl. *et* Cham. is a Mexican pine tree widely planted around the world, with close to one million hectares (Tadesse and Fidalgo-Fonseca, 2022), due to its fast growth, straight trunk, lack of knots, wood quality, and easy silvicultural management (Velázquez *et al*., 2004). It is intensively used in plantations with improved trees in countries in Africa and America (Kanzler *et al*., 2012), although in Mexico, wild forests are primarily used. This has led to the extinction of superior trees in the purest *P. patula* stands (Aparicio-Rentería *et al*., 2014).

Due to the genetic deterioration and the practical difficulties of supplying seeds for reforestation, isolated efforts have been made to establish seed orchards in the past 20 years (Salaya-Domínguez *et al*., 2012), which consist in the planting of genetically superior trees that are resistant to pests and diseases, able to adapt to harsh environments (such as degraded soils and extreme climates), and have a high commercial value

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(Ohira *et al*., 2009). After selecting the best parents based on the progeny trials, the massive multiplication of these individuals is carried out to incorporate genetic gain into the plantations or natural stands (Bonga, 2016).

There are several cloning techniques (grafting, layering, rooting of cuttings, and *in vitro* cultivation); however, propagation via cuttings is used because it is very affordable on an economic scale (Trueman, 2006). Unfortunately, for most conifers, the ontogenic age of donor plants or mother plants is a limiting factor (Mitchell *et al*., 2004a). Wendling *et al*. (2014) define ontogenic maturity as the process of development in woody plants that implies the start of reproductive maturity, with a reduction in both the growth rate and the rooting of cuttings. Morphological (Menzies *et al*., 2000), physiological (Fraga *et al*., 2002), hormonal (Valdés *et al*., 2002; Valdés *et al*., 2004), and genetic (Alvarez *et al*., 2016) changes are involved in this phase. In a review on the effect of ontogenic age on the rooting of conifer cuttings, Mitchell *et al.* (2004a) found that the percentage of rooting ranges between 25 and 100 % in juvenile material and between 1 and 75 % in mature material. In addition, a loss in root quality has been reported for *Dalbergia melanoxylon* (Amri *et al*., 2010), *Grewia optiva* (Husen, 2012), and other species as the ontogenic age progresses.

In order to reduce the negative effect of ontogenic maturation, different methods and techniques have been implemented to obtain young sprouts from the mother plants (Mitchell and Jones, 2006; Wendling *et al*., 2014). One of them is the formation of propagation hedges in which the mother plant is kept between 15 and 20 cm tall by frequently pruning to favor the emission of new sprouts from lateral buds and delay the maturing process (Mitchell *et al*., 2004b). Despite this, in different *Pinus* species, loss of rooting capacity has been reported, varying between 35 and 64 % (Mitchell *et al*., 2004a). In the case of *P. patula*, Mitchell *et al.* (2004b) reported a reduction of 35 to 80 % in rooting, as well as in root quality (number of roots, diameter, and dry mass of the root), in hedges between one and four years of age. The reduction in the quality of the roots leads to a lower growth in the height and diameter of the plants rooted in the field.

In Mexico, there are few studies and advances regarding this topic. Aparicio-Rentería (2014) was one of the first to address the topic of the production of *P. patula* cuttings, obtaining weighted rootings of 81 % when using 21-month-old hedges without the use of auxins in forest soil and mine sand substrate (1:2). Despite obtaining satisfactory results, they do not provide any details on their methodology. On the other hand, Rivera-Rodríguez *et al.* (2016) evaluated three factors (substrate, auxins, and ontogenic age) of the ontogenic effect on rooting, and they reported a reduction of 10 to 57.5 % for the ages of 12 and 24 months, respectively, without the application of auxins. These authors proposed the establishment of cuttings in a rooting chamber created by them with controlled nebulization and a night/day thermal system under two substrates (perlite and a mixture of vermiculite and peat moss); however, they showed humidity problems, which had a negative impact on the percentage of rooting.

In order to establish more favorable conditions for the establishment of cuttings, Escamilla-Hernández *et al.* (2021) proposed an alternative design based on 220 cm^3

containers (tubes) with the recirculation of water by bagging to avoid excess humidity in the substrate, testing alternative sawdust- and bark-based mixtures, and they obtained a rooting of 77 % in cuttings from 10-month-old hedges, without the use of auxins, with a survival rate of 100 %. This was an important advance for the establishment of cuttings, which helps investigate other factors.

The ontogenic process is an important factor since the juvenile phase is very short; after the second year, the rooting of *P. patula* starts reducing significantly (Mitchell *et al.*, 2004b). Whether the ontogenic maturity process is observed before 12 months is currently unknown. If this were the case, the different physiological, biochemical, and genetic studies would have to focus on earlier ages to better explain the ontogenic maturity process; otherwise, the possibility of maximizing the stage of production of cuttings opens up. The aim of this study was to evaluate the percentage and quality of the rooting of cuttings obtained from 7-, 10-, and 13-month-old *P. patula* hedges.

MATERIALS AND METHODS

Hedge establishment and management

The investigation was conducted in a greenhouse of the forest nursery of the Colegio de Postgraduados, located in Texcoco, State of Mexico, Mexico (19° 27′ 38.25′′ N, 98° 54′ 23.91′′ O), at an altitude of 2240 m. The propagation hedges were established from a seed lot of the first-generation "Reserva Forestal Multifuncional" clonal seed orchard in Aquixtla, Puebla, Mexico. The lot included the *P. patula* seeds of the eleven best clones, according to the evaluations of the progeny trials carried out by Salaya-Domínguez *et al.* (2012).

To generate 7-, 10-, and 13-month-old plants to create hedges and ensure that the three ages were examined at the same time while constructing the experiment, they were planted in a staggered fashion with a 3-month gap. The first was planted on June 6th (E_3), the second on September 6th (E_2), and the last one on December 6th (E_1) of 2017. The plants were sown directly in 220 cm^3 containers (tubes). A total of 300 plants were sown, 100 per treatment.

The initial pruning was carried out on the main stem to remove the apical part (3 cm) when the secondary leaves emerged (Escamilla-Hernández *et al.*, 2020); this criterion was consistent throughout all plant ages. The youngest plants (E_1) were pruned at an age of four and a half months with an average height of 14 cm; the medium-aged plants (E_2), after five months and with an average height of 10 cm, and the oldest plants (E_3), after seven months and with an average age of 16 cm. Differences in plant heights are due to the season of their production; the growth of the younger plants (E_1) was higher in March and April, along with their development, whereas E_2 plants were produced in less favorable environmental conditions.

Fifteen days after pruning, the mother plants were transplanted into 4 L pots in a substrate mixture formed by composted pine bark, peat moss, vermiculite, and perlite

(60:15:15:10, v/v/v/v) with 7 g L^{-1} of Osmocote® controlled release fertilizer (15-9-12, N-P-K) with a release period of eight to nine months. The mother plants (hedges) were kept under greenhouse conditions, beneath a 50 % shade cloth (Escamilla-Hernández *et al*., 2020).

All hedges were adjusted by pruning five primary branches to control the architecture of the plant. To synchronize the production of cuttings with the moment of the evaluation, several prunes were made for every ontogenic age. After forming the hedges, the E_1 plants were no longer trimmed, whereas the E_2 hedges were pruned for the second time after seven months, and the E_3 hedges were pruned another two times (at nine and 10 months). To obtain homogenous, quality sprouts, four sprouts were left on the main stem and two on each main branch (Figure 1). The criterion at the moment of harvest was determined based on the phenological characteristics and not on the chronological age of the sprout, according to Menzies *et al.* (2000). The age of the sprouts in the E_1 hedges was two and a half months, and in the E_2 and E_3 hedges, three months.

Figure 1. Management of *Pinus patula* hedges to obtain cuttings. E_3, E_2, and E_1: mother plants aged 13, 10, and 7 months, respectively.

Obtaining and managing of cuttings

To establish the experiment, 50 hedges were selected from every treatment, and the sprouts generated on the main stem and the pruned branches of each hedge were harvested. To do this, a Felco F2 hand pruner was used. The disinfection process was carried out for every hedge. The pruner was disinfected with 96° ethyl alcohol before cutting. Next, Captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) was applied at a dose of 1.5 g L^{-1} at the freshly pruned areas of the plant. The average length of the cuttings was 9 cm (Rivera-Rodríguez *et al*., 2016), measured using a graduated ruler to the closest millimeter. The average basal diameter was between 2 and 2.5 mm, and it was measured using a digital vernier with an accuracy of 0.01 mm.

The phenological characteristics of the cuttings were described for every treatment at the moment of harvesting for their evaluation. The cuttings from the E_1 hedges presented more juvenile characteristics (flexible stem, primary leaves, and the presence of axillary buds), whereas those from E_3 displayed a greater degree of development (less flexible stem, primary and secondary leaves, without axillary buds). The cuttings from the E_2 hedges displayed intermediate characteristics (flexible stem, primary leaves, start of secondary leaves, and the presence of axillary buds), as described by Menzies *et al*. (2000). The needles were removed from the buds harvested in the first 3 cm from the base, and they were disinfected using Captan in doses of 1.5 g L^{-1} for 15 min (Figure 2).

Figure 2. Characteristics of *Pinus patula* sprouts for the rooting of cuttings from mother plants E_3 (13 months), E_2 (10 months), and E_1 (7 months).

Treatments and experimental design

The experiment was conducted in mid-July 2018. The three hedge ages (E1, E2, and E3) were assessed using a complete random block design with four replications. One hundred cuttings were used per treatment and 25 per experimental unit. The cuttings were transplanted in individual 220 cm^3 containers that were placed in trays with 25 cells at 3 cm of depth into the substrate, and they were sprayed with fungicide solution (thiabendazole 1.5 g L^{-1}) and insecticide (Imidacloprid 1.5 g L^{-1}). To maintain the relative humidity at saturation, each tray was covered in a 90 cm wide by 120 cm tall plastic bag and placed in a rustic greenhouse. The rooting substrate used in the containers was a mixture of fresh pine sawdust and composted pine bark (90:10 v/v). The bark was sieved through a 0.5 cm mesh. The substrates were disinfected using Tecto ® fungicide in a dose of 1.5 g L^{-1} of water.

Air and substrate temperatures were taken with a HOBO sensor at 15-minute intervals. The mean monthly temperature varied between 18 and 20.7 °C during the rooting period (July–November, 2018). However, the daytime temperature in that period varied between 10.8 °C (average minimum) and 36.3 °C (average maximum). The monthly mean temperature of the substrate varied between 17.5 and 20.8 °C during the same period, with average minimum and maximum values of 11.5 °C and 31 °C, respectively.

Variables evaluated

Twenty weeks after the experiment was established, the cuttings were removed from the substrate, and the following characteristics were evaluated: survival, rooting, the presence of callus, the number and length of primary roots, and the presence of secondary roots. Live cuttings were considered to be those that displayed no necrotized tissue anywhere. The cuttings with at least one root of 1 mm in length were considered rooted (Rivera-Rodríguez *et al.*, 2016). The presence of callus was identified as the bulking of meristematic tissue at the base of the stem, resulting from cell division (Rasmussen *et al.*, 2009). Primary roots are those that originate at the base or lateral part of the stem, and secondary roots are those that form from the primary roots, with a minimum length of 0.5 cm (Hartmann *et al.*, 2014). The primary roots were measured using a ruler graduated in centimeters to the nearest millimeter. From the characteristics evaluated in every cutting, the percentage of live cuttings with callus and root was obtained for each experimental unit. Considering only rooted cuttings, the average number and length of primary roots per cutting were obtained, as well as the percentage of cuttings with secondary roots.

Statistical analysis

A one-way analysis of variance was carried out using the Mixed procedure of the SAS program, version 9.4 (SAS Institute, 2004). In cases where a significant effect of the age of the mother plant was found, Tukey's mean comparison test with $p = 0.05$ was carried out. The model used in the analysis of variance was as follows:

$$Y_{ij} = \mu + B_i + T_j + \varepsilon_{ij}$$

where Y_{ij} is the observed value of the variable in the i-th experimental unit of the j-th treatment, μ is the effect of the general mean, B_i is the random effect of the i-th block, T_j is the fixed effect of the j-th treatment, and ε_{ij} is the experimental error. All the response variables, except for the root number and length, were transformed with the arcsine function before the analysis of variance, and the average values were then transformed to their original scale.

RESULTS AND DISCUSSION

There were significant differences between treatments in the survival of the cuttings ($p = 0.029$). However, in the rooting variables, presence of callus, number of primary roots, root length, and presence of secondary roots, no significant effect was found ($p \geq 0.05$) of the age of the mother plant (Table 1).

Table 1. Results of the analysis of variance of the characteristics evaluated in the trial for the rooting of *Pinus patula* cuttings obtained from hedges aged 7, 10, and 13 months.

Variable	Mean	GL	F	P
Survival (%)	98.3	2	6.76	0.029
Rooted cuttings (%)	75.6	2	3.35	0.082
Cuttings with calluses (%)	22.1	2	1.42	0.291
Primary roots (number)	1.8	2	0.15	0.865
Root length (cm)	7.1	2	0.06	0.943
Rooted cuttings with secondary roots (%)	84.9	2	1.49	0.298

The survival rate of the cuttings was 100 % in the treatments of younger plants (E_1 and E_2) (Figure 3A), while the cuttings from the more aged plants (E_3) displayed a survival rate of 95 %. The death rate in the cuttings from E_3 was due mainly to management aspects and/or specific conditions of the trial, since the lower percentage of survival in them was found in one of the replications, with 84 %.

Rooting efficiency

The rooting percentage in the whole trial was 75.6 % (Table 1). Although this value varied between 67.3 and 81 % in the treatments, the differences were not significant between them (Figure 3B). These results are comparable to the values obtained by Mitchell *et al.* (2004b) in *P. patula* (80 %) with one-year-old mother plants and Majada *et al*. (2011) in *P. pinaster* (84.7 %) with 11-month-old plants. The weighted percentage of rooting reached (75.6 %) indicates an achievement for the establishment of *P. patula* cuttings in Mexico, since it approximates the percentages reported in countries with production systems by cuttings in *Pinus* (Trueman, 2006).

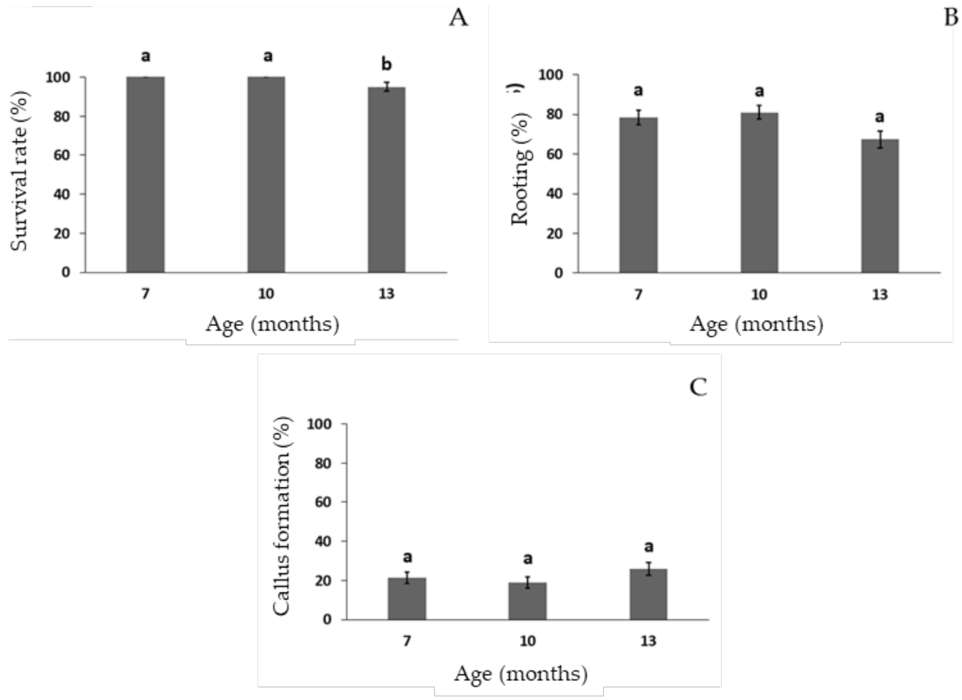

Figure 3. Effect of the ontogenic age (7, 10, and 13 months) of the mother plant. A: survival; B: rooting; C: presence of callus**.**

In the ages evaluated (7, 10, and 13 months), no effect of the ontogenic age was observed, as reported by Wendling *et al.* (2014) in conifers, who mention that the older the plant, the less rooting it will have. These results are encouraging, since most production systems by cuttings begin at 12 months with a rooting percentage of 80 % for *P. patula* and decrease after the third year with a rooting percentage of 70 % until they reach 35 % after four years (Mitchell *et al.,* 2004b). As mentioned earlier, the production system for cuttings is short. With these results, it is possible to broaden the cutting production cycle, starting at 7 months.

On the other hand, in the three evaluated ages of the plant, cuttings displayed morphological characteristics such as stem, leaf, and axillary bud types (Figure 2), considered juvenile traits by Menzies *et al.* (2000). This fact contributes towards the identification of juvenile characteristics as a practical and inexpensive tool, unlike others such as physiological, hormonal, or genetic ones (Fraga *et al*., 2002; Valdés *et al*., 2004; Alvarez *et al*., 2016). It is worth mentioning that in the same hedge, buds with different degrees of maturity can be found due to the position of the buds in the hedge (apical or basal) or due to their management in the nursery (water stress, excess or insufficient nutrients, and extreme temperature changes) (Wendling *et al.,* 2014).

A high weighted rooting percentage (75.6 %) without the use of rooting hormones indicates that the cuttings have adequate levels of endogenous auxins (Osterc *et al*., 2009). Despite this, a 14 % reduction was observed in the rooting ability between E_2 (81 %) and E_3 (67 %) (Figure 3B), probably due to a slight effect of the maturity of the mother plant, which may be related to a decrease in auxins (Wendling *et al.*, 2014). For these cases, the use of external stimulants on the base of the cutting could increase the percentage of rooting (Rosier *et al*., 2004).

On average for the entire trial, 22.1 % of the cuttings formed a callus on the base, with a variation between 19 and 26 % in the three ages (Figure 3C); the lower values were displayed in E_1 and E_2 (22 and 19 %) and the highest in E_3 (26 %). This variable is complementary to the percentage of rooting; that is, as the percentage of calluses decreases, the percentage of rooting increases, and vice versa (Hartmann *et al*., 2014). Rivera-Rodríguez *et al.* (2016) reported 42.5 % of calluses in mother plants aged 12 months, in complement with 57.5 % of rooting.

Rooting quality

The hedge's ontogenic age had no effect on rooting quality; the number of primary roots per cutting remained between 1.8 and 1.9; root length was between 7 and 7.3 cm; and between 82 and 88 % of the rooted cuttings presented secondary roots (Table 2). These values are similar to those recorded by Escamilla-Hernández *et al.* (2020) in 10-month-old *P. patula* hedges, where they reported values of 2.1 for the number of roots, 6.7 cm in length, and 76.1 % rooted cuttings with secondary roots. Majada *et al.* (2011), in a four-month evaluation, reported an average of 2.9 primary roots and 3.3 cm for root length in *P. pinaster* cuttings obtained from 11-month-old hedges without the application of auxins. In another study, when *P. patula* cuttings were analyzed after three and a half months, an average of 1.4 primary roots were found per cutting, with a length of 3.8 cm and 36.7 % of cuttings with secondary roots (Rivera-Rodríguez *et al*., 2016).

Table 2. Average values (± standard error), by age of the mother plant, of the characteristics of the roots evaluated in the trial for the rooting of *Pinus patula* cuttings.

Age of mother plant (months)	Primary roots		Cuttings with secondary roots (%)
	Number	Length (cm)	
7	1.9 ± 0.16 a	7.0 ± 0.60 a	84.9 ± 3.1 a
10	1.8 ± 0.16 a	7.0 ± 0.60 a	87.7 ± 2.8 a
13	1.8 ± 0.16 a	7.3 ± 0.60 a	82.1 ± 3.3 a

Average values followed by the same letter do not differ significantly (Tukey, $p = 0.05$).

Although these studies evaluated the quality of the rooting in less time, the data are similar to those obtained in a sample of 30 cuttings per treatment evaluated after four months in this study. This indicates that the roots from the cuttings aged 7, 10, and 13 months display favorable attributes for greater rooting efficiency and quality (Figure

4). These mother plants help obtain a better distribution in the root architecture to make water and nutrient absorption more efficient and give adequate mechanical support in the field (Davis and Jacobs, 2005; Mitchell *et al.*, 2005).

Figure 4. Rooting of *Pinus patula* cuttings with three ontogenic ages (7, 10, and 13 months, E1, E2, and E3, respectively).

CONCLUSIONS

No important ontogenic effects were observed in the capacity of the rootings or on the quality of the roots in the cuttings from 7-, 10-, and 13-month-old mother plants. This indicates that, in less than a year, the hedge produces sprouts with distinctive juvenile characteristics that can be used as a practical and inexpensive tool to identify juvenile material for *Pinus patula* cuttings propagation.

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