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COMBINATION OF GRAFTING TECHNIQUES TO CLONE SUPERIOR GENOTYPES OF *Pinus patula* Schiede ex Schltdl. *et* Cham.

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ABSTRACT

Grafting in species of *Pinus* is used to establish asexual seed orchards. Generally, grafting success is low, therefore, it is required to study the influencing factors. Such as the technique and the contact surface of the cambium that depends on the length of the cut on the scion and the rootstock. The objective of this study was to evaluate graft success, growth, and needle development on *Pinus patula* grafts as functions of the grafting technique and cutting length. It was expected that a specific combination of technique and cutting length would allow predicting the graft success. The techniques of top cleft, side-veneer and splice grafting were used with three cutting lengths (1.5, 2.0, and 2.5 cm) in a completely randomized design with factorial array. The data were analysed with an analysis of variance and treatment means were compared with the Tukey test ($p \leq 0.05$). Sixty days after grafting, technique influenced success; top cleft grafting had the highest value (80%) but was not statistically different from side veneer grafting (73.3%). There was, however, statistical difference between top cleft and splice grafting (56.7%, $p \leq 0.05$). There were no differences among the three cutting lengths in terms of the three variables evaluated. The effect of the interaction showed that top cleft and side veneer grafts with the cutting length of 1.5 cm expressed the greatest growth (32.4 and 32.2 mm, respectively); followed by side veneer grafting with the cutting length of 2.0 cm (30.4). The study demonstrated that the most viable techniques to increase grafting success in *P. patula* are the top cleft and side veneer techniques. Cutting length of the scion and the rootstock can be set within the range 1.5 to 2.5 cm

Keywords: vegetative propagation, graft success, rootstock, scion, seed orchards.

INTRODUCTION

Pinus patula Schiede ex Schltdl. *et* Cham. is a species native to Mexico. It has high economic importance because of high productive potential, which has made this species a subject for breeding programs (Flores *et al*., 2019). Those programs include establishing asexual seed orchards (ASO) to maintain a sustained supply of genetically high-quality seed for high-yield plantations (Aparicio-Rentería *et al.*, 2013; Hernández *et al*., 2016).

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The ASO of the genus *Pinus* have been established by cloning superior trees through grafting for almost half a century (McKeand *et al*., 2003). This technique allows integrally multiplying traits of superior mature-tree genotypes that have low or no capability of developing adventitious roots (Hartmann *et al.,* 2014).

However, although propagation by grafting is achieved in several species of the genus *Pinus*, there are still problems of low grafting success caused by diverse factors. Such as, internal anatomy, health, taxonomic and genetic affinity between the scion and rootstock, bud phenology, rootstock age, grafting technique, environmental conditions, management, and the grafter skills (Pérez-Luna *et al*., 2019; Barrera-Ramírez *et al*., 2020).

Grafting techniques most commonly used on conifer species are top cleft and side veneer (Muñoz *et al.,* 2013). Recently, these techniques have been applied on the Mexican species *Pinus engelmannii* Carr. (Pérez-Luna *et al*., 2019), *Pinus rzedowskii* Madrigal & M. Caball. (Solorio-Barragán *et al*., 2021), and *Pinus pseudostrobus* var. *oaxacana* (Mirov) Harrison (Barrera-Ramírez *et al*., 2020). With *P. patula*, application of side veneer grafts has been documented with 29% survival (Aparicio-Rentería *et al*., 2013). Also, Villaseñor and Carrera (1980) evaluated the two techniques, resulting in higher success (63%) with top cleft grafting.

Splice grafting has been little applied in conifers despite its simplicity and rapid execution. This technique requires only a slanted cut of equal length and angle on the scion and the rootstock. For this reason, it is one of the most used methods in horticulture with a high percentage of success (Pardo-Alonso *et al*., 2020).

The internal anatomy of the scion and the rootstock is another aspect to consider in grafting success. Particularly, the contact surface at the cambium between rootstock and scion is key in the joining process of the graft because of its capacity for cell formation and differentiation in reconnecting vascular tissues (Castro-Garibay *et al*., 2017). Pardo-Alonso *et al*. (2019) pointed out that besides grafting stems (scion-rootstock) with similar diameter, the surface area of the cut and the angle must be considered. Under this premise, it can be expected that the longer the cut the larger the contact area, and the greater graft success. Several lengths limited by the diameter of the scion have been suggested (Muñoz *et al*., 2013; Pérez-Luna *et al*., 2019); thus, it is necessary to determine what cutting length better favours the graft joining in a given species.

This research proposed to find the most viable technique and cutting length for grafting in *P. patula*. The objective of the study was to evaluate graft success, growth, and development on *P. patula* grafts as functions regarding three grafting techniques (top cleft, side veneer, and splicing) and three cutting lengths on the scion and the rootstock (1.5, 2.0, and 2.5 cm). The hypothesis was that a larger contact surface (generated by the longest cut) between the cambium regions of rootstock and scion would cause the greater adherence and mechanical support of the graft with a faster reconnection of the vascular conduits, therefore allowing to predict graft success in *P. patula*.

MATERIALS AND METHODS

The *P. patula* seedlings used as rootstock were grown from seed from Ixtacamaxtitlán, Puebla. Once germinated, seedlings were transplanted to 6-L black polyethylene bags, one plant per bag, filled with a mixture of sawdust, pine bark, vermiculite (Agrolita, State of Mexico, Mexico), and peat (Kekkilä Professional, Vantaa, Finland) in a proportion of 70-20-5-5, respectively.

To the mixture, eight-month controlled release fertilizer Multicote® (18-6-12 + micronutrients) (Haifa, Israel) was added at a dose of 8 g L^{-1} substrate. In addition, in the weekly watering, a dose of 1 g soluble fertilizer Peters Professional® (20-20-20 + micronutrients) L^{-1} water (Everris, Ohio, USA) was applied, and every two weeks, alternately, two solutions of fungicide were applied, Ridomil Gold Bravo SC® (1 mL L^{-1}) (Syngenta, USA) and Tecto 60® (1 g L^{-1}) (Syngenta, USA) to prevent infections. Plants were kept in nursery conditions for one year, in October 2019, when they exhibited desirable characteristics for grafting; these are, healthy, 77 to 79 cm in height, 8.02 to 8.04 in diameter (Figure 1B).

Buds (for scions) were then collected from a four-year-old *P. patula* progeny test, located at the Agua Azul site in Ejido Peñuelas Pueblo Nuevo, Chignahuapan, Puebla. The scion-donor trees were selected for their outstanding growth, in both height and diameter and for their straight stem. These selected individuals belong to the families with the best performance in the trial (8, 27, 50, 81, and 85). When they were collected, buds were found in a phenological state of active growth (11 to 15 cm in length and 5.8 to 6.0 mm in diameter), with closed scales, without needles, healthy and vigorous (Figure 1A and 1C).

After they were collected, buds were taken to the forest nursery at Colegio de Postgraduados, Campus Montecillo, located at 19° 27′ 34.8″ N and 98° 54′ 15.8″ W at an altitude of 2249 m, to set up the experiment. In order to prevent tissue dehydration during transport, buds were moistened with tap water, wrapped in paper, and placed in polyethylene bags in a cooler with ice. When they arrived at the nursery, they were refrigerated at 4 °C, and the following day they were hydrated by submerging them in a solution of foliar fertilizer Bayfolan® S (Bayer, State of Mexico, Mexico), 2 g L^{-1} water,

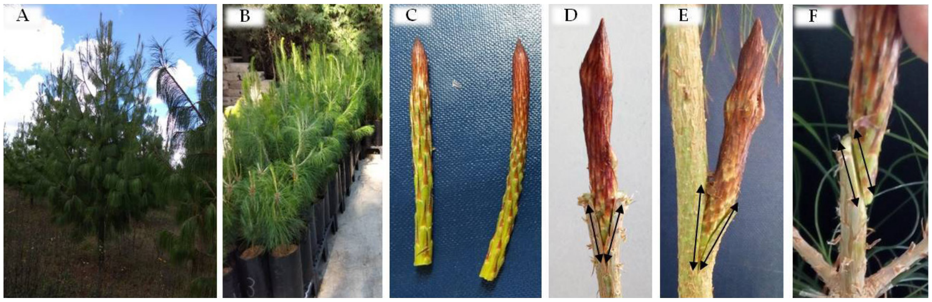

Figure 1. Characteristics of the *Pinus patula* plant material used, and the grafting techniques evaluated in the experiment. A: scion-donor trees; B: rootstock plants; C: scions; D: top cleft graft; E: side veneer; F: splice graft. Arrows indicate the cuts in each grafting technique.

for 10 min. They were then washed with tap water, liquid soap, and 1 mL L^{-1} sodium hypochlorite to eliminate dust and surface resin. After this, they were submerged in a solution of tap water and fungicide powder Captan 50® (ADAMA, Jalisco, México), 1.5 g L^{-1}, for 20 min. Finally, the buds were cut to a length of 7.0 cm to obtain scions with uniform size.

Treatments—grafting techniques

The experiment established three types of graft (top cleft, side veneer and splice grafting). For the top cleft graft, the stem was first cut horizontally (decapitation) above the last whorl of the rootstock. Needles in the main stem were then removed, and the decapitated area was cleaned with a solution of water and quaternary ammonium salts (Pursue®, Michigan, USA), 2 mL L^{-1}. Immediately in this decapitated area, a vertical cut of variable length depending on the treatment (1.5, 2.0, or 2.5 cm) was made with a cutter to create the slit. At the base of the scions, two cuts were made with a scalpel and a number-12 blade in the form of a wedge, aiming to obtain only one plane on each side. Once the cuts were made in the stem of the rootstock and at the base of the scion, the cambium zones of both were matched when the scion was inserted into the slit in the rootstock (Figure 1D). Immediately, to seal the grafted area and prevent displacement, a plastic stripe (“thin grafting tape” 1 cm wide and 50 μm thick) was wrapped several times around the area. After placing the tape, it was pressed firmly and continuously to favour the joining of the internal structures. Finally, a transparent polyethylene bag (15 - 25 cm) was placed and closed over the graft to generate and maintain a humid microenvironment, protecting the graft from dehydration.

The side veneer graft was done 10 cm above the base of the rootstock stem (without decapitation). In this area, with the cutter, a slit was made at a certain degree of inclination, while at the base of the scion the cut was made in the shape of a wedge with the three different cutting lengths described for the top cleft graft. The scion was then inserted into the slit in the rootstock (Figure 1E). Placement of the transparent polyethylene bag and the tape at the point of joint was also similar to that for the top cleft grafting technique.

For the splice graft, handling of the rootstock and the scion was similar to that for the top cleft graft in almost all the steps, differing only in the cut at the base of the scion and in the upper part of the rootstock. In this case, the cut was diagonal with the same length and angle to match the two structures, also, the three cutting lengths described for the other two types of grafts were also applied. Finally, the joint was tied, and the polyethylene bag was positioned as in the two previous techniques (Figure 1F).

The grafts were kept in greenhouse conditions with manual irrigation and partial temperature control, plastic roof and anti-aphid screen that protected them against wind, rain, hail, and low temperatures. To allow adequate ventilation, the grafts were placed on plastic bases, leaving a space between the graft and the floor. Using a data-logger sensor Temp/Hum HOBO® (Model U12-O12, Onset, USA), temperature (9.0 – 30.1 °C) and relative humidity (39.3 – 89.8%) were recorded. The polyethylene bags were removed gradually from the grafts that exhibited success. In the third week, a small cut was made at the corners of the bag; in the fifth week, the top of the bag was opened completely. The plastic at the point of union was not removed until week 12 to assure the internal connections between the scion and the rootstock, as well as the complete scarring and callus formation.

The experiment was set up in a completely randomized design with factorial arrangement. The two factors evaluated were grafting technique with three levels (top cleft, side veneer and splice grafting), and the cutting length with three levels (1.5, 2.0, and 2.5 cm) on rootstock and scion, with ten replications, generating a total of 90 grafts. Sixty days after grafting, three variables were evaluated: 1, graft success; 2, grafted scion elongation; and 3, initial needle development. Graft success was determined when scion presented elongation, needle growth, vigour, turgidity, and the characteristic green colour of live buds. The scions that turned brown, were dehydrated, and were not turgid or vigorous were considered as "not successful". Scion growth was evaluated by subtracting the initial length of the scion (7 cm) from its length 60 d after grafting. Successful grafts were recorded as the percentage of scions that had developed needles longer than 1 cm.

The data obtained from the variables evaluated were subjected to analysis of variance with R version 3.6.1, and then to the Tukey test for multiple comparison of means ($p \leq 0.05$). When the assumptions of normality were not met, the data on success and needle development were transformed with the function [T = arcsine ($\sqrt{Y}$)] before performing the analysis of variance and later they were re-transformed to the original units with the function [$Y = 100\ \text{sine}^2\ (T)$] (Cuevas-Cruz *et al*., 2015). A graph to depict the interaction (grafting technique × cutting length) was constructed for the variable that presented statistical difference.

$$Y_{ijk} = \mu + T_i + L_j + TL_{ij} + \epsilon_{ijk}$$

Y_{ijk} = value of the response variable corresponding to replication k of level i of T and level j of L; μ = general mean; T_i = effect of the grafting technique; L_j = effect of cutting length; TL_{ij} = grafting technique × cutting length interaction; ϵ_{ijk} = experimental error; i = top cleft, side veneer or splice; j = 1.5, 2.0 and 2.5 cm; k = 1, 2,…, 10th replication.

RESULTS AND DISCUSSION

After 60 d, when the experiment ended, more than 50 % of all the grafts were successful, with growth in length of more than 17 mm, and more than 60 % of the grafts developed needles (Table 1). The percentage of successful grafts is considered acceptable for conifers since, in general, for species of this group, successful grafts of less than 50 % have been reported (Muñoz *et al*., 2013). Moreover, growth in length and development of needles in the grafts demonstrate grafting viability (Table 1). In grafts of *P. pseudostrobus*, growth and needle development indicate the establishment of new cambium tissue between the two parts, which reactivates physiological functions to initiate development and growth of a complete plant then constituted by two genotypes (Barrera-Ramírez *et al*., 2020).

Table 1. Analysis of variance and test of means (±standard error) of the variables graft success, elongation (cm), and needle development for the factors grafting technique (GT), cutting length (CL), and their interaction (GT × CL) in grafts of *Pinus patula*.

Factor	DF	Graft success (%)	Growth (mm)	Needle development (%)
Grafting technique (GT)	2	0.022†	0.749ns	0.354ns
Top cleft		80.0 ± 5.8 a	17.1 ± 5.5 a	66.9 ± 2.6 a
Side veneer		73.3 ± 3.3 ab	17.6 ± 3.0 a	69.0 ± 10.4 a
Splice		56.7 ± 3.3 b	17.6 ± 5.2 a	45.6 ± 19.3 a
Average		70.0 ± 4.1	17.6 ± 2.7	60.5 ± 7.4
Cutting length (CL)	2	0.070ns	0.075ns	0.138ns
1.5 cm		66.7 ± 3.3 a	25.5 ± 7.2 a	80.2 ± 4.4 a
2.0 cm		66.7 ± 8.8 a	17.7 ± 3.5 a	51.3 ± 15.9 a
2.5 cm		76.7 ± 8.8 a	10.2 ± 1.7 a	50.0 ± 9.6 a
Average		70.0 ± 4.1	17.6 ± 2.7	60.5 ± 7.4
GT x CL	4	0.210ns	0.040†	0.529ns
CV (%)		16.5	57.5	34.5

Mean values of treatments with different letters in a column within each factor studied indicate statistical difference. DF: degrees of freedom. CV: coefficient of variation. Statistical differences when †$p \leq 0.05$ and ns, not statistically different.

The statistical analysis of the grafting techniques (GT) evaluated showed that only graft success had statistical difference ($p \leq 0.05$), while for cutting length (CL) there were no differences in the three variables evaluated. For the interaction between the two factors (GT x CL), only the variable graft growth was different (Table 1)

Effect of the grafting technique

Graft success

Of the three grafting techniques evaluated, top cleft grafting obtained the highest percentages of success. With this technique, success increased more than 20%, relative to that observed with splicing, which had the lowest percentages, while side veneer grafts had intermediate values (Table 1). Mugerwa and Okullo (2010) obtained similar results with grafts of *Pinus caribaea* var. *hondurensis*; the top cleft type favoured success of the grafted scion by 25%, relative to splicing. The authors attributed the success of top cleft grafts to a more rapid, stronger joining that allowed transport of water and nutrients to the scion.

In conifer species satisfactory results have been obtained with both top cleft and side veneer grafting (Barrera-Ramírez *et al*., 2020; Pérez-Luna *et al*., 2020), although side-veneer grafting has been more frequently applied (Blada and Panea, 2011). The success of both techniques is related to the internal and external anatomy of the tissues at the joint between the scion and the rootstock (Castro-Garibay *et al*., 2017). Villaseñor and Carrera (1980) reported 80% success with top cleft grafts and 25% less with side veneer grafts for *P. patula*. For the same species, Aparicio-Rentería *et al*. (2013) obtained 38%

less success for side veneer grafts, compared with the results of this study, arguing that the low success was due to extreme climatic factors, mainly temperature and humidity. The election of one of the two techniques (top cleft or side veneer) in *P. patula* will depend on the final objective; both have advantages and disadvantages to be considered. The techniques of top cleft and side veneer grafting have generally been applied for the establishment of asexual seed orchards (Aparicio-Rentería *et al*., 2013). Particularly, side veneer grafting could also be considered when the objective is to manage hedges for the production of adventitious shoots since the genotype of interest is located closer to the roots, which favours maintaining juvenile characteristics (Wendling *et al*., 2014).

On the other hand, the lower success of splice grafting is because the technique is only one diagonal cut (therefore just one plane) on both the scion and the rootstock (Pardo-Alonso *et al*., 2020), and the surfaces of the cambium (scion-rootstock) only overlap and are not fixed in place as in the case of the top cleft and side veneer grafts, in which the scion is inserted into a slit with two exposed planes (Hartmann *et al*., 2014). Indeed, the scion is placed under the pressure that the two planes of the rootstock exert, favouring contact of both cambium regions (Mugerwa and Okullo, 2010). The splicing technique is more fragile at the joint and more susceptible to movement, increasing the risk of the parts of the graft displacing and interrupting the internal connections.

Also, the area of cambium contact is smaller, but this structure is responsible and key for a successful joining (Pina *et al*., 2017; Gautier *et al*., 2019). However, the splice graft could be another alternative for cloning *P. patula*, particularly for small rootstock and scions. It has been recommended for stems smaller than 5 mm in diameter, for which other types of cuts are difficult (Lee *et al*., 2010). This technique has been applied to obtain grafts at a greater scale in species of the genera *Abies* and *Pinus* using scions obtained from hedges of superior genotypes grafted onto rootstock less than one year old (Pérez-Luna *et al*., 2020).

Currently, this technique is widely used for mass production of scions for grafting tomato (Pardo-Alonso *et al*., 2020). However, because the joint in that case is more fragile, the use of special silicon clips should be considered to maintain continuous firm pressure and better alignment to guarantee contact of the vascular systems of both parts (Lee *et al*., 2010; Pardo-Alonso *et al*., 2020). The plastic material used in this study to secure the joint could have negatively influenced the pressure required to maintain the scion joined to the rootstock. Blada and Panea (2011) recommend using the most appropriate material to secure the parts of the graft to guarantee the scarring.

Growth in length and needle development in the grafted scion

Grafting type had no effect on the variables of growth in length and needle development of the grafted scion (Table 1). This indicates that the grafts, regardless of the technique used, responded favourably. Once the grafts successfully joined, physiological reactivation of the bud was immediately manifested in initial growth and needle development on the grafted scion (Muñoz *et al*., 2013). Greenwood *et al.*

(2010) showed that successful grafts present immediate development and growth in scions grafted onto *Picea rubens* Sarg. In a study with *P. pseudostrobus*, however, there was a difference in growth of 2.2 cm between side veneer and top cleft grafting techniques. The authors considered that the best growth of the side veneer grafts occurred because of their location near the roots thus allowing better flow for water and nutrients (Barrera-Ramírez *et al*., 2020).

In this study with *P. patula*, low growth in length (only 17 mm; Table 1) with the three grafting techniques evaluated was likely due to the season of the year, given that data on this variable were recorded in December. In this season active growth of the buds of this species decreases because of both a shorter photoperiod and lower temperatures (Viveros-Viveros and Vargas-Hernández, 2007; Pérez-Luna *et al*., 2019).

Despite no effect of the grafting technique on the percentage of plants with developed needles was detected (Table 1), it is important to consider that after the graft succeeds the growing needles will be the organs responsible for generating the carbohydrates necessary for the vital processes, future growth, and survival of the grafted plant (Lal, 2018). These needles are more indispensable during establishment of the side veneer grafts since, for this technique, all of the rootstock's aerial part must be eliminated and, in the end, only the needles remain to achieve development of the grafted scion (Barrera-Ramírez *et al*., 2020).

Effect of cutting length

Graft success, growth, and needle development of the graft

The different cutting lengths on the rootstock and the scion were not statistically different in the considered variables (Table 1). It was expected that the longest cutting length (2.5 cm) would favour a higher percentage of success than the other two cuts (1.5 and 2.0 cm). In some studies, cutting length has been important for grafting success. In *Mangifera indica* L. the size of the cut (> 4 cm) was determinant for graft success (Mng´omba, 2013). Blada and Panea (2011) found that that the highest percentage of graft success was achieved with larger cuts, which generated larger contact surface (4 to 5 cm) between the cambium of the rootstock and that of the scion on side veneer grafts of *Picea pungens* Engelm. var. *glauca* Regel. These authors argued that the large contact surface influenced development of the callus, the cell structure necessary for the reconnecting tissue (scarring). Scarring is the first step in the formation of new conveyance structures between the parts the make up the graft (Gautier *et al*., 2019).

Nevertheless, graft success does not depend only on the cutting length but also on the species and type of graft. For side veneer grafts of *P. pseudostrobus*, a cutting length of 3 – 5 cm has been recommended (Pérez-Luna *et al*., 2019). Whilst for *P. caribaea*, cutting length needs being shorter than 2.5 cm in top cleft grafting or than 2 cm in splicing (Mugerwa and Okullo, 2010). Based on the results of this research, cuts from 1.5 to 2.5 cm (with no statistical difference detected by length) are appropriate to expect more than 65% success in grafts of *P. patula* (Table 1). This means that, within that interval of cutting length, the area of contact of the cambium was sufficient to achieve efficient

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scarring (Figure 2E, F and G). A cutting length less than this interval could result in a mechanically weak union between the parts of the graft, affecting the formation of the callus and of new vascular tissues by excessively reducing the contact area of the cambium (Hartmann *et al*., 2014; Pina *et al*., 2017).

To increase the percentage of success obtained in the *P. patula* grafts, other factors that also affect grafting success should be considered. First, the morphology and the phenological state of the buds used as scions at the moment of collection; since in conifers these vary considerably among genera, species, provenance or genotypes (Castro-Garibay *et al*., 2017; Flores *et al*., 2019); Second, the angle of the cut and diameter of scion and rootstock that permit larger contact surface and a better match between the two areas of union of the graft, which increases the probability of the two vascular tissues reconnecting (Pardo-Alonso *et al*., 2019).

The scion successfully resumes its growth and development when the vascular connection is established with functional xylem and phloem to obtain water and nutrients, given the process involves high water demand (Hartmann *et al*., 2014; Guadaño *et al*., 2016). An indicator of possible incompatibility is the lack of graft development, which can occur when vascular connection is insufficient, discontinuous or because of phloem degeneration in the graft area, causing an imbalance in water and nutrient transport that affects growth of the graft (Martínez-Ballesta *et al.*, 2010; Pina *et al*., 2017).

Effect of interactions between factors

The interaction between factors (grafting type and cutting length) generated differences in growth of the grafts (Table 1). The highest growth values (> 30 mm) were obtained with the following combinations: 1) top cleft x 1.5 cm cut, 2) splice x 1.5 cm cut, and 3) side veneer graft x 2.0 cm cut (Figure 3).

The best results in growth were obtained with shorter cuts (1.5 cm), with which the contact area between the rootstock and the scion is smaller, combined with the grafts that were performed near the terminal bud of the rootstock (top cleft and splice)

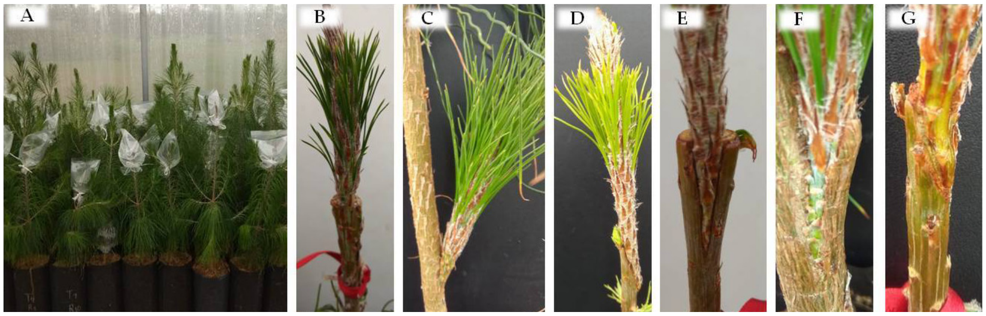
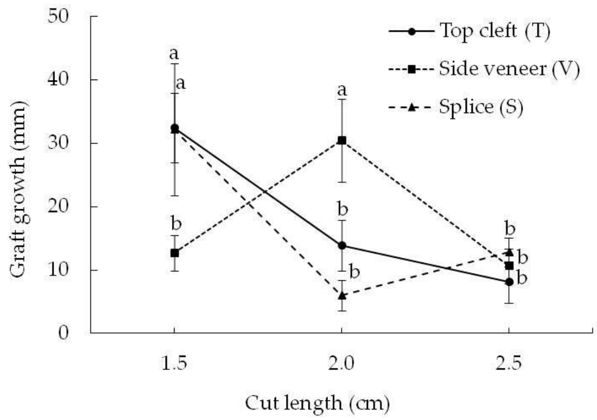


Figure 2. Experimental setup and successful *Pinus patula* grafts. A: grafts covered with plastic bags to prevent dehydration; B, C, D: top cleft graft, side veneer graft, and splice graft respectively 60 d after insertion; E, F, G: callus forming on top cleft, side veneer, and splice grafts at 90 d.



GT	CL	$P \leq 0.05$
T	1.5	<0.0001
S	1.5	<0.0001
V	2.0	<0.0001
T	2.0	<0.0001
S	2.5	<0.0001
V	1.5	<0.0001
V	2.5	<0.0001
T	2.5	0.0006
S	2.0	0.0448

Figure 3. Means of graft elongation (± standard error) in *Pinus patula* and values of *p* for the combination on each level of the grafting technique (GT), top cleft (T), side veneer (V) and splice grafting (S) with each level of the factor cutting length (CL): 1.5, 2.0, and 2.5 cm. Different letters indicate significant differences (Tukey, $p \leq 0.05$).

(Figure 3). These interactions suggest that the grafts that were done in the apical region of the plants achieve better reconnection of tissues when the cuts are smaller, and this is reflected in the growth of the grafted scion. It is likely that hormones play an important role in these interactions. It is known that auxins accumulate near the apex of the plants where they are synthesized (apical meristem) and are responsible for vascular formation and differentiation, as well as for scarring during the union of parenchymatous tissues in the graft (Goldschmidt, 2014; Nanda and Melnyk, 2018).

In this research, it was observed that the process of scarring was faster in lesions made by small cuts. The faster the union of the tissues through scarring, the faster the cell-to-cell communication via plasmodesma (membrane channels) and thus the faster the reactivation of the physiology of the grafted plant. This allows directing the available resources and stimulating both growth and development of the graft as if it were a single individual (Goldschmidt, 2014; Hartmann *et al*., 2014). Therefore, it can be expected that the faster the callus forms, the faster the growth of the graft shall begin.

CONCLUSIONS

Grafting technique and cutting length are determining factors for success, growth, and needle development in *Pinus patula* grafts. The study demonstrated that the most viable techniques for increasing success of *P. patula* grafts are top cleft and side veneer grafting, which are also the most commonly used grafting techniques with conifers.

Cutting length in the evaluated range did not define success of the graft, and thus applying a cut between 1.5 and 2.5 cm can be suggested for future grafting trials. When cutting length is combined with grafting technique, favourable combinations were obtained in graft growth, in particular, top cleft grafting with 1.5 cm of cutting length.

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