Revista Mexicana de Ciencias Forestales Vol. 14 (75)
Enero - Febrero (2023)

DOI: 10.29298/rmcf.v14i75.1255

Article

Efecto del riego, la fertilización y el contenedor en la respuesta a la injertación de plántulas de *Pinus patula* Schltdl. & Cham.

Effect of irrigation, fertilization and the container on the response to grafting of *Pinus patula* Schltdl. & Cham. Seedlings

Edgar David López Avendaño[1], Miguel Ángel López López[1*], Carlos Ramírez Herrera[1], Manuel Aguilera Rodríguez[1]

Fecha de recepción/Reception date: 17 de febrero de 2022
Fecha de aceptación/Acceptance date: 20 de noviembre del 2022

[1]Posgrado en Ciencias Forestales, Colegio de Postgraduados, Campus Montecillo. México.

[*]Autor para correspondencia; correo-e: lopezma@colpos.mx

[*]Corresponding author; e-mail: lopezma@colpos.mx

Abstract

Grafting success depends on multiple factors, including species-dependent ones. However, there are few studies on the effects of management of rootstock and the composite (grafted) *Pinus patula*, seedlings on graft performance. The objective of this study was to evaluate the grafting success and development of *P. patula* shoot grafts based on factors associated with management of both the rootstock and the composite (grafted) plant. In a 2x2x2 factorial experiment, the rootstocks were subjected to two types of container, two levels of irrigation and two levels of fertilization one month before grafting, which went on with the treatments until the end of the experimental period. Grafting success (*PI*), graft basal diameter (*DBI*), graft length (*LI*), graft robustness index (*IR*), graft basal diameter increase (*IDBI*) and graft length increase (*ILI*) were evaluated. Mann-Whitney tests and analyses of variance followed by Tukey tests (α=0.05) were applied. The factors tested did not affect statistically or directly *PI*, whose general mean value was 72.5 %. The container type and levels of watering and fertilization significantly affected all the assessed morphological variables.
Key words: Slow release fertilizer, graft, rootstock, grafting success, asexual reproduction, saturation watering.

Resumen

El éxito de la injertación depende de múltiples factores, algunos especie-dependientes. Sin embargo, existen pocos estudios en *Pinus patula* relativos a los efectos del manejo del patrón y de la planta compuesta (injertada) sobre el desempeño del injerto. El objetivo del presente estudio fue evaluar el prendimiento y desarrollo de injertos de brotes de *Pinus patula* en función de factores asociados tanto al patrón, como a la planta injertada. En un experimento factorial 2×2×2, los patrones se sometieron a dos tipos de envase, dos niveles de riego y dos niveles de fertilización un mes antes de la injertación, y se continuó con los tratamientos hasta el término del período experimental. Se evaluó el prendimiento de los injertos (*PI*), el diámetro basal del injerto (*DBI*), la longitud del injerto (*LI*), el índice de robustez del injerto (*IR*), el incremento en diámetro basal del injerto (*IDBI*) y el incremento de la longitud del injerto (*ILI*). Se utilizaron pruebas de *Mann-Whitney* y de análisis de varianza, seguidos de pruebas de comparación de medias de *Tukey* (α=0.05). Los factores probados no influyeron

estadística ni directamente en el *PI*, cuyo valor general promedio fue de 72.5 %. Los tipos de contenedor y los niveles de riego y fertilización sí afectaron significativamente las variables morfológicas evaluadas.
Palabras clave: Fertilizante de liberación lenta, injerto, planta patrón, prendimiento, reproducción asexual, riego a saturación.

Article

Introduction

Pinus patula Schltdl. & Cham. is an endemic species to central Mexico (Perry, 1991; Farjon and Styles, 1997). It owns qualities that make it one of the most important species in this country provided its high productivity and abundance in forests (Dvorak, 2002; Rivera-Rodríguez *et al.*, 2016). In addition, their trees are fast growing, have good natural pruning and high quality wood (Velázquez *et al.*, 2004).

Recently, forestry researchers in Mexico have shown interest in developing forest genetic improvement programs from selected parents (Camcore, 2008), using grafts as a method of clonal propagation. The cloning of forest trees through this procedure allows multiplying genotypes of mature trees with characteristics of economic and ecological interest as part of forest genetic improvement (Wang, 2011; Vargas-Hernández and Vargas-Abonce, 2016; Pérez-Luna *et al*., 2020).

The composite plant is made up of the scion and the rootstock, which together form a new plant with different genotypes in which the scion, shoot or bud donated by the tree has desirable genetic characteristics and constitutes the component of the upper part of the graft, while the rootstock is its lower part that provides the root system, as well as water and nutrients for the development of the graft (Wright, 1976; Mudge *et al.*, 2009; Ranjith and Ilango, 2017; Kita *et al.*, 2018). One of the most widely used techniques for this procedure in coniferous species is the terminal cleft graft, also known as terminal graft (Muñoz *et al.*, 2013).

1. The success of grafting depends on the factors that condition it, among which are the type and technique of grafting, the compatibility between scion and rootstock, the quality and origin of the rootstock, the vigor and origin of the bud, the position of the bud in the crown of the donor trees, grafting season, the experience of the grafter, the genetic, taxonomic and anatomical characteristics of the rootstock and scion, the connection of the cellular tissue between the rootstock and the scion, and the presence or absence of growth regulating hormones (Villaseñor and Carrera, 1980; Moore, 1984; Jayawickrama *et al.*, 1991; Lott *et al.*, 2003; Valdés *et al.*, 2003; Pina and Errea, 2005; Hibbert-Frey *et al.*, 2011; Darikova *et al.*, 2013; Muñoz *et al.*, 2013; Goldschmidt, 2014; Pérez-Luna *et al.*, 2019; Pérez-Luna *et al.*, 2020).

To submit plants to a grafting cycle it is necessary to produce high quality rootstocks; however, there are few studies on techniques in this regard with pine species, which leaves this activity unattended even knowing the importance of the rootstock on the graft success and growth (Hibbert-Frey *et al*., 2010; Darikova *et al.*, 2013; Kita *et al.*, 2018).

The extensive literature on the topic reveals that the pattern has effects on multiple characteristics of the scion, such as success, development, vigor, fruiting and growth habit, among others (Jayawickrama *et al.*, 1991; Verdugo-Vásquez *et al*., 2021). However, most of the studies analyze the effects of the genetic characteristics of the rootstock and there are very few that address aspects of its management (Copes, 1980; Barrera-Ramírez *et al.*, 2021). However, there is evidence that the vigor of the rootstock is related to the future behavior of the grafted plant and that management practices of the rootstock that influence its vigor probably affect the grafting and the performance of the grafted plant.

Thus, the objective of the present study was to evaluate the grafting and the growth of the grafted plant or composite plant of *Pinus patula*, depending on factors associated, both to the rootstock and to the grafted plant, by testing types of container, fertilization levels and irrigation levels. The null hypothesis is that the tested factors do not affect the success or the morphology of the composite plant.

Materials and Methods

Study area and origin of scions

Scions from six nine-year-old trees with average diameter and height of 20.61 cm and 16.25 m, respectively, were used. The trees belonged to a forest plantation that was established in August, 2011 on the *Casa Redonda* property of the *Palo Bendito ejido*, *Huayacocotla* municipality, in the state of *Veracruz*, Mexico, between 20°27'22” N and 98°29'00” W, at an altitude of 2 460 m.

The selection of the trees was based, essentially, on the phenological state of the shoots, that the trees were free of pests and apparent diseases, and that they showed good phenotypic characteristics such as self-pruning, straightness of the stem and absence of bifurcations, in addition to outstanding diameter (sociological dominance). The shoots collected on January 14th, 2021, were still in the dormant stage, with an average length of 6 cm.

The shoots were collected from the outer middle part of the crown, where the recent vegetative growth appears; they were collected and grouped to later store them in bags labeled with the corresponding tree number; they were stored in a cooler with cans with frozen water, covered with a damp cloth to keep them at a low temperature and avoid stress and loss of turgor. The shoots were grafted 24 h after being cut.

Origin and preparation of rootstocks

Work was handled with nine-month-old *P. patula* plant rootstocks, 7.1 mm in basal diameter and 56.3 cm in height, from the *Pueblo Nuevo* Forest Nursery, located in *Peñuelas Pueblo Nuevo* community, *Chignahuapan* municipality, in the state of *Puebla* (19°57'36" N and 98°06'26.20" W, at 2 680 masl).

The seed came from a seed orchard located in the vicinity of the nursery itself. The plants were produced in polystyrene trays with 77 cavities 170 cm^3 in capacity under 50 % shade mesh conditions. The specimens were grown in a mixture of sawdust, peat moss, perlite (Multiperl®) and vermiculite (Agrolita®) substrate in a 70:10:10:10 (vol:vol) ratio. The nutritional management consisted of the use of Osmocote® 18-06-12, in doses of 7 g L^{-1}, in addition to Micromax® at a rate of 200 g m^{-3}.

One month before grafting the plants, they were transplanted into two types of containers: 1.9 L pots and 310 cm^3 tubes. The substrate consisted of a homogeneous mixture of peat moss, PRO-MIX FLX®, perlite and vermiculite in a 6:3:1 ratio, respectively. Half of the standard plants of each type of container (30 plants) were added 2 g L^{-1} of Osmocote Scotts® 14-14-14, with 8 months release (light fertilization) and the other half (30 plants), 7 g L^{-1} of the same material. To this second group, a nutrient solution from Landis *et al.* (1989) every 15 days (heavy fertilization) until the end of the experimental period, to ensure a sufficient nutritional supply (full fertilization). It was added the aforementioned nutrient solution contains all the macro and micronutrients, and was formed by using regionally available soluble fertilizer materials (Table 1). Irrigation was applied every third day; a group of 60 plants was irrigated at 100 % saturation and another only at 40% saturation.

Table 1. Nutrient solution suggested by Landis *et al.* (1989) and fertilizer materials used.

Mineral nutrient	Concentration (ppm)	Fertilizer material used
	Macronutrient	
N	50	Urea
P	60	Phosphoric acid
K	150	Potassium sulfate
Ca	80	Sagaquel Ca®
Mg	40	Sagaquel Mg®
S	60	Potassium sulfate + copper sulfate
	Micronutrient	
Fe	4	Sagaquel Fe®
Mn	0.8	Sagaquel Mn®
Zn	0.32	Sagaquel Zn®
Cu	0.15	Copper sulfate
B	0.5	Nutriboro®

Grafting procedure

Grafting was carried out during the second week of January 2021 at the facilities of the *Montecillo* Campus Forest Nursery, *Colegio de Postgraduados*, *Texcoco*, State of Mexico (19°27'46.8" N, 98°54'23" W; 2 240 masl). The grafting technique implemented in the experiment was the terminal cleft technique described by Muñoz *et al.* (2011 and 2013), which has produced better results than other techniques in *P. patula* (González-Jiménez *et al.*, 2022).

Prior to grafting, the scions were washed for three times: 1) immersion in water with a prepared solution of commercial liquid soap and 1 % diluted chlorine and were manually shaken for 5 minutes to ensure the removal of dust, resin and organisms that could be on its surface, 2) rinsed with running water and submerged again in a second solution prepared with Captan® 1.5 g L^{-1} for 15 minutes to prevent and avoid subsequent phytosanitary problems, and 3) rinsed again with running water and deposited in a cooler to keep the shoots cool and turgid before grafting scion by scion.

The terminal grafting technique consisted of making a transversal cut in the rootstock, at a height of 15 cm, after eliminating the needles or fascicles present three cm below the height of the cut; subsequently, a longitudinal cut of 3 to 5 cm was made right in the center of the stem of the rootstock, so that the scion could be inserted into this slit, to which two diagonal cuts were previously made on opposite sides of its base (the size pattern slit), leaving the scion in a "v" shape.

The scion was inserted into the slit of the rootstock, in such a way that the cambium of both coincided. Once the union was made, it was fixed with Parafilm® tape throughout the wound, to avoid phytosanitary problems in the graft area, or dehydration of the tissues at the union point. Finally, a transparent plastic bag (30-40 days) was placed covering the entire scion, including the Parafilm® tying, to create an atmosphere of adequate humidity and temperature for the graft. The bag was gradually withdrawn until the graft was "successful" and adapted to greenhouse conditions. Once the grafting process was finished, the grafted plants were accommodated in a space inside the greenhouse in a completely random manner and the complementary irrigation and fertilization activities were implemented according to the corresponding treatments.

Irrigation and soluble fertilization

Irrigation was carried out every third day from the month prior to grafting (December 11, 2020) until the conclusion of the experimental phase (April 20, 2021) and at all times the pH was adjusted to 5.5. On each irrigation date, four grafted plants were randomly selected for each type of container (1.9 L pots and 310 cm^3 tubes) and the amount of water needed (in grams) to saturate their substrates was determined by weighing them. The average amount necessary (of the four containers) to saturate the substrate was incorporated into all containers with a 100 % saturation irrigation regime. Only 40 % of that quantity was applied to the containers whose irrigation regime 40 % irrigation.

For the treatments that received soluble fertilization, the nutrient solution suggested by Landis *et al.* (1989) (Table 1), which was applied every 15 days as part of the complementary fertilization to the group of plants that received 7 g L^{-1} of slow-release fertilizer, was added so that, as a whole, it constituted a complete fertilization.

In addition, in the week that no nutrient solution was applied, a Captan® 1.5 g L^{-1} solution was prepared to irrigate all the plants with the proportion of water that corresponded to them according to the irrigation regime, in order to prevent phytosanitary problems. that could influence the success of the graft.

Assessed variables

The graft success (*PI*) was evaluated 70 days after grafting was carried out, on the live scions only. Graft basal diameter (*DBI*) and graft length (*LI*) were also measured at 70 and 100 days after grafting. From these measurements, the robustness index (*IR*) and the increments in basal graft diameter (*IDBI*) and graft length (*ILI*) were derived.

The *DBI* measurements were obtained with a CD67-S6"PM Mitutoyo digital vernier caliper, and the *LI* was measured with a conventional 30 cm ruler. The *DBI* was measured immediately above the grafting point, while the *LI* was measured from this point to the apical bud of the graft. The *IR* was obtained by dividing the *DBI/LI*; the *IDBI* based on the basal diameters corresponding to the first and second evaluation, that is:

$$IDBI = DBI_{(100\ días)} - DBI_{(70\ días)}$$

In a similar way, the *ILI* was calculated as the difference in length growth between the second and the first evaluation:

$$ILI = LI_{(100\ días)} - LI_{(70\ días)}.$$

Experimental design and statistical analysis

A complete randomized experimental design was used with a 2×2×2 factorial arrangement: two types of container (310 mL tube and 1.9 L pot), two irrigation levels (100 % and 40 % irrigation of saturation) and two fertilization levels (2 g L^{-1} of Osmocote® 14-14-14 only and 7 g L^{-1} Osmocote® 14-14-14 + nutrient solution from Landis *et al.* [1989]). The combination of the levels of the three factors tested resulted in a total of eight treatments, which were replicated 15 times, and the experimental unit was represented by a grafted plant.

An analysis of variance (ANOVA) was performed to determine the effect of the factors and the interactions on the *DBI*, *LI*, *IR*, *IDBI* and *ILI* with the data from the evaluation at 100 days.

The experimental design model was:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkl} \qquad (1)$$

Where:

Y_{ijkl} = Value of the response variable of level *i* of *A*, level *j* of *B*, level *k* of *C*

μ = General mean

A_i = Effect of the *i* level of the *A* factor (container)

B_j = Effect of *j* level of the *B* factor (irrigation)

C_k = Effect of the *k* level of the *C* factor (fertilization)

AB_{ij} = $A \times B$ interaction corresponding to the *i* level of *A* and the *j* level of *B*

AC_{ik} = $A \times C$ interaction corresponding to the *i* level of *A* and the *k* level of *C*

BC_{jk} = $B \times C$ interaction corresponding the *j* level of *B* and the *k* level of *C*

ABC_{ijk} = $A \times B \times C$ interaction corresponding to the *i* level of *A*, the *j* level of *B* and the *k* level of *C*

ε_{ijkl} = Experimental error corresponding to the *l* replication of the *i* level of *A*, the *j* level of *B* and the *k* level of *C*

In order to analyze graft success (*PI*), due to the fact that the assumptions of normality and homogeneity of variance were not met, even testing several transformations of the variable, the non-parametric Mann-Whitney test was applied to detect the effects of the factors studied. A comparison of means was performed by Tukey test, with a confidence level of α=0.05. Statistical analyzes were performed with version 9.0 of the statistical software Statistical Analysis System (SAS, 2002).

Results and Discussion

Grafting success

After 70 days from the day of grafting, the average grafting success(*PI*) was 72.5 %; which is relatively high, compared to that obtained by Barrera-Ramírez *et al*. (2021), who registered a *PI* of 41 % in *Pinus pseudostrobus* Lindl., but close to that achieved by Muñoz *et al.* (2013) in that same species (*P. pseudostrobus*; 82.8 %). Despite this percentage, the Mann-Whitney statistical test indicated that the tested factors did not significantly affect the process (Table 2).

Table 2. Mann-Whitney test for the graft success of terminal cleft grafts of *Pinus patula* Schltdl. & Cham.

Factor	Sum of scores	Mean score	Pr<Z
Container			0.4881
Pot	151.0	12.58	
Tube	149.0	12.42	
Fertilization			0.4406
Complete	147.0	12.25	
Light	153.0	12.75	
Irrigation			0.3057
100 % saturation	159.0	13.25	
40 % saturation	141.0	11.75	

Hildebrant (2017) mentioned that the rootstock container should be 5-10 cm in diameter by 10-15 cm deep to provide the plant with enough space for root growth. However, the present study shows that success is similar with a container volume of 310 mL than with one of 1.9 L. The absence of the effect of container volume is probably due to the fact that none of the container sizes limited root growth, provided the relatively small size of the rootstocks.

In regard to fertilization, Barrera-Ramírez *et al.* (2021) also did not recognize significant effects of rootstock fertilization on graft success in *Pinus pseudostrobus* Lind. var. *oaxacana* (Mirov) S. G. Harrison; instead, they determined that the succes is affected by factors associated with genetic affinity (origin of the material), phenology (grafting time) and type of graft. Studies by Barnett and Miller (1994), Yin *et al.* (2012) and Goldschmidt (2014) confirm the effects of the genetic affinity of the rootstock and the scion on graft success. These authors point out that a greater genetic compatibility between the components of the graft implies greater anatomical, morphological, physiological and biochemical affinity and a greater probability of successful grafting.

In the study described here, the amount of water supply before and after the grafting operation did not affect graft success ($Pr<0.3057$, Table 2). It is highly probable that this lack of effect is due to the decrease in the transpiration rate of the scion, achieved by placing a transparent polyethylene bag during grafting. Dabirian and Miles (2017) demonstrated that the reduction of the transpiration rate in the scion, in this case through the application of antiperspirant substances, effectively improves the success rates in watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai). In the grafting of forest species, the use of transparent bags to cover the scions and reduce transpiration is common (López, 2020; Pérez-Luna *et al.*, 2020; González-Jiménez *et al*., 2022). In this work, the transpiration rates of the scions were not assessed, but it is possible to deduce that they were reduced, in such a way that the two irrigation levels tested could be sufficient to adequately supply water to the buds.

Morphological variables

Graft basal diameter (*DBI*) and graft length (*LI*) showed significant differences ($p \leq 0.05$) between container types, fertilization levels, and irrigation levels, but not for interactions (Table 3), so that the proposed null hypothesis is rejected. This suggests that the individual effects of one factor on these variables are independent of the level taken by the other factors, and that the effects of such factors are additive (Berrington and Cox, 2007).

Table 3. Analysis of variance of the effect of two types of container, two fertilization regimes, two irrigation regimes and their interactions on morphological variables of *Pinus patula* Schltdl. & Cham. grafts.

Source of variation	**Basal diameter of the graft (*DBI*)**	**Length of the graft (*LI*)**	**Robustness index (*IR*)**	**Diameter increase (*IDBI*)**	**Length increase (*ILI*)**
Type of container	0.0004*	<0.0001*	0.1840	0.6394	0.0091*
Fertilization regime	0.0026*	0.0292*	0.4248	0.0079*	0.0193*
Irrigation regime	0.0103*	0.0006*	0.0216*	0.7204	0.1433
Type of container × Fertilization regime	0.8367	0.1122	0.1010	0.0746	0.0082*
Type of container × Irrigation regime	0.9468	0.7554	0.9602	0.3010	0.3047
Fertilization regime × Irrigation regime	0.8478	0.1696	0.2249	0.7933	0.0742
Type of container × Fertilization regime × Irrigation regime	0.4061	0.4268	0.0589	0.8597	0.0988

The importance of preparing the rootstocks before subjecting them to grafting lies mainly in the fact that they are responsible for providing the root system that in turn will provide water and nutrients to the graft (Ranjith and Ilango, 2017; Kita *et al.*, 2018). The development of the root system depends on the space available to grow, and according to Landis (1990), the type of container (size and color) directly affects the temperature of the substrate and this in turn, affects the development of the root. In addition to determining the growth space for the root, the volume of the container constitutes the size of the water and nutrient reservoirs for the plant (Pershey, 2014). Consequently, the type of container affects not only the root system of the composite plant, but also the growth in diameter and height of the plants (Castro-Garibay *et al*., 2016).

Copes (1980) mentioned that the vigor of the rootstocks is determinant for the subsequent growth of the graft; so that when the rootstock is small or not very vigorous, the graft frequently presents a plagiotropic growth, in contrast to the orthotropic growth of grafts on vigorous and well-rooted rootstocks. The vigor of the rootstock is determined, among other factors, by the size of the container (Landis, 1990), which possibly explains the greater growth of the graft in the 1.5 L container, compared to the 310 mL container in the present study (Table 4).

Table 4. Comparison of Tukey means (α=0.05) for morphological and quality variables in *Pinus patula* Schltdl. & Cham. grafts.

Factor	Level	*DBI*	*LI*	*IR*	*IDBI*	*ILI*
Container	Pot	6.1709 a	28.191 a	0.27445 a	0.41093 a	10.0047 a
	Tube	5.535 b	20.483 b	0.32683 a	0.44571 a	7.4762 b
Fertilizer	7 g L^{-1} + nutritive solution	6.1072 a	26.021 a	0.28577 a	0.52837 a	9.8256 a
	2 g L^{-1}	5.6002 b	22.705 a	0.31525 a	0.32548 b	7.6595 b
Irrigation	100 %	6.0222 a	27.236 a	0.2604 b	0.40378 a	9.2244 a
	40 %	5.6705 b	21.173 b	0.34526 a	0.4555 a	8.2275 a

DBI = Basal diameter of the graft; *LI* = Length of the graft; *IR* = Robustness index; *IDBI* = Diameter increase; *ILI* = Length increase.

Once the graft has got successful, we have a complete (composite) plant whose growth is affected by the same factors that define the growth of a non-grafted plant, in addition to others associated with the composite plant itself, such as hormonal aspects and permanence or not of the physical compatibility between rootstock and graft (Verdugo-Vásquez *et al*., 2021). However, in the present study a firm union between rootstock and scion was observed without callous deformations until the time of evaluation of the experiment. The use of slow release mineral fertilizers increases the longitudinal growth of the plant (Cañellas *et al.*, 1999), depending on the amount and type of nutrients it contains. For this reason, to produce rootstocks, Pérez-Luna *et al.* (2021) used, with good graft survival results, 10 g L^{-1} of Multicote® 6: 18-6-12 (NPK) + micronutrients, while Barrera-Ramírez *et al.* (2021) applied three different fertilization doses: 3, 5 and 8 kg of controlled release fertilizer Multicote® 15-7-15 (NPK) + 2 MgO + micronutrients per cubic meter of substrate, and achieved better results with the highest fertilizer dose.

In the present study, the level of fertilization influenced the growth in basal graft diameter (*DBI*) since it increased when complete fertilization was applied (7 g L^{-1} of Osmocote 14-14-14 + nutritive solution from Landis *et al.* (1989) (Table 4). However, the same did not occur with graft length (*LI*) since there were no statistically significant differences in shoot elongation, nor did it occur with *IR*. On the other hand, the increases (between the first and second evaluation) in diameter and elongation of the shoot aumented with complete fertilization, in coincidence with what was reported in the study conducted by Mutabaruka *et al.* (2015) in *Castanea sativa* Mill.

The irrigation rate received by the grafts marked significant differences in *DBI* (5.6705 *vs* 6.0222 mm, with 40 and 100 % saturation, respectively) and *LI* (21.173 *vs* 27.236 cm, with 40 and 100 % saturation, respectively) with grafts growing the most when the substrate was saturated to 100 %. Despite these results, Hibbert-Frey *et al.* (2011) concluded that water availability did not affect scion growth when grafting *Abies fraseri* (Pursh) Poir. shoots on *Abies bornmuelleriana* Mattf.

rootstocks, although in this case, the interspecificity of the graft may have influenced the result.

The graft robustness index (*IR*) showed a significant difference ($p \leq 0.05$) between irrigation rates (Table 3). The *IR* is an indicator of the resistance of the plant to desiccation by wind, its survival and growth in dry locations, furthermore, it is a criterion that must be considered in the production of quality plants. Saenz *et al.* (2014) mentioned that to improve the *IR* it is necessary to produce plants in large containers to favor root development. In grafting projects, this variable has not been included, however, it will be necessary to establish the grafts in the field and determine their behavior in relation to the index.

The increase in basal graft diameter (*IDBI*) was only significantly affected ($p \leq 0.05$) by the fertilization regime while the increase in graft length (*ILI*), by the type of container, fertilization, and the double interaction type of container × fertilization regime, which indicates that the effect of the container on the length of the graft is affected by the fertilization regime and vice versa.

When performing the comparison of Tukey means (α=0.05; Table 4), statistical differences were found between levels of the factors studied. Growth in both diameter and graft length is higher when using rootstock in larger container, full fertilization, and heavy irrigation (additive effects of factors tested) (Berrington and Cox, 2007). This should be considered if increased growth rates of the composite (grafted) plants, especially in diameter and length is to be pursued.

Conclusions

Based on the variation induced by the various treatments applied, the terminal fissure grafting technique in *Pinus patula* during the winter season, registers an average success of 72.5 %. The graft success percentage is not affected by the tested levels of irrigation, fertilization and container.

The growth in diameter and length of the graft is influenced by the irrigation regime, the fertilization regime and the dimensions of the container; the combination of greater volume of the container, complete fertilization and saturation irrigation every third day, produces greater development of the graft.

The robustness index (*IR*) is only influenced by irrigation, the increase in basal diameter (*IDBI*) is affected by fertilization and the increase in graft length (*ILI*) by container size and fertilization, as well as the double interaction type of container × fertilization regime.

Acknowledgements

To the National Council of Science and Technology (*Conacyt*), for the financial support provided with grant number 1010725 to carry out the master's studies of the first author. To M. of Sc. Benito González Jiménez for his support in the grafting stage.

Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Edgar David López Avendaño: planning and conducting nursery research, data collection and statistical analysis, structure and writing of the manuscript; Miguel Ángel López López: research planning and monitoring during the experiment, analysis and validation of data, correction of the manuscript; Carlos Ramírez Herrera: advice on the project, collection of plant material in the field and correction of the manuscript; Manuel Aguilera Rodríguez: advice on the project and correction of the manuscript.

References

Barnett, J. R. and H. Miller. 1994. The effect of applied heat on graft union formation in dormant *Picea sitchensis* (Bong.) carr. Journal of Experimental Botany 45(1):135-143. Doi: 10.1093/jxb/45.1.135.

Barrera-Ramírez, R., J. J. Vargas-Hernández, R. López-Aguillón, H. J. Muñoz-Flores, E. J. Treviño-Garza and O. A. Aguirre-Calderón. 2021. Impact of external and internal factors on successful grafting of *Pinus pseudostrobus* var. *oaxacana* (Mirov) Harrison. Revista Chapingo Serie Ciencias Forestales y del Ambiente 27(2):243-256. Doi: 10.5154/r.rchscfa.2020.05.037.

Berrington de G., A. and D. R. Cox. 2007. Interpretation of interactions: a review. The Annals of Applied Statistics 1(2):371-385. Doi: 10.1214/07-AOAS124.

Camcore. 2008. ¿Estamos más cerca de entender la ascendencia de las poblaciones de "la variante Patula" en la Sierra Madre del Sur? Boletín de noticias Camcore para México y Centroamérica 2(2):1. https://camcore.cnr.ncsu.edu/files/2015/04/boletincamcore2008_2abril.pdf. (27 de abril de 2022).

Cañellas, I., L. Finat, A. Bachiller y G. Montero. 1999. Comportamiento de planta de

Pinus pinea en vivero y campo: ensayos de técnicas de cultivo de planta, fertilización y aplicación de herbicidas. Investigación Agraria, Producción y Protección Vegetales 8(2):335-359. https://revistas.inia.es/index.php/fs/article/view/619/616. (14 de diciembre de 2021).

Castro-Garibay, S. L., A. Aldrete; J. López-Upton y V. M. Ordáz-Chaparro. 2018. Efecto del envase, sustrato y fertilización en el crecimiento de *Pinus greggii* var. australis en vivero. Agrociencia 52(1):115–127. https://www.scielo.org.mx/pdf/agro/v52n1/1405-3195-agro-52-01-115.pdf. (14 de octubre de 2021).

Copes, D. L. 1980. Effect of rootstock vigor on leader elongation, branch growth, and plagiotropism in 4- and 8-year-old Douglas-fir grafts. Tree Planters' Notes 31(1):11-14. https://rngr.net/publications/tpn/31-1/31_1_11_14.pdf. (12 de febrero de 2022).

Dabirian, S. and C. A. Miles. 2017. Increasing survival of splice-grafted watermenlon seedlings using a sucrose application. HortScience 52(4):579-583. Doi: 10.21273/HORTSCI11667-16.

Darikova, Y. A., E. A. Vaganov, G. V. Kuznetsova and A. M. Grachev. 2013. Changes in the anatomical structure of tree rings of the rootstock and scion in the heterografts of Siberian pine. Trees 27:1621-1631. Doi: 10.1007/s00468-013-0909-6.

Dvorak, W. S. 2002. *Pinus patula* Schiede & Schltdl. & Cham. In: Vozzo, J. A. (edit.). Tropical tree seed manual. Agricultural Handbook 721. United State Department of Agriculture, Forest Service. Washington, DC, USA. pp. 632-635.

Farjon, A. and B. T. Styles. 1997. *Pinus* (pinacea). Flora Neotropica, Monograph 75. Organization for Flora Neotropica and New York Botanical Garden. New York, NY, USA. 293 p.

Goldschmidt, E. E. 2014. Plant grafting: new mechanisms, evolutionary implications. Frontiers in Plant Science 5:1-9. Doi: 10.3389/fpls.2014.00727.

González-Jiménez, B., M. Jiménez-Casas, J. López-Upton, M. Á. López-López and R. Rodríguez-Laguna. 2022. Combination of grafting techniques to clone superior genotypes of *Pinus patula Schiede* ex Schltdl. *et* Cham. Agrociencia 56(5):993-1017. Doi: 10.47163/agrociencia.v56i5.2582.

Hibbert-Frey, H., J. Frampton, F. A. Blazich, D. Hundley and L. E. Hinesley. 2011. Grafting fraser fir (A*bies fraseri*): Effect of scion origin (crown position and branch order). HortScience 46(1):91-94. Doi: 10.21273/HORTSCI.46.1.91.

Hildebrant, T. 2017. *Conifer propagation*. American Conifer Society. https://conifersociety.org/conifers/articles/conifer-propagation-101/. (21 de febrero de 2022).

Jayawickrama, K. J. S., J. B. Jett and S. E. McKeand. 1991. Rootstock effects in grafted conifers: A review. New Forest 5:157–173. Doi: 10.1007/BF00029306.

Kita, K., H. Kon, W. Ishizuka, E. Agathokleous and M. Kuromaru. 2018. Survival rate and shoot growth of grafted Dahurian larch (*Larix gmelinii* var. japonica): a comparison between Japanese larch (*L. kaempferi*) and F_1 hybrid larch (*L. gmelinii* var. japonica × *L. kaempferi*) rootstocks. Silvae Genetic 67(1):111–116. Doi: 10.2478/sg-2018-0016.

Landis, T. D., R. W. Tinus, S. E. McDonald and J. P. Barnett. 1989. The container tree nursery manual. Seedling nutrition and irrigation. Vol. 4. Agriculture Handbook. 674. U. S. Department of Agriculture, Forest Service. Wahington, DC, USA. 119 p.

Landis, T. D. Containers: Types an functions. In: Landis, T. D., R. W. Tinus, S. E. McDonald and J. P. Barnett. 1990. The container tree nursery manual. Containers and growing media. Vol. 2. Agriculture Handbook 674. U. S. Department of Agriculture, Forest Service. Washington, DC, USA. pp. 1-39.

López L., J. Á. 2020. Manejo de un huerto semillero y banco clonal de *Pinus douglasiana* Martínez en Jalisco. Fideicomiso para la Administración del Programa de

Desarrollo Forestal del Estado de Jalisco (Fiprodefo). Guadalajara, Jal., México. 210 p.

Lott, L. H., L. M. Lott, M. Stine, T. L. Kubisiak and C. D. Nelson. 2003. Top grafting longleaf × slash pine F1 hybrids on mature longleaf and slash pine interstocks. In: Tree Improvement and Genetics-27th Sothern Forest Tree Improvement Conference. Oklahoma State University. Oklahoma, OK, USA. pp. 96–101.

Moore, R. 1984. A model for graft compatibility-incompatibility in higher plants. American Journal of Botany 71(5):752–758. Doi: 10.1002/j.1537-2197.1984.tb14182.x.

Mudge., K., J. Janick, S. Scofield and E. E. Goldschmidt. 2009. A history of grafting. Horticultural Reviews 35:437-493. Doi: 10.1002/9780470593776.ch9.

Muñoz F., H. J., G. Orozco G., V. M. Coria A., R. Toledo B. y H. Aguilar G. 2011. Validación de dos métodos de injerto de *Pinus pseudostrobus* Lindl., en Michoacán. Folleto técnico Núm. 24. INIFAP-Campo Experimental Uruapan. Uruapan, Mich., México. 43 p.

Muñoz F., H. J., J. Á. Prieto R., A. Flores G., T. Pineda O. y E. Morales G. 2013. Técnicas de injertado "enchapado lateral" y "fisura terminal" en *Pinus pseudostrobus* Lindl. Folleto técnico Núm. 68. INIFAP-Campo Experimental Valle del Guadiana. Durango, Dgo., México. 48 p.

Mutabaruka, C., H. F. Cook and G. P. Buckley. 2015. Effects of drought and nutrient deficiency on grafts originating from sound and shaken sweet chestnut trees (*Castanea sativa* Mill.). iForest-Biogeosciences and Forestry 9(1):109-114. Doi: 10.3832/ifor1572-008.

Pérez-Luna, A., J. Á. Prieto-Ruíz, J. López-Upton, A. Carrillo-Parra, … and J. C. Hernández-Díaz. 2019. Some factors involved in the success of side veneer grafting of *Pinus engelmannii* Carr. Forests 10(2):112-129. Doi: 10.3390/f10020112.

Pérez-Luna, A., C. Wehenkel, J. Á. Prieto-Ruíz, J. López-Upton, … and J. C. Hernández-Díaz. 2020. Grafting in conifers: A review. Pakistan Journal of Botany 52(4):1369–1378. Doi: 10.30848/PJB2020-4(10).

Pérez-Luna, A., J. C. Hernández-Díaz, C. Wehenkel, S. L. Simental-Rodríguez, J. Hernández-Velasco and J. Á. Prieto-Ruíz. 2021. Graft survival of *Pinus engelmannii* Carr. in relation to two grafting techniques with dormant and sprouting buds. PeerJ 9:e12182. Doi: 10.7717/peerj.12182.

Perry, J. P. 1991. The pines of Mexico and Central American. Timber Press. Austin, TX, USA. 231 p.

Pershey, N. A. 2014. Reducing water use, runoff volume, and nutrient movement for container nursery production by scheduling irrigation based on plant daily water use. Master of Science Thesis, Horticulture, Michigan State University. East Lansing, MI, USA. 151 p.

Pina, A. and P. Errea. 2005. A review of new advances in mechanism of graft compatibility–incompatibility. Scientia Horticulturae 106(1):1–11. Doi: 10.1016/j.scienta.2005.04.003.

Ranjith, K. and R. V. J. Ilango. 2017. Impact of grafting methods, scion materials and number of scions on graft success, vigour and flowering of top worked plants in tea (*Camellia* spp.). Scientia Horticulturae 220:139–146. Doi: 10.1016/j.scienta.2017.03.039.

Rivera-Rodríguez, M. O., J. J. Vargas-Hernández, J. López-Upton, Á. Villegas-Monter y M. Jiménez-Casas. 2016. Enraizamiento de estacas de *Pinus patula*. Revista Fitotecnia Mexicana 39(4):385-392. https://www.scielo.org.mx/pdf/rfm/v39n4/0187-7380-rfm-39-04-00385.pdf. (22 de abril de 2022).

Sáenz R., J. T., H. J. Muñoz F., C. M. Á. Pérez D., A. Rueda S. y J. Hernández R. 2014. Calidad de planta de tres especies de pino en el vivero "Morelia", estado de

Michoacán. Revista Mexicana de Ciencias Forestales 5(26):98-111. Doi: 10.29298/rmcf.v5i26.293.

Statical Analysis System (SAS). 2002. SAS 9.0. Cary, NC, USA. SAS Institute.

Valdés, A. E., M. L. Centeno and B. Fernández. 2003. Changes in the branching pattern of *Pinus radiata* derived from grafting are supported by variations in the hormonal content. Plant Science 165(6):1397-1401. Doi: 10.1016/j.plantsci.2003.08.003.

Vargas-Hernández, J. J. and J. I. Vargas-Abonce. 2016. Effect of giberellic acid ($GA_{4/7}$) and girdling on induction of reproductive structures in *Pinus patula*. Forest

Systems 25(2):e063. Doi: 10.5424/fs/2016252-09254.

Velázquez M., A., Á. Pérez y G. Llanderal O. 2004. Monografía de *Pinus patula*. Secretaría de Medio Ambiente y Recursos Naturales (Semarnat), Comisión Nacional Forestal (Conafor) y Colegio de Postgraduados (Colpos). Coyoacán, D. F., México 124 p.

Verdugo-Vázquez, N., G. Gutiérrez-Gamboa, I. Díaz-Gálvez, A. Ibacache and A. Zurita-Silva. 2021. Modifications induced by rootstocks on yield, vigor and nutritional status on *Vitis vinifera* Cv Syrah under hyper-arid conditions in Northern Chile. Agronomy 11(5):979-983. Doi: 10.3390/agronomy11050979.

Villaseñor, R. R. y M. V. S. Carrera G. 1980. Tres ensayos de injertado en *Pinus patula* Schl. et Cham. Ciencia Forestal en México 5(23):21-36.

Wang, Y. 2011. Plant grafting and its application in biological research. Chinese Science Bulletin 56(33):3511–3517. Doi: 10.1007/s11434-011-4816-1.

Wright, J. W. 1976. Introduction to Forest Genetics. Academic Press, Inc. Los Ángeles, CA, USA. 463 p.

Yin, H., B. Yan, J. Sun, P. Jia, … H. Liu. 2012. Graft-union development: a delicate process that involves cell-cell communication between scion and stock for local auxin accumulation. Journal of Experimental Botany 63(11):4219-4232. Doi: 10.1093/jxb/ers109.