Article

Incompatibility of the capulín (*Prunus serotina* ssp. capuli (Cav.) McVaugh) as rootstock of the sweet cherry tree (*Prunus avium* L.)

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Abstract

The capulín (*Prunus serotina* ssp. capuli (Cav.) McVough 1951) is a subspecies of *P. serotina* Ehnr. of Mesoamerican origin used mainly as fruit. Although from the 80's there were reports of a collection of capulín from central Mexico, called San Martín, which showed compatibility with the cherry tree (*Prunus avium* L.) possibly because it was a diploid capulín, this possible quality had not been the subject of a study that the cherry tree is little cultivated in the country. This situation is changing due to the release of new cherry tree varieties with low winter cold requirements. It returned to the idea of testing the materials of capulines that had been described as possible patterns of the cherry tree given the quality of the first one to adapt to the soils in Mexico and the susceptibility of the traditional rootstocks of the cherry tree to cancer (*Dibotryon* sp.) or blight of fire (*Phytophtora* sp.) mainly. In this investigation, remarkable compatibility of the Chapingo capulín with the cherry tree was not found and by looking for the causes analyzing both species by means of flow cytometry we found that the San Martin capulín is tetraploid and not diploid as it was supposed. The above is added to the possible production of phenolic compounds that is common in *Prunus*, producing a translocalized incompatibility that we observe in most of the grafted plants.

Keywords: *Prunus*, cytometry, Mexico, propagation.

Reception date: May 2018
Acceptance date: June 2018

Introduction

The capulín (*Prunus serotina* ssp. capuli (Cav.) VcVaugh) has been used in the country with multiple purposes, among which are: consumption of fresh fruit, roasted seeds and forestry (Rzendowski and Calderon, 2005; Fresnedo *et al*., 2011). McVaugh (1951), classified the capulín as one of the five subspecies of *Prunus serotina* Ehnr. based on their morphological characters and distribution zones. Avendaño-Gómez (2015); Rzendowski and Calderón (2005); Rohrer (2014) affirm that the capulines are the only subspecies really domesticated and that their range of distribution was extended by human influence to what the American territory comprises from the south of Canada, to the south of Bolivia between 1 200 and 3 200 meters above sea level. According to Halarewicz *et al*. (2017), this species is now a powerful invader in Europe. Fresnedo *et al*. (2011) studied the morphology of the capulines of the center-west of Mexico and found a structuring of the variation where the capulines of the center of the country seem to be domesticated as suggested by Avendaño-Gómez (2015).

Muratalla (1984) reported new perspectives of use of the capulín when introducing some varieties of sweet cherry by grafting them in collections of capulín coming from Puebla, Mexico. Of his essays, only one collection presented compatibility with the Cristobalina cherry cv. and named it the San Martin capulín. From this experience also note that the type of slit graft gave the best results, either using the capulín as a cherry tree pattern or using the capulín as an intergraft. When looking for a use of capulines in Central America, Navarro *et al*. (1996) in Honduras tested different types of grafting and times in capulines and distinguished that the simple English graft in October produced a success of more than 60% of grafted plants.

As noted by Olmstead *et al*. (2006); Gainza *et al*. (2015), modern production of cherry trees needs high density plantings, use of rootstocks that control vigor and increase precocity with low management costs and economically viable for producers. In the search for a rootstock for the new varieties of cherry tree with low cold requirement, the objective of our study was to test the San Martin capulín as a cherry tree rootstock given its adaptation to the soils in Mexico and the susceptibility of the traditional rootstocks of the cherry to cancer (*Dibotryon* sp.) or fire blight (*Phytophtora* sp.) mainly. The above in the interest of exploring the cultivation of cherry trees in the country.

Materials and methods

The research was carried out in the facilities of the Center Regional University Center West of the Autonomous University Chapingo (CRUCO) in Morelia, Michoacan. The CRUCO is located at latitude 19° 52’ and longitude 101° 02’ with a height of 1 920 masl.

Vegetal material

Seeds were multiplied by individuals from a collection identified by Muratalla (1984) as San Martin de (*Prunus serotina* ssp. capuli). The 160 capulín trees were established in the CRUCO field at a distance of 1 m between trees and 2 m between rows. In the Table 1 shows the characteristics of the San Martin type capulín.

Table 1. Origin, environment and taxonomic identity reported for the San Martín de *Prunus serotina* ssp. collection. capuli that served as a rootstock.

Key code	Population and State	Ambient		Taxonomic identity reported
		Geographical location	Conditions	
Mex	Chapingo, Mexico	19° 29' 58'' N 98° 52' 44'' W 2270 masl	C(w0) wb(i)g Collection in experimental fruit orchard Encino	*Prunus serotina* ssp. capuli (Cav.) McVaugh

The trees of capulín that were grafted with cherry tree presented a year of age from its germination, plants were selected that presented homogenous characteristics as for height and diameter presenting approximately 2.5 cm of diameter and 120 cm of height, the plants were free of diseases. The simple slit grafting method recommended by Muratalla (1984) was used and Stella, Van, Lapin, Brooks and Bourlat varieties were grafted in an equal number of repetitions. Finally, the graft was tied with a green plastic tape 1 cm wide and 20 cm long, leaving the yolk free for free growth Figure 1.

Figure 1. Grafting of cherry tree (*Prunus avium* L. in Capulín (*Prunus serotina* ssp. Capuli (Cav.) McVaugh.

Variables evaluated

Next, the variables that were evaluated are recorded in Table 2.

Table 2. Variables evaluated in the *Prunus serotina* capuli/*Prunus avium* grafts.

Organ	Morphological descriptor	Abbreviation	Unit of measurement
Complete tree	Height of the plant	ADP	(m)
Stem and branches	Stem diameter pattern	DDTP	(cm)
	Stem diameter graft 1	DDTI1	(cm)
	Stem diameter graft 2	DDTI2	(cm)
	Number of branches	NDR	Number
Leaves	Number of sheets	NDH	Number
	Width of leaves	ADH	(cm)
	Length of leaves	LDH	(cm)
Percent engraftment		PDP	Number

Estimation of nuclear DNA content by flow cytometry

Isolation of cell nuclei

The protocol for the isolation of cell nuclei recommended by Arumuganathan and Earle (1991) with modifications served to analyze the young leaves of the plants. Then the summary of the protocol: place in a Petri dish a small amount of tissue from the study plant and the reference standard used (approximately 30 mg of each tissue). Place both tissues one on top of the other to standardize a possible effect of cytosolic compounds. Add 1 mL of the buffer (LB01, tris MgCl2, etc) to the Petri dish.

Immediately cut both tissues with a knife or scalpel (use in each sample analyzed a new blade) in very thin slices in the chosen buffer and incubate on ice for at least 15 minutes. Mix the homogenate by pipetting up and down several times (avoid the formation of air bubbles). Filter the suspension through a 42 μg nylon mesh in a microcentrifuge tube. Add the fluorochrome (in the case of DAPI a concentration of 4 μg mL^{-1} is normally used, while in the case of IP and EB it is 50 μg mL^{-1} together with 50 μg mL^{-1} of RNase), and shake gently. Incubate the sample in ice and darkness before analysis (15 min to 1 h), occasionally shaking the tubes.

Flow cytometry analysis

The samples were analyzed in the laboratory in an Attune® acoustic flow cytometer configuration of argon blue/violet laser at an intensity of 50 mW at 450 nm/20 mW at 488 nm respectively (Life Techologies, San Diego CA, USA). For this, propidium iodide fluorochrome (PI Sigma - Aldrich) to stain the isolated plant nuclei and was used as a tissue reference species of *Hordeum vulgare* "Sultan" (5.19 pg of DNA) from the germplasm bank of the International Maize and Wheat Center (CIMMYT)-Mexico. The measurements were made to each individual to make the representation of the *taxa* evaluated. In each performed analysis, at least 10 000 events and a sample volume of 200-300 μL were measured. The coefficient of variation in each analysis was less than 5. The genome size of each sample was estimated according to the formula:

DNA 2C (pg)= value G1 shows X= the standard genome size (5.19 pg)/G1 value of the standard.

Results and discussion

The capulín *P. serotina* ssp. capuli and the cherry tree cultivars Stella, Van, Lapin, Brooks and Bourlat did not develop a graft union that could be considered compatible. There was no notable difference between the cherry tree cultivars, the individuals with pruning, two were of the Stella variety, two of Brooks and two of the Lapin variety. In Figure 2, the percent yield obtained in general for the grafts of cherry tree cultivars on capulín is verified. The arrest was 3.75% of all grafted individuals.

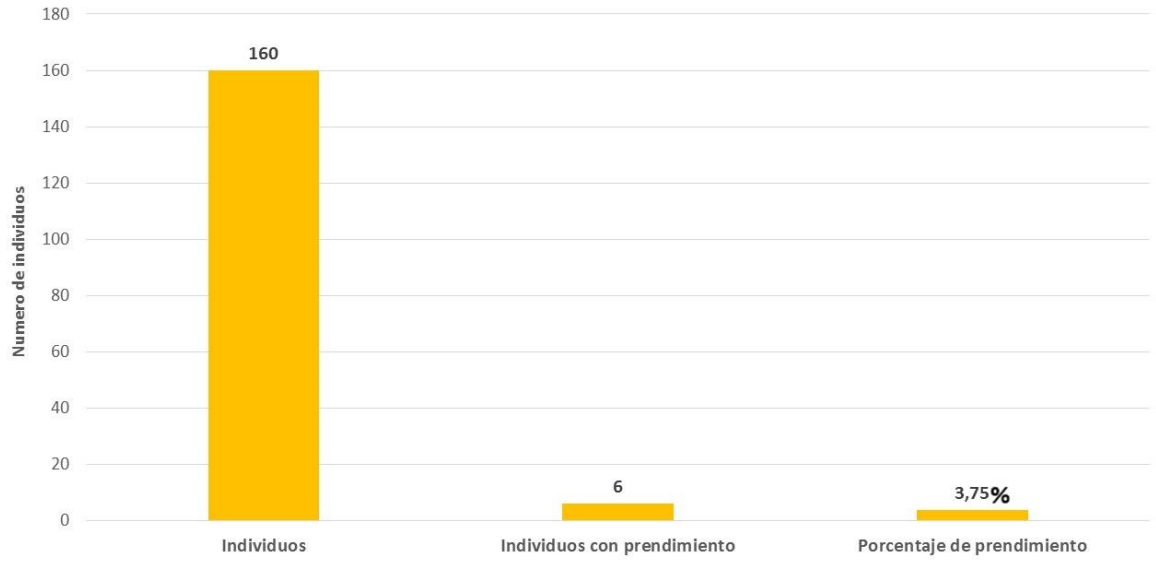

Figure 2. Percentage of cherry tree harvested graft in capulín.

Individual development record

In the Figure 3 shows the development of grafted individuals taking into account the means of the eight variables measured.

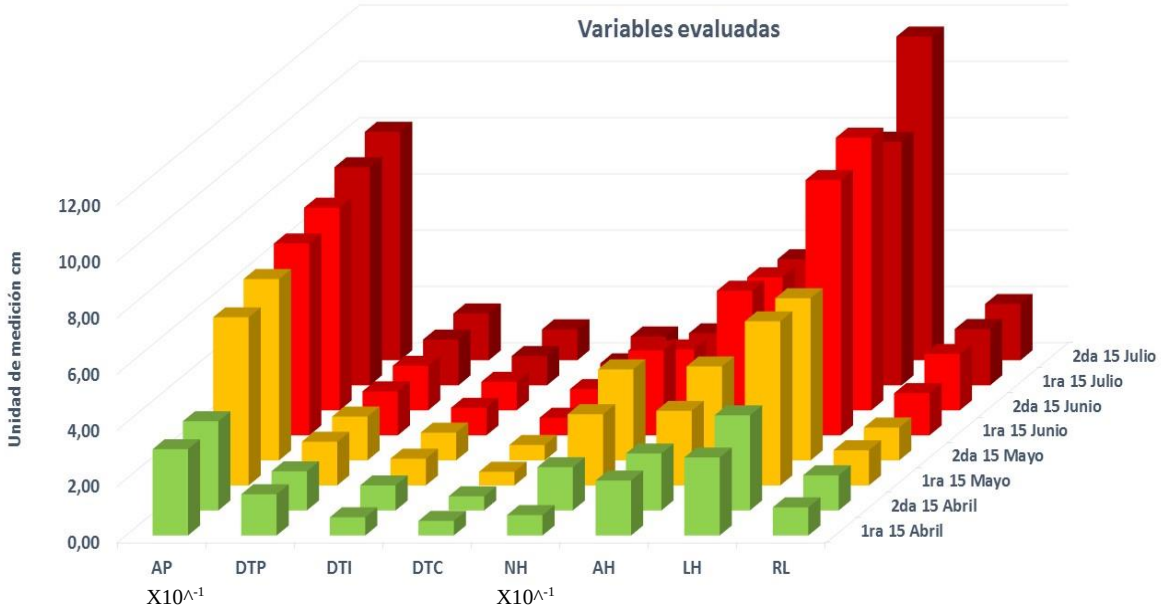

Figure 3. Development of grafted individuals of cherry tree on capulín

The incompatibility between the capulín and the cherry tree was revealed by the number of individuals with arrest (6 individuals representing 3.75% of the total grafted individuals). This did not allow the application of inferential tests and soon the development of the surviving plants is

described which also allows us to take elements to understand the incompatibility of much of plants. Thus, it was important to note that plant height (AP) presented an upward development except in the second half of April where individuals showed lower growth, it was resumed from the first half of May and remained up to 5 cm for fifteen days. In the end the growth reached up to 81 cm on average.

In the same way, the stem diameter (DT) has an ascending development, but in the second half of April it decreased. The stem diameter of the graft (DTI) presented a similar pattern reaching a final development of 1.10 cm. In the variable number of leaves (NH) the individuals initially presented on average around 7 leaves and at the end of the period they had an average of 20 leaves. The width of leaf (AH) presented a development generally on average of 0.5 cm in each fortnight reaching a final growth of 5.55 cm. The leaf length (LH) presented a development in its general averages of 2 cm every fortnight. The lateral branches (RL) of the individuals could be observed from the first half of April and at the end of the registration of the individuals, on average they came to present 2 lateral branches in the second half of July.

The registration of the diameters of the individual's stem diameter pattern (DTP) and stem diameter of the cherry tree (DTC) in the surviving individuals describes a modest but favorable progression.

In the Figure 4 shows the means of the variables evaluated of the individuals who survived. The figure refers to the fact that the variables with the highest development were plant height (AP), a development higher than 66 cm x $10^{\wedge-1}$ is obtained in its general mean, number of leaves (NH) in which the individuals presented a maximum of 20 leaves in its record x $10^{\wedge-1}$, leaf width (ADH) variable in which the surviving individuals came to observe an average development of 3.8 cm and finally the variable leaf length (LDH) the general average obtained by the surviving individuals present a development of 7.33 cm.

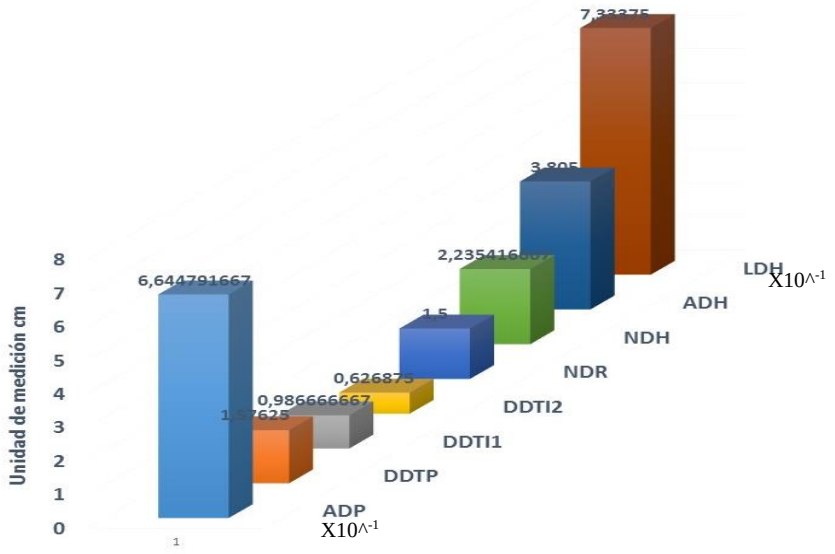

Figure 4. Average of the variables of living individuals.

The results of this study coincide with what was pointed out by Gainza *et al*. (2015) who recognizes the translocalized incompatibility in *Prunus* as one that during the first year of grafting presents a defoliation, discoloration of the leaf and a subsequent non-development associated with a carbohydrate translocation block mainly.

In the different cultivated species of the *Prunus* genus, there are few high-end rootstocks, due to the incompatibility present between rootstock and graft, preventing a strong and lasting functional union. Incompatibility in the genus *Prunus* is very present in species such as cherry, almond, apricot, peach and plum (Gainza *et al*., 2015).

Usenik (2006) mentioned that recent data show that several biochemical pathways are affected during the formation of the union of a graft. In *Prunus*, the metabolism of phenolic compounds is common. According to Olmstead (2006); Gainza *et al*. (2014), mention that small amounts of phenols can be extremely sufficient to produce limiting dysfunctions at the local level at the interface between two or more cells. Meanwhile, on the other hand, studies using callus cultures of *Prunus avium* L. have shown that podaflavine (phenol typical of *Prunus* species) interferes with the permeability of tissues, resulting in damage to the membrane. In incompatible graft unions, there is mobility of phenols from the vacuole in the cytoplasm, causing stress that results in growth dysfunction, which is probably caused by the inhibition of the lignin pathway (Kueger *et al*., 2012). In addition, concentrations of catechins and proanthocyanidins, these flavonoids, increase under stress and grafting is no exception (Pina and Errea, 2005).

Sorce *et al*. (2002); Koepke and Dhingra (2013); Souza (2015) mention that in a complex disorder such as incompatibility there is a biochemical background also complex and dependent on the specific genetic interactions between the cells of the pattern and graft. The success of the union depends primarily on the compatibility of the graft union to allow a rapid development of the vascular connections between the pattern and the graft (Olmstead *et al*., 2006) allowing this in turn the rapid resumption pattern and graft and the vascular regeneration of the tissues of the xylem and phloem of both parts through a cellular differentiation (Gainza, 2014; Souza, 2015).

Genome sizes

Next, the histograms obtained from the estimation of the nuclear DNA content of our evaluated species are presented, allowing us to determine the genome size of *P. avium* L. and *P. serotina* ssp. capuli (Cav.) McVaugh. All cherry tree cultivars presented the same value.

In the genetic field Dickson *et al*. (1992) indicate variations of ploidy in the capulín ranging from diploid to hexaploid. Downey and Iezzoni (2000), mention that *Prunus serotina* is a tetraploid species and Pairon and Jaquemond (2005) based on an analysis with DNA markers type microsatellites determined it as allotetraploid. Various authors such as Beck *et al*., (2014); Guzmán *et al*. (2018) and Pairon and Jacquemart (2005) have revealed a narrow genetic diversity in the species. This is the first study that determines the genome size of *P. serotina* ssp. capuli that coincides with the value of 1C= 0.5 pg that Dickson *et al*. (1992) reported for the species *P. serotina* Ehrn. and with the value of 1C= 0.35 pg that Arumuganathan and Earle

(1991) reported for *P. avium* L. In Figure 5, the results obtained from the flow cytometric analysis are observed where it can be seen that the sweet cherry tree (*P. avium* L.) has a smaller genome size San Martin capulín (*P. serotina* ssp. *capuli*) possibly this difference is related to the low engraftment of the graft.

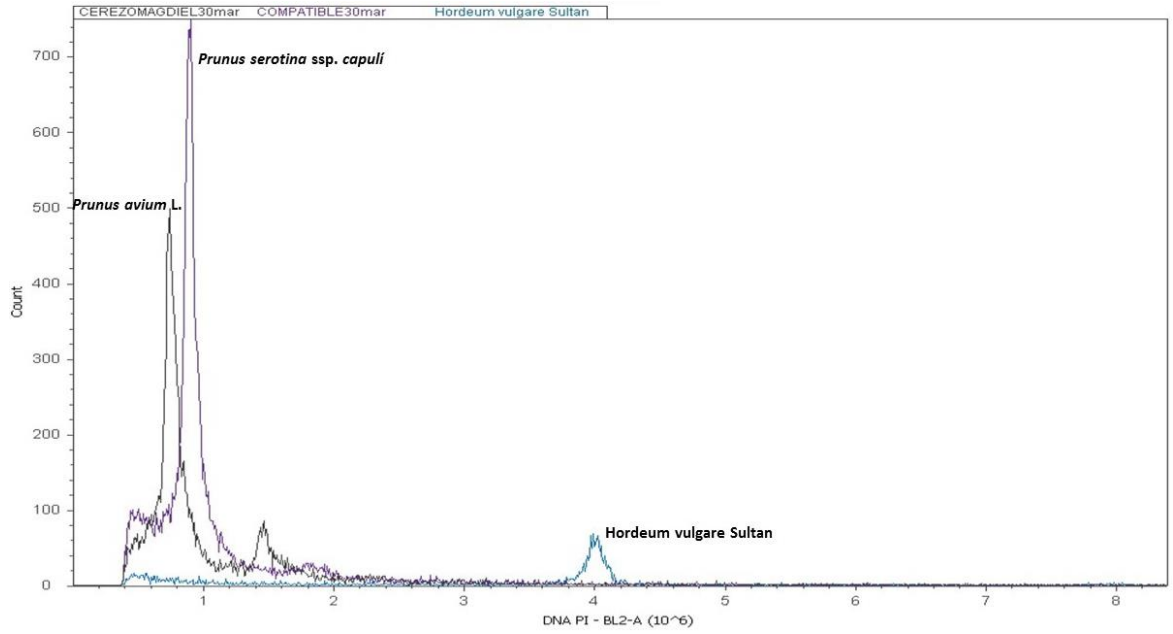

Figure 5. Histogram of the estimation of the DNA content by flow cytometry of the cherry tree (*cv* Stella) and the capulín type San Martín.

Considering the genetic and anatomical factors that affect the compatibility of the capulín and the cherry tree, the analysis of the size of the genome can be important to understand this relationship. Possibly one of the causes of the incompatibility of the capulín as a rootstock of the cherry tree may be associated with the genetic factors that are translated into anatomical characters as indicated by Souza *et al*. (2014) in a similar study in *Psidium*. In this study, differences in genome sizes can be linked to the difficulty of fusion and cell exchange because the tissue of each species continues to reproduce by mitosis maintaining its own genome number and genetic dosages in the metabolic pathways and this leads to non-union of tissues. Thus, the technique of flow cytometry can help in the early stages of the evaluation of the fruit utility of the other subspecies of *P. serotina* Ehrn.

Conclusion

The cultivars Stella, Van, Lapin, Brooks and Bourlat de Cerezo (*Prunus avium* L.) present an incompatibility presumably translocalised by being grafted onto the capulín (*Prunus serotina* ssp. capuli (Cav.) McVough) identified as type San Martín by Muratalla (1984). Possibly one of the causes of the incompatibility of the capulín as a cherry tree rootstock may be associated with the genetic factors that result in defoliation, discoloration of the leaf and a subsequent non-development associated with a possible blockage of carbohydrate translocation mainly. The determination of the genome size by flow cytometry can help in the first stages of the evaluation of the fruit utility of the other subspecies of *P. serotina* Ehrn.

Acknowledgments

The first author received a grant from CONACYT, for doctoral studies at UACH. The financing was provided by the project: CB 2011 169334 of CONACYT- Mexico and by DCRU of UACH. We appreciate the suggestions of the M.C. Alfonso Muratalla of the CP and the facilities of Dr. Peaubelle of the HCU Lyon-Sud, Lyon France.

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