Interspecific grafting of *Pinus patula*

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ABSTRACT

Objective: To evaluate the compatibility of *Pinus patula* grafts on rootstocks of seven pine species.

Design/Methodology/Approach: *P. patula* scions were grafted on *P. greggii*, *P. teocote*, *P. pseudostrobus*, *P. cembroides*, *P. ayacahuite*, *P. hartwegii*, and *P. patula* rootstocks. The seven treatments were established in a randomized complete four block design; survival and growth were evaluated and recorded during the experiment.

Results: At eight months, *P. patula* and *P. teocote* rootstocks recorded the highest survival rate (35%), while no *P. cembroides* graft survived. *P. patula* rootstocks recorded the highest graft growth, surpassing the grafts on *P. pseudostrobus*, *P. ayacahuite* and *P. hartwegii*, by 30, 78, and 90%, respectively.

Study Limitations/Implications: The rootstocks of *P. cembroides*, *P. hartwegii*, and *P. ayacahuite* were not compatible with *P. patula* grafting. This situation reduces the number of potential species that can be used to clone *P. patula* genotypes.

Finding/Conclusions: The survival and graft growth were more successful on rootstocks of species phylogenetically closer to *P. patula*.

Keywords: cloning, compatibility, rootstock, phylogenetic relationship.

INTRODUCTION

A graft is the union between two organs of plants from different origin, composed of a scion (aerial part) and a rootstock (root system) (Darikova *et al.*, 2011). The parts of the graft can come from the same (intraspecific) or different (interspecific) species. Interspecific grafts have been successfully used to increase productivity and resistance to adverse factors. In addition, they have great potential for forestry (Han *et al.*, 2019). Intraspecific grafts are usually more compatible (Hartmann *et al.*, 2014). However, variable results have been obtained with interspecific grafts (Melnyk, 2017). Pérez-Luna *et al.* (2020) grafted *Pinus engelmannii* Carr. with the same species and with the *P. engelmannii* × *P. arizonica* Engelm. var. *arizonica* hybrid. In this case, the survival of the first graft was higher on the hybrid.

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This type of grafts increases productivity, the capacity to adapt to different environments, and the resistance to pests, diseases, and stress caused by abiotic factors (drought, salinity, excess, or deficit of water); in addition, it accelerates or increases fruiting, improves graft compatibility, or promotes a smaller size that favors the harvesting of cones in asexual seed orchards (Castro-Garibay *et al*., 2017; Han *et al*., 2019). In Mexico, grafting has the potential to seek favorable combinations and to improve the compatibility between pine species, because 49 (40%) out of the approximately 120 species of the world can be found in its territory (Gernandt and Pérez-de la Rosa, 2014).

Grafting scions from superior genotypes of *Pinus patula* Schiede ex Schltdl. *et* Cham. var. *patula* on rootstocks of other pine species would increase the productive potential of the species, because it can be established in asexual seed orchards used for genetic improvement programs. Research is fundamental to overcome interspecies compatibility, one of the main problems in this practice. The aim of this study was to determine the initial compatibility of *P. patula* scions with rootstocks of *P. patula*, *P. greggii* var. *australis* Donahue *et* López, *P. teocote* Schiede ex Schltdl. *et* Cham., *P. pseudostrobus* Lindl., *P. cembroides* Zucc., *P. ayacahuite* Ehren., and *P. hartwegii* Lindl. The first three species belong to the Oocarpae subsection; meanwhile, *P. pseudostrobus* and *P. hartwegii* are part of the Ponderosae subsection of the *Pinus* subgenus. Finally, *P. ayacahuite* and *P. cembroides* are more taxonomically distant (subgenus *Strobus*) from the scion species (Gernandt *et al*., 2005; Lira, 2020).

The objective of this study was to evaluate the initial compatibility of the *P. patula* graft on rootstocks of seven different pine species, in order to identify the most compatible rootstocks, regarding the survival and growth of the grafts.

MATERIALS AND METHODS

The experiment was established at the Colegio de Postgraduados, Campus Montecillo, located at 19° 27’ 34.8” N and 98° 54’ 15.8” W, at 2,249 m.a.s.l. Four months before grafting, the rootstock plants of the seven species were transplanted into 1 L plastic containers, using a substrate made of 60-20-20 peat moss, perlite, and vermiculite, with Multicote® 18-6-12+2MgO+ME at a dose of 8 g L^{-1}. The rootstocks were between 18 and 24 months old at the time of grafting and each species had its own morphological characteristics (Table 1).

Table 1. Origin, age, and morphological characteristics of the pine species used as rootstock plants in interspecific grafts of *Pinus patula*.

Rootstock species	Age (months)	Origin	Height (cm)	Diameter (mm)
P. patula	18	Chignahuapan, Puebla	58.7±1.1	7.1±0.2
P. greggii	18	Chignahuapan, Puebla	60.0±1.3	7.3±0.5
P. teocote	18	Acaxochitlán, Hidalgo	43.6±1.0	5.3±0.1
P. pseudostrobus	24	Tulancingo, Hidalgo	35.4±1.0	7.7±0.5
P. cembroides	24	Zimapán, Hidalgo	22.3±0.5	7.9±0.3
P. ayacahuite	24	Huayacocotla, Veracruz	51.8±2.5	8.4±0.3
P. hartwegii	24	Cofre de Perote, Veracruz	24.6±1.1	9.8±0.4

In March 2021, buds were collected from a 16-year-old select *P. patula* tree (G40). This tree had been chosen in 2018, because it stood up in the thinned sexual seed orchard, as a result of its height (16.9 m), normal diameter (26.8 cm), and stem straightness. This seed orchard is located at 19° 57' 36.09" N and 98° 06' 18.92" W, at 2,592 m.a.s.l., in the Peñuelas Pueblo Nuevo ejido, Chignahuapan, Puebla (Figure 1A). The buds were actively growing; nevertheless, they had not yet formed needles. They were vigorous and healthy, with a 20.0 cm average length and 4.7 mm average diameter (Figure 1B).

The buds were indistinctly collected from all over the tree crown. After their collection and until the moment of grafting, the buds were handled following the lateral technique described by González-Jiménez *et al.* (2022). However, depending on the morphology of the species evaluated, the height of the grafting varied from 5 to 10 cm from the base of the rootstock stem. Post-grafting management activities included: opening of the bag between weeks 3 to 5; removing the plastic at the junction point after two and a half months; pruning the aerial part of the rootstock at 2, 3, and 4 months after grafting, getting rid of approximately a third part of the crown each date, until only the grafted bud remained as the leader of the plant. The grafts were kept under 50% shade mesh. In average, irrigation was applied three times per week, using 1 g L^{-1} of Peters Profesional® 20-20-20 general purpose fertilizer.

The experiment was established under a randomized complete block design with four repetitions. Seven treatments were established in each block; the treatments matched the seven rootstock species used in this experiment (T1: *P. patula*, T2: *P. greggii*, T3: *P. teocote*, T4: *P. pseudostrobus*, T5: *P. cembroides*, T6: *P. ayacahuite*, and T7: *P. hartwegii*). The experimental unit consisted of five grafts, resulting in a total of 140 grafts. The variables evaluated were: 1) survival eight months after grafting (%); 2) growth of the length of the graft (cm); and 3) evaluation of the length of needles (cm). The growth of the length of the graft was determined based on the difference between the initial length of the scion (7 cm) and its length six months after grafting. The length of the needles was evaluated through the selection of four needles from each graft; afterwards, their length was measured from the union of the fascicle to the tip, obtaining the average per graft. Both evaluations were carried out on the same date.

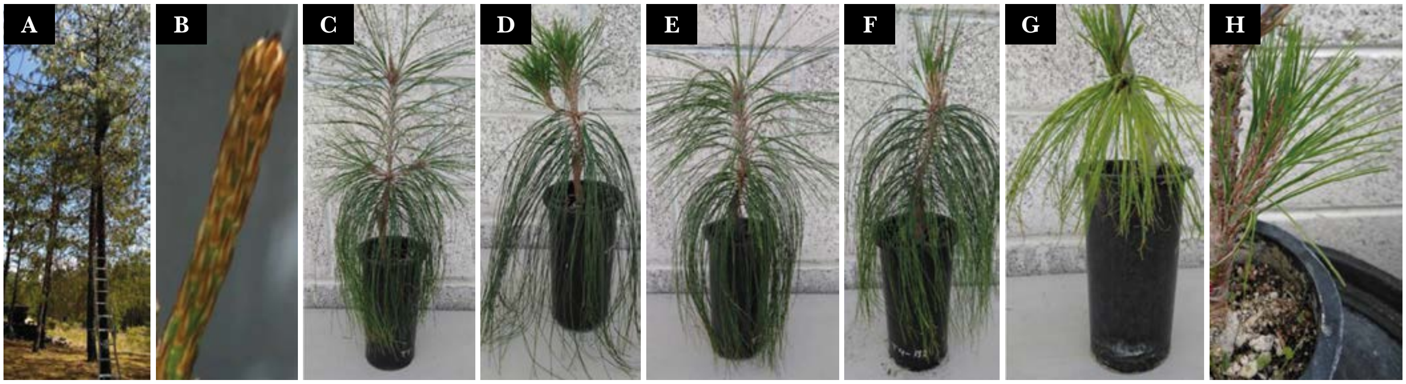

Figure 1. Interspecific grafts of *Pinus patula*. A: *Pinus patula* G40 scion donor tree; B: buds used as scions for grafting; C: eight-month-old grafts on *P. patula* rootstocks; D: *P. greggii*; E: *P. teocote*; F: *P. pseudostrobus*; G: *P. ayacahuite*; and H: *P. hartwegii*.

An analysis of variance was carried out to detect statistical differences between treatments; subsequently, the Tukey Multiple Comparison Test was performed, using the Mixed procedure of the Statistical Analysis System 9.4 software (SAS, 2013). The data of the variables (percentage) did not meet the assumption of normality; consequently, they were transformed using the $\left[T = \text{arcsine}\left(\sqrt{Y}\right)\right]$ function, before the analysis of variance was carried out. Subsequently, they were retransformed using the $\left[Y = 100\ \text{sine}^2\left(T\right)\right]$ function.

$$Y_{ij} = \mu + \tau_i + B_j + \varepsilon_{ij}$$

Where: i=1, 2,...t; j=1, 2,...r; t=number of treatments; j=number of blocks = number of repetitions; Y_{ij}=value of the response variable matching repetition j of the treatment i; μ=overall mean; i=effect of the treatment i; B_j=effect of the block j; ε_{ij}=experimental error, i=T1, T2, T3,...; j=1, 2, 3,... repetitions.

RESULTS AND DISCUSSION

Survival reduced to 22.5% by the final evaluation. The grafted scions recorded a length and needle growth of 2.1 and 8.7 cm, respectively. The analysis of variance recorded significant differences ($p \leq 0.05$) between treatments in the three variables (Table 2).

Graft survival

The highest graft mortality took place 60 days after the grafting. This situation could be the result of a possible physiological and anatomical incompatibility caused by the differences between rootstock species and the scions, as well as environmental factors. As a consequence of the atypically abundant rainfall in the area (May), excessive moisture inside the bag that covered the graft caused phytosanitary problems at the point of the union of the graft. However, survival remained relatively constant after this period. Likewise, Pérez-

Table 2. Mean values and standard error (±) in the experimental grafting of *Pinus patula* scions on rootstocks of seven different pine species.

Treatment	Survival (%)	Graft growth (cm)	Needle length (cm)
	0.0001*	**0.0001***	**0.0001***
P. patula	35±5.0 a	13±1.7 a	21.4±0.4 b
P. greggii	30±5.8 a	9.3±0.1 ab	29.9±1.2 a
P. teocote	35±5.0 a	9.5±0.7 ab	21.9±1.5 b
P. pseudostrobus	15±5.0 ab	9.1±0.4 b	22.5±0.6 b
P. cembroides	0.0±0.0 b	-	-
P. ayacahuite	5±5.0 b	2.8±0.5 c	11.9±0.3 c
P. hartwegii	15±5.0 ab	1.3±0.4 c	8.1±2.9 c
Average	22.5±4.6	7.5±1.5	1.3±1.6

*p≤0.05; means with different letters in each column indicate statistical differences between treatments (Tukey, 0.05).

Luna *et al.* (2020) recorded the stabilization of the survival of *P. engelmannii* × *P. arizonica* var. *arizonica* grafts on *P. engelmannii* rootstocks, 60 days after the grafting. Environmental factors are decisive during the "joint" period. Consequently, a special infrastructure is required for graft production. The nursery should be covered with shade mesh and a retractable plastic greenhouse roof, in order to protect the grafts from rain and hail. This roof can be opened or closed in order to keep the appropriate temperature, depending on the environmental conditions.

The percentage of graft survival variably reduced according to the rootstock species. *P. ayacahuite* recorded the highest mortality (95%), while *P. patula*, *P. teocote*, and *P. greggii* were less susceptible (65-70%) to the factors that influenced the mortality of the grafts during this period (Table 2). The grafts of *P. patula* on rootstocks of the same species recorded the highest survival value among the treatments (Table 2). Regarding the intraspecific grafts, a higher compatibility can usually be expected, as a result of their taxonomic affinity (Darikova *et al.*, 2011). Solorio-Barragán *et al.* (2021) grafted *P. rzedowskii* on five rootstock species, likewise achieving the highest survival of intraspecific grafts.

P. teocote and *P. greggii* had the grafts with the highest survival among scions grafted on rootstocks from a different species (Figure 1D and 1E). Gernandt *et al.* (2005) performed phylogenetic reconstructions with molecular data from DNA regions of chloroplasts and nucleus, placing *P. patula* in the same subsection as *P. teocote* and *P. greggii*; consequently these species are phylogenetically related. Therefore, the hypothesis is that the anatomical characteristics of these species may have evolved in a similar way. Consequently, there is a higher probability to obtain grafts compatible with these two pine species that have be used as rootstock plants for *P. patula* scions. In addition, Solorio-Barragán *et al.* (2021) reported a high compatibility when species of the same subsection (*Cembroides*) were grafted: scions of *P. rzedowskii* on *P. pinceana* Gordon & Glend.

On the one hand, *P. greggii* used as rootstock plant to graft scion from *P. patula* has some of the following characteristics: precocious flowering, high growth rates (height and diameter), and the potential to adapt to limiting humidity conditions and poor soils (Ruiz-Farfán *et al.*, 2015). On the other hand, *P. teocote* is another alternative that can be used as a rootstock for *P. patula*. This pine species is widely distributed in Mexico and could provide resistance to water deficit or tolerance to low-fertility soils. In addition, it is highly responsive when cut, which could favor the "succsess" percentage. Consequently, this species could be potentially used as rootstock for *P. patula*, as a result of the circular and continuous shape of its vascular cambium (Gernandt and Pérez-de la Rosa, 2014; Castro-Garibay *et al.*, 2017). No information on *P. patula* grafts has been documented on these two rootstock species; therefore, trials to determine its influence on the scion through their interaction must be carried out (Han *et al.*, 2019).

Studies aimed to determine the use of rootstocks of some species that are better adapted to adverse conditions have been carried out. Han *et al.* (2019) grafted *Populus cathayana* Rehder on *Populus deltoides* Bart. ex Marsh rootstocks which are more resistance to drought. The aim of that study was to increase the efficient use of water and soil nutrients. Guadaño *et al.* (2016) grafted *Pinus pinea* L. on *Pinus halepensis* Mill. because it is more resistant to drought and better adapted to limestone soils.

The rootstocks of *P. pseudostrobus* and *P. hartwegii* recorded a low survival rate (15%). Although the rootstock of these species had little compatibility, functional grafts were indeed obtained (Figure 1F and 1H). Although they belong to the same subgenus (*Pinus*), they are from a different subsection (Ponderosae), which can explain the results obtained (Gernandt *et al.*, 2005). Likewise, Solorio-Barragán *et al.* (2021) found compatibility and a higher survival rate (50%) when grafting *P. rzedowskii* scions on *P. ayacahuite* var. *veitchii*. Both species belong to the same subgenus, but to a different subsection. This situation shows that a successful graft is more difficult in some pine species, perhaps as a result of factors related to the cambium characteristics and their internal conduction structures (Castro-Garibay *et al.*, 2017). *P. ayacahuite* rootstocks recorded the lowest survival rate (Figure 1G). Meanwhile, *P. cembroides* rootstocks had no compatibility, probably because both rootstock species are the most taxonomically distant from *P. patula* and they belong to another subgenus (*Strobus*) (Gernandt *et al.*, 2005).

Graft growth

The scion successfully resumes its growth and development when the vascular connection is reestablished and consequently it can receive water and nutrients (Hartmann *et al.*, 2014). The highest average value was obtained with the rootstocks of the same species (13 cm), while with *P. pseudostrobus*, *P. ayacahuite*, and *P. hartwegii*, it recorded lower growth differences (30, 78, and 90%, respectively) (Table 2). These results match other intraspecific grafting experiments, where a better response was obtained, as a consequence of the anatomical and histological affinity of the parts of the graft (Castro-Garibay *et al.*, 2017).

Although, *P. patula* rootstocks obtained a higher value than *P. greggii* and *P. teocote*, there were not statistical differences between them, because their difference in growth fell into the range of 3.7 cm. The taxonomic closeness of these two species with *P. patula* (same subsection) could be related to their similar growth (Gernandt *et al.*, 2005). Since the evaluations of this study were limited to the first six months after the grafting took place, the growth of these scion-rootstock graft combinations should be evaluated after several years, in order to determine if the contrasts are permanent.

P. pseudostrobus is a relatively fast growing species and it is associated with the natural distribution range of *P. patula* (Perry, 1991). However, grafts on *P. pseudostrobus* rootstocks grew less than on the same species (*P. patula*). An example of the application of interspecific grafts is the use of dwarfing rootstocks (Gautier *et al.*, 2019). Low-height grafts can be kept in asexual seed orchards, facilitating their management and cone harvesting (Jayawickrama *et al.*, 1991). Grafting of *P. patula* on *P. radiata* resulted in a lower growth; however, strobile production did not diminish (Castro-Garibay *et al.*, 2017).

P. ayacahuite and *P. hartwegii* did not record a favorable graft growth (<3 cm) (Table 2). The particular growing habits of *P. hartwegii* may have influence this response: this pine species has one of the lowest growth rates. The growth of this species is mainly limited by the environmental conditions (low temperatures and low precipitation) that prevail in its native subalpine ecosystems. Additionally, after germination, the seedlings have a grassy state, they stop growing (height), and enter an apparent dormancy state that lasts between two and six years (Rivera *et al.*, 2021). These characteristic may have influenced the growing habits of the grafted *P. patula* scion.

Needle length

The influence that the rootstock species exerts on the growing habits of the graft needles could be identified, because the scions belong to a single genotype. The use of different rootstocks may provide differences in phenotype or architecture of the scion (Gautier *et al.*, 2019; Han *et al.*, 2019). The development of needles is an indication of graft "succsess". When a functional xylem is formed, the growth of these structures begins, as a result of the great water demand involved in the process (Guadaño *et al.*, 2016). In addition, these organs are responsible for all the photosynthesis and transpiration processes required by the new plant (González-Jiménez *et al.*, 2022).

The grafts with *P. greggii* rootstocks recorded greater needle growth (29.9 cm) than both species. In ungrafted adult trees, *P. greggii* and *P. patula* develop 10-15 and 15-25 cm long needles, respectively (Perry, 1991). This combination recorded shorter length differences of 28, 27, 25, 60, and 73%, with *P. patula*, *P. teocote*, *P. pseudostrobus*, *P. ayacahuite*, and *P. hartwegii*, respectively (Table 2). Overall, larger needles have a greater photosynthetic area and increase productivity, which is related to plant growth (He *et al.*, 2020). Consequently, the grafts on *P. ayacahuite* and *P. hartwegii* rootstocks recorded the lowest needle growth and lowest growth regarding graft length (Figure 1G and 1H), possibly because the rootstocks can alter the photosynthesis rate of the grafted scion (Han *et al.*, 2019).

The length of needles in ungrafted plants of *P. ayacahuite* (10-18 cm) and *P. hartwegii* (8-16 cm) is shorter than the length of the needles of *P. patula* during the adult state of ungrafted trees (Perry, 1991). These morphological differences regarding the length of the needles must be a consequence of the phylogenetical distance between these two rootstock species and *P. patula*. Therefore, their different growing habits are assumed to be the result of their separate evolution (Gernandt *et al.*, 2005). This same growing habit was observed in the scions of *P. patula* grafted on rootstocks of these two species, which developed shorter needles than those recorded on rootstocks of the same species.These two rootstock species exerted a stronger influence on the average growth of needles than the scion. Melnyk (2017) suggested selecting the appropriate rootstock that influences the desired size and vigor of the graft, because the rootstock exerts an important control over the scion.

CONCLUSIONS

The rootstock species with a closer phylogenetical relation with *Pinus patula* recorded a higher graft survival rate. Different rootstock species have particular effects on the growing habit of the grafted scions of *P. patula*.

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