POLLEN TUBE GROWTH IN MANDARIN

CRECIMIENTO DEL TUBO POLÍNICO EN MANDARINA

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SUMMARY

Pollen tube growth was studied in cvs. Monica, Reyna, Dancy, and Satsuma mandarin (*Citrus reticulata* unshiu Marc). Pollen tubes were stained with lacmoid and observed with a white light microscope. Lacmoid stained the callose brilliant blue. Pollen grains deposited on the stigma varied from 367 to 4170 among the four cultivars. Pollen germination, however, varied from 1 to 4 %, probably having effects on the ovule fertilization. The pollen load on the stigma of 'Satsuma,' both under of open and self-pollination conditions, was low probably due to male sterility. In compatible pollinations the pollen tubes reached the ovary in only 2 days, while in self-pollinated 'Monica' and 'Satsuma' pollen tubes were not observed in the locules after 8 d. According to the development and number of pollen tubes, cvs. Reyna, Dancy and Satsuma could be good pollenizers for cv. Monica.

Index words: *Citrus* Unshiu Marc, pollen tube, self-incompatibility.

RESUMEN

Se estudió el desarrollo del tubo polínico en los cvs. de mandarino Monica, Reyna, Dancy y Satsuma. Los tubos polínicos se tiñeron con lacmoide y se observaron con un microscopio de luz blanca. El lacmoide tiñó la calosa de un color azul brillante. El número de granos de polen depositados sobre el estigma de los cuatro cultivares fue de 367 a 4170, pero los porcentajes de germinación fueron de apenas 1 a 4 %, lo que probablemente afectó en la fertilización de los óvulos. La cantidad de polen depositada sobre el estigma del mandarino 'Satsuma', tanto en autopolinización como en polinización abierta fue baja, lo que se atribuye a esterilidad masculina. En las polinizaciones compatibles los tubos polínicos llegaron al ovario en 2 d, mientras que en las autopolinizaciones de 'Mónica' y 'Satsuma' aún no se observaban tubos polínicos en los lóculos después de 8 d. De acuerdo con el desarrollo y número de tubos polínicos, los cvs. Reyna, Dancy y Satsuma podrían ser buenos polinizadores para el cv. Mónica.

Palabras clave: *Citrus reticulata* unshiu Marc, tubo polínico, autoincompatibilidad.

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INTRODUCTION

In most angiosperms, fruit set depends on seed development following the processes of pollination and fertilization. However, some species may be able to form seedlessness fruits as the result of parthenocarpy or stenospermocarpy (fertilization followed by post-zygotic abortion).

In citrus fruits seedlessness can be caused by sterility, and percentages of functional pollen vary greatly among species and cultivars. The 'Washington' navel orange (*Citrus sinenesis* [L.] Osbeck) produces no viable pollen, while in 'Satsuma' mandarin (*Citrus unshiu* Marc.) the number of pollen mother cells is low and most of the pollen grains degenerate, especially at temperatures below 19 °C (Soost and Roose, 1996).

In addition to genetic sterility in *Citrus*, self-incompatibility and cross-incompatibility may exist. Helsop-Harrison (1975) refers to self-incompatibility as "the partial or complete incapacity of a pollen-fertile hermaphrodite or monoecious angiosperm to set viable seed on self-pollination." If incompatibility is associated with floral morphological changes, it is said to be heteromorphic, but if those changes do not exist the incompatibility is homomorphic, which can be sporophytic or gametophytic.

The sporophytic system has been reported in relatively few horticultural species; in most cases it results in inhibition of pollen germination on the stigma. Although in some species the pollen germinates, it never reaches the ovule. Other characteristics associated with this type of incompatibility are trinucleate pollen with a short storage capacity, and poor *in vitro* germination (Brewbaker, 1967), and flowers with dry stigmas (Helsop-Harrison and Shivanna, 1977).

The gametophytic system is the most frequently reported in fruit trees, which results in retarded pollen tube growth following self-pollination (Pandey, 1958; Knox and Singh, 1987). The tubes cease growth in the style and show a characteristic appearance, featuring swollen tips and terminal deposition of callose. Other abnormalities observed are burst pollen tips, irregular callose deposition in pollen tube walls, and spiralling of pollen tubes (Kahn and DeMason, 1986). The gametophyte mechanism is also associated with the production of binucleate pollen, with good storage capacity, which germinates well *in vitro* (Brewbaker, 1967), and flowers with wet stigmas (Helsop-Harrison and Shivanna, 1977). One or more of these characteristics is commonly used as circumstancial or corroborative evidence for this type of incompatibility.

Self-incompatibility causes unfruitfulness in citrus with a low capacity for parthenocarpy. All *Citrus maxima* (*C. grandis*) tested cultivars have been self-incompatible, and several hybrids are incompatible. Self-incompatibility has been reported for several cultivars in Japan (Soost and Roose, 1996). Other researchers have reported self-incompatibility for some cultivars of the species *C. limon*, *C. limettoides*, and *C. sinensis* (Frost and Soost, 1968); however, several cultivars of *C. sinensis* also have a high degree of pollen sterility (Soost and Roose, 1996).

'Clementina' tangerine 'Robinson' hybrid tangerine [*C. reticulata* x (*C. paradisi* x *C. reticulata*)] (Soost, 1965), and tangelos (*C. paradisi* x *C. reticulata*) 'Orlando' and 'Mineola', both grapefruit hybrids 'Duncan' with 'Dancy' tangerine, are self-incompatible (Ton and Krezdorn, 1966). Hybrids between self-incompatible cultivars are also self-incompatible, and sometimes cross-incompatible (Soost and Roose, 1996).

The aim of this study was to determine whether the pollen loads deposited naturally on the stigmas of four mandarin cultivars are sufficient for adequate ovule fertilization. Also to determine whether there is gametophytic self-incompatibility in the cultivars studied, and to select the best pollenizer for 'Monica' mandarin.

MATERIALS AND METHODS

The study was conducted in orchards of Álamo, Veracruz, México (20° 32' NL and 97° 28' WL), where the mean temperature is 22 °C to 26 °C and annual precipitation is 1124 mm, distributed between June and October. We studied 7-year-old mandarine trees grafted on rootstocks of sour orange (*Citrus aurantium* L.), planted at of 7 x 7 m spacing. During this research the mean temperature was approximately 30 °C at midday (12:00 h), which increased gradually up to 40 °C (at 15:00 h).

Pollination

From eight trees of each of the cultivars ('Monica', 'Dancy', 'Reyna' and 'Satsuma'), 20 flowers just before opening (floral buds) per tree were selected to quantify the pollen load and the growth of pollen tubes once the flowers opened. On four trees per cultivar, 20 floral buds per tree were covered with bags to quantify pollen load and the growth of pollen tubes in conditions of self-pollination.

For cross-pollinations, flowers to provide pollen from cvs. Monica, Dancy, Reyna and Satsuma, were collected just before opening. The 'Monica' mandarin was used as female, using well developed flowers and buds, and 120 floral buds per tree (eight trees) were emasculated. Pollination was carried out between 9:00 and 14:00 h (40 with pollen from each pollenizer).

Pollen grains and tubes

In all cases, pistils were collected 2, 4, 6, and 8 d after the pollination treatments (5 to 10 pistils per collection and per cultivar). The samples were fixed in 2:1 (100 % ethanol:glacial acetic acid). They were then washed in 70 % ethanol, washed in distilled water, softened for 72 h in sodium hydroxide 0.8 N, and finally washed again in distilled water during 24 h.

For observation, the pistils were cut into four sections (stigma, upper part of the style, lower part of the style, and ovary). The stigma was also cut into eight equal sections and the number of pollen grains was quantified in each to estimate the total. Pollen tubes were observed using a mixture of 0.5 % lacmoid (Sigma L-7512) plus 5 % sodium bicarbonate dissolved in 30 % ethanol, and then quantified. The observations were made with a Zeiss white light transmission microscope. Data were submitted to analysis of variance under a completely randomized design, and means were compared with the Tukey's test (Statistical Analysis System,1997).

RESULTS

Pollen grains and tubes

With the technique used the stigma was light brown, the pollen grain walls were dull brown, while the cytoplasm was colorless. Callose of the pollen tubes stained bright blue, and because they were squashed during the process, the stigma tissue was not preserved integrally (Figure 1A). The callose plugs formed in the pollen tubes varied in size and appeared discontinuously throughout the tube length (Figure 1B).

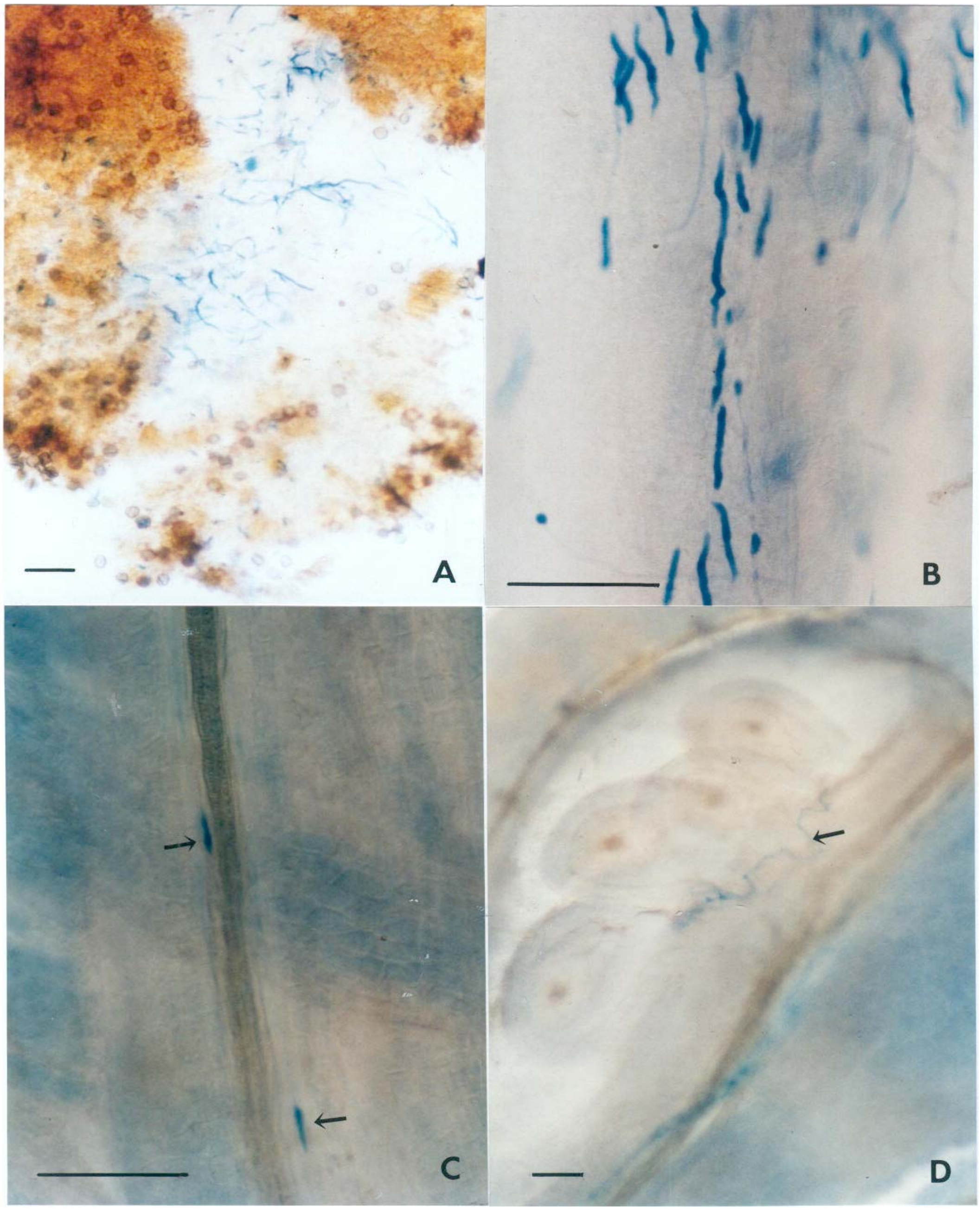

Figure 1. Pollen grains and tubes in mandarin. (A) Pollen tubes on stigma; the tissue was not preserved integrally during squashing; (B) Callose plugs in the pollen tubes varied in size and appeared discontinuously throughout the tube length; (C) Pollen tubes growing along the carpel vascular bundles, arrows indicate callose plugs; (D) Pollen tubes in the ovary, some them spiralling (arrow) before penetrating the ovules through the micropyle. Scale bars = 0.1 mm

After the pollen tubes had penetrated the style, the callose plugs looked like long, bright blue strips and the entire length of each tube was more easily observed when fewer tubes were seen in the ovary (Table 1). Style tissue appeared light grainy blue, while the vascular bundles were colorless to gray. The pollen tubes grew through style canals, but when the styles were squashed they seem to have grown along the carpel vascular bundles (Figure 1C).

When reaching the ovary, some pollen tubes penetrate the locules and others are detoured through the carpel walls. Some tubes which have penetrated the locules are spiralled before they reach and penetrate the ovules through the micropyle. The ovular integuments appear colorless and the nucellus as a dull brown (Figure 1D).

Pollinations

In all compatible pollinations the pollen tubes reached the ovary in only 2 d after treatments. In the open pollinations, even when there was enough pollen on the stigma to fertilize the ovules, only 1 to 4 % of the grains germinated. Four days after anthesis there were less pollen grains on the stigma of 'Satsuma', than those found in 2 d. In the upper part of the style there were more pollen tubes than in the lower part, but only a few reached the ovary. In all cases, the number of pollen tubes decreased from the upper part of the style toward the base, so that only 11 to 19 tubes per pistil reached the ovary (Table 1).

Table 1. Number of pollen grains and tubes in mandarin under conditions of openpollination, 4 d after anthesis.

Cultivar	Average number of pollen grains on stigma	Average number of pollen tubes in style		Average number of pollen tubes in ovary
		Upper style	Lower style	
Monica	2 488.0 a[ξ]	26.93 c[ξ]	15.26 b[ξ]	18.89 a[ξ]
Dancy	2 635.3 a	55.21 ab	30.53 a	19.63 a
Reyna	2 600.0 a	47.06 b	30.62 a	10.50 b
Satsuma	1 867.6 b	69.79 a	22.16 b	11.84 b
LSD	585.6	15.80	7.92	5.20

[ξ]Values in columns followed by the same letter are not significantly different by Tukey's test (P≤0.01).

Under conditions of self-pollination, the pollen loads on the stigma were smaller (Table 2) compared to those found in open pollination (Table 1); that is because bags on the self-pollinated flowers excluded pollinator insects. Four days after anthesis the cv. Monica had a larger number of pollen grains on the stigma, while 'Satsuma' had a very small number. Although in 'Monica' there were more pollen grains on the stigma than in the others cultivars, only a small number of tubes (0.63 on average) penetrated the ovary wall, and none penetrated the locules. In contrast, in 'Dancy' and 'Reyna' a large part of the tubes observed in the upper style reached the ovary. In 'Satsuma' none of the tubes reached the ovary, thus showing some self-incompatibility.

Table 2. Number of pollen grains and tubes in self-pollinated pistils of mandarin, 4 d after anthesis.

Cultivar	Average number of pollen grains on stigma	Average number of pollen tubes in style		Average number of pollen tubes in ovary
		Upper style	Lower style	
Monica	1 809.4 a[ξ]	13.96 c[ξ]	7.70 c[ξ]	0.63 c[ξ]
Dancy	1 262.5 ab	41.34 a	25.97 a	13.91 a
Reyna	972.8 ab	27.13 b	19.60 b	5.47 b
Satsuma	367.4 b	21.67 bc	18.27 b	0.00 c
LSD	1 093.5	8.89	5.78	3.49

[ξ] Values in columns followed by the same letter are not significantly different by Tukey's test (P≤0.01).

In cross-pollinations, all the cultivars were compatible with 'Monica'; the best crosses were with 'Reyna' and 'Satsuma'; 'Dancy', considered as a good pollen donor, presented a low pollen germination on the stigma (Table 3). In observations made every other day, a very uniform trend was maintained. The highest number of pollen tubes was observed 6 d after pollination; even so, the number of pollen tubes in the ovary can be considered low, since a pistil has an average of ten carpels each with two rows of ovules attached to the central axis (Table 4).

Table 3. Number of pollen grains and tubes in pistils of 'Monica' mandarin, using three pollenizers, 4 d after pollination.

Pollenizer	Average number of pollen grains on stigma	Average number of pollen tubes in style		Average number of pollen tubes in ovary
		Upper style	Lower style	
Dancy	4 170.6 a[ξ]	19.45 c[ξ]	14.22 b[ξ]	16.65 b[ξ]
Reyna	2 210.0 b	44.30 a	29.67 a	27.96 a
Satsuma	3 110.6 ab	29.15 b	23.54 c	25.83 a
LSD	1 122.0	9.24	6.40	4.37

[ξ] Values in columns followed by the same letter are not significantly different by Tukey's test (P≤0.01).

Table 4. Number of pollen tubes in the ovary of 'Monica' mandarin, using three pollen donors.

Cultivar	Days after pollination			
	2	4	6	8
Dancy	17.90 b[ξ]	16.65 b[ξ]	23.20 b[ξ]	20.85 b[ξ]
Reyna	27.67 a	27.96 a	41.82 a	35.06 a
Satsuma	5.16 c	25.83 a	28.11 b	26.28 b
DMS	3.33	4.37	4.30	3.53

[ξ]Values in columns followed by the same letter are not significantly different by Tukey's test (P≤0.01).

DISCUSSION

Research about self-incompatibility has concentrated on herbaceous species, because they are easier to handle and have shorter generation times. However, in fruit trees the phenomenon has economic importance and breeding implications. Incompatibility has generally been evaluated through seed formation, but pollen tubes have been rarely quantified or their abnormalities recorded.

In our study the number of pollen grains indicates that pollination is not a limiting factor for fruit set. However, pollen germination is very low (1 to 4 %), which may lead to a deficiency in ovule fertilization.

Competition among male gametes can occur before and after their germination. Stephenson and Bertin (1983) consider that competition during the progamic phase is the most important. However, when the pollen loads are abundant and from the same genotype, it is possible that before germination competition is greater. Pollen tube growth, fusion of the nuclei, and initial development of the embryo are process involved after germination. Late or ovarian self-incompatibility in initial embryo development (Sage *et al*., 1994) is often difficult to differentiate from embryo abortion caused by other factors.

Both in compatible and incompatible pollinations, the number of pollen tubes decreases from the upper part of the style toward the ovary. Generally, most pollen tubes penetrate the stigma, but very few of them reach the ovary. Sedgley (1976) suggested that many of the non-growing tubes have swollen tips, a common phenomenon occurring *in vitro*, when substrates decline and products of pollen metabolism increase. Another possible explanation is that pollen tubes secrete an inhibitor which acts on the slower-growing tubes and arrests their growth. In citrus thousands of pollen grains may be deposited and germinate on the stigma, but only 0-40 tubes reach the ovary, while in avocado (*Persea americana* Mill.) only one or two tubes reach the ovary from 60 pollen grains germinating on the stigma.

In self-pollinated flowers of 'Monica' and 'Satsuma' the pollen tubes did not penetrate the locules, then suggesting a degree of self-incompatibility, as indicated by Ton and Krezdorn (1966) for 'Clementina' tangerine and 'Orlando' tangelo (toronja x mandarina). In self-pollinated gynoecia of cv. 'Orlando'; Khan and DeMason (1986) found that the primary site of pollen tube inhibition was the stigma, since no tube grew beyond the upper part of the style. It is probable that our results differ from these cases because of a higher degree of self-compatibility.

Some of the abnormalities reported by Khan and DeMason (1986), such as heavy callose deposition at the pollen tube tips and burst pollen tips, were not detected in our study. We think that in mandarin the site of inhibition occur at the base of the style, in the ovary or in the ovules, as it was proposed by Sage *et al*. (1994). In 'Monica' the pollen tubes reached the base of the style, but instead of penetrating the locules, they continued to grow through the carpel walls to the receptacle, thus indicating that there was no recognition between the pollen tubes and the ovules.

According to Ton and Krezdorn (1966), in citrus the self-incompatibility is of the gametophytic type, and correlated to binucleate pollen, with long storage capacity, and inhibition of the pollen tubes at some site between the stigma and the ovary (Brewbaker, 1957).

It has been established that the growth rate of pollen tubes is influenced by environmental conditions, mainly temperature (Papademetriou, 1975, Socias i Company *et al*., 1976; Sedgley and Anells, 1981; Vasilakakis and Porlingis, 1984). According to Sedgley (1977), in avocado the rate of pollen tube growth increased with a rise in temperature, but under a regime of 33/28 °C (day/night) some abnormalities were observed. At temperature regime of 25/20 °C, the pollen tubes reached the ovary in 18 h. In pear (*Pyrus communis* L.) the pollen tubes reached the base of the style in 24 h at 21 °C, in 72 h at 15.5 °C, and in 120 h at 10 °C (Papademetriou, 1975). In date palm (*Phoenix dactylifera* L.), at 25 to 28 °C, most tubes reached the base of the ovary in 6 h and at 15 °C they took more than 8 h (Reuveni *et al*., 1986).

In Veracruz, México, the pollen tubes reached the ovary only 2 d after pollination, whereas Khan and DeMason (1986) found that some pollen tubes of 'Orlando' tangelo penetrated the ovules of 'Dancy' tangerine in 9 to 12 d after pollination. In compatible crosses of citrus, Ton and Krezdorn (1966) observed penetration of ovules by pollen tubes within 6 to 8 d; these researchers claim that after this period the ovule fertilization would not have been possible because abscission starts after 8 d. Presumably the differences among all these results are due to environmental temperatures.

CONCLUSIONS

The number of pollen grains on the stigma indicate that pollination is not a limiting factor for fruit set in mandarin at Veracruz, México. The cv. Satsuma had the lowest number of pollen grains on the stigma under conditions of self-pollination and open pollination, probably due to male sterility.

The 'Monica' and 'Satsuma' mandarins showed some degree of self-incompatibility, and 'Reyna,' 'Satsuma,' and 'Dancy' are promising pollenizers for 'Monica' mandarin.

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