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## Pollen Morphology and Reproductive Performances in *Opuntia ficus-indica* (L.) Mill.

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### Abstract

The fruits of *Opuntia ficus-indica* (L.) Mill., the most worldwide cultivated species of Cactaceae family, contain viable seeds (100-250) of large size. This characteristic influences the commercial quality of the fruit. The study of floral biology in *Opuntia* allows to understand the behaviour of the plant during reproductive phase and perhaps to distinguish clones with different fruits behaviour (with seed and seedless). The study resulted in a palynological characterization by optical and scanning microscopy (SEM), followed by a qualitative analysis on the male line with pollen viability and in vitro germination tests, in addition to a pollen-pistil interaction survey. The plant material collected, during June-July 2010, consisted of clonal samples of the same genetic origin collected from populations characterizing the Sicilian cultivation area and distinguished in seedly cultivar 'Gialla' (yellow) and 'Rossa' (red) and seedless cultivar 'Bianca' (white). The palynological characterization revealed that *Opuntia* pollen has a radio-symmetric shape with a reticulated surface, poly-panto-porate with a perimeter from circular to polygonal. Moreover, the membrane of the pore appears finely granulated. Scanning microscopy reveals that the pollen has an intectated, reticulated and hetero-brochated exine with about 20-24 pores with a thin membrane. The average viability of the pollen was 91.2% ( $\pm 4.6$ ) in 'Bianca' cultivar, 82.40% ( $\pm 9.88$ ) for both seedly cultivars. In vitro germination rate was 28.76% ( $\pm 6.59$ ) in the white cultivar, 27.85% ( $\pm 5.15$ ) as overall average in 'Gialla' and 'Rossa' cultivar. In vivo germination rate in the seedless cultivar (75.77%  $\pm 5.32$ ) was higher than what was that recorded in the other two (53.5%  $\pm 6.5$ ).

### INTRODUCTION

Cactus (*Opuntia ficus-indica* (L.) Mill.), commonly known as cactus pear, belongs to Cactaceae family, subfamily Opuntioideae which is reported to contain about 130 genera and nearly 1500 species and that were originally native to the New World (Singh, 2003). This species, the most worldwide cultivated, is characterized by its edible fruits that contain viable seeds (100-250 per fruit). The pulp of the fruit develops from the funicular envelopes of the seeds which contribute also to the development of the seed coat (Mejía and Cantwell, 2003). The viable seeds are morphologically large and their presence greatly restricts the commercial quality and limits fruits acceptability in many markets because of the hard seed coat.

The three main cultivars of *Opuntia* characterizing the Sicilian cultivation area are clone's populations of the same genetic origin known as 'Gialla' (yellow), 'Rossa' (red) and 'Bianca' (white) (Barbera et al., 1992; Mondragon-Jacobo and Bordelon, 1996). Their fruits present various colours due to the combination of two pigments present in different concentrations in fruit pulp and skin (Butera et al., 2002).

The yellow cultivar or 'Gialla' is the most abundant; it represents 80 and 90% of Sicilian orchards (Butera et al., 2002). Presumably, it is the most productive, able to be handled and well-liked by consumers (Barbera et al., 1992). 'Rossa' cultivar is less

productive than the yellow one and is planted in less than 10% of the orchard. Finally, 'Bianca' cultivar is present in only 2% of familiar orchards. It is not so well spread because its fruits are unable to be handled. It was already reported in 1827, as a cultivar characterized by a reduced number of normal seeds but this aptitude is not well known yet (Mondragon-Jacobo and Bordelon, 1996).

Some plant species have shown particular fruit development behaviour known as seedlessness or parthenocarpy. It is the natural or the artificially induced or the genetically modified production of fruit without fertilization (Vardi et al., 2008). This ability has been reported by prior researches in many species such as *Citrus* (Crane, 1969) particularly in Mandarin (Talon et al., 1990) where it was demonstrated that the potential for setting parthenocarpic fruits is mainly influenced by hormonal status of the fruit in the latter stages of cell division and early stages of enlargement.

Also some experiments have been conducted on different *Opuntia* clones in order to distinguish varied fruit development behaviour. Normally sized parthenocarpic fruits containing empty seeds were obtained from fertilized flowers treated by Gibberellin and Auxin in pre-anthesis (Gil and Espinosa, 1980), but for some clones of *Opuntia*, the occurrence of pollination is not necessary to obtain seedless prickly fruits in fact, Weiss et al. (1993) reported vegetative parthenocarpy in some *Opuntia ficus-indica* (L.) clones.

The study of floral biology in *Opuntia* will allow to understand better the performances of the plant during the reproductive phase and afterwards to highlight eventual particular fruit development trend (parthenocarpy) in some clones.

In this preliminary work, we tried to analyze pollen morphological features of *Opuntia ficus-indica*. In addition, we studied the male line through pollen quality tests: viability and in vitro pollen germination. Subsequently, we analyzed the pollen tube growth behaviour, through the pistil, investigating deeply on the fertilization processes.

## **MATERIALS AND METHODS**

The plant material consisted on the spring flush flowers of *Opuntia ficus-indica* clonal samples of the cultivar 'Giulla' (yellow), 'Rossa' (red) and 'Bianca' (white) which characterize the Sicilian cultivation area. At anthesis, the flowers were harvested at random several times between June to July 2010 from the cladodes (Fig. 1) to collect pollen samples. They were used for the palynological study to assess pollen performances through viability and in vitro pollen germination tests. Finally, the pistils were collected two days after anthesis to analyse the pollen tube growth along the style, close to the ovary and near the micropyle.

### **Pollen Morphology**

For the analysis of pollen morphology, we used optical microscopy (OM) and scanning electron microscopy (SEM). For OM observations, fresh pollen was collected and powdered on a thin glycerinated fucsin colorant layer (10 g of gelatin + 60 ml water + 55 ml glycerol + 2 g of fucsin in phenol) deposited on a slide and covered by cover glass and observed through DMLB, Leika, Wetzlar, Germany light microscope.

For SEM observations, pollen collected from mature anthers was processed as described by Ahmad et al. (2010): pollen sample was fixed in FAA (formalin acetic acid alcohol) overnight at 4°C. After three washes in sodium cacodylate buffer (0.1 M, pH = 7). Then it was post fixed in 1% osmium tetroxide for 2 h at 4°C. This was followed by three washes in sodium cacodylate buffer to remove osmium. After that it was dehydrated in an ascending alcohol gradient (50 → 100°) and dried in an oven at 28°C for 12 h to evaporate the alcohol contained in the sample.

### **Pollen Viability**

Pollen viability was assessed according to Heslop-Harrison and Heslop-Harrison (1970). Fresh pollen was collected at anthesis and treated with fluorescein diacetate (FDA) (10 mg) dissolved in acetone (5 ml) and diluted in 10% sucrose solution (Reale et al., 2009). A small amount of pollen was suspended in a drop of the prepared solution on

a slide that was covered by a cover glass and observed using epifluorescence microscope with BP 450-490 exciter filter and LP 515 barrier filter.

### **In Vitro Pollen Germination**

Mature pollen grains were collected from flowers at anthesis and immediately putted in the germination medium reported by Brewbaker and Kwack (1963) made up of 100 ppm  $H_3BO_4$ ; 300 ppm  $Ca(NO_3)_2 \cdot 4H_2O$ ; 200 ppm  $MgSO_4$ ; 100 ppm  $KNO_3$  in a 40% sucrose solution (Sgromo et al., 2010). Pollen grains were placed on the surface of 10 ml of medium in plastic Petri dishes of 60 mm diameter. After 24 h of incubation in darkness at 25°C without shaking, the pollen was observed using a light microscope (DMLB, Leica, Wetzlar, Germany). Percentage of germination was calculated from counts of at least 100 pollens for each cultivar from each dish with at least 5 replicates.

### **Pollen Tube Growth and Fertilization Processes**

Under free pollination conditions, five to ten flowers of each cultivar were tagged prior to anthesis. Pistils and ovules were prepared for fluorescence microscopy 60 h after flower opening as follow: they were soaked in a 1% sodium carbonate solution for 1 h, washed with distilled water, softened by autoclaving in 1% sodium sulphite solution for 20 min (Jefferies and Belcher, 1971), and stained with a 0.1% solution of aniline blue in 0.1 N  $K_3PO_4$  for 4 h (Cuevas et al., 1994).

Aniline blue induced the fluorescence of the callose plugs located along the pollen tubes so they can be clearly identified and followed. Squashed preparations of pistils and ovules were observed under a DMLB Leica microscope with UV light source using a BP 515-560 exciter filter and an LP 590 barrier filter.

Percentage of in vivo pollen germination was calculated from counts of at least 100 pollen grains on the stigma observed under microscope. Furthermore, percentage of pollen tube along the style and near the micropyle was calculated.

## **RESULTS AND DISCUSSION**

During the period between June to July 2010, *Opuntia ficus-indica* (L.) fresh pollen was collected from flowers at anthesis in order to conduct palynology study using light and SEM microscopy.

Moreover, pollen reproduction performances were evaluated using laboratory tests of pollen viability and in vitro germination.

Additionally, pistils and ovules from the flowers were collected two days after anthesis under free pollination conditions, prepared for microscopy observation in order to investigate the fertilization processes and eventually identify some particular fruit development behaviour.

### **Pollen Morphology**

The observations realized by the optical microscopy allowed to appreciate the general characteristics of *Opuntia ficus-indica* (L.) pollen grain. It presented a spherical, apolar radio-symmetrical form with a reticulated and a poly-panto-porated surface, with a circular polygonal perimeter (6-8 sides).

The ultrastructure of the pollen grain was analysed by means of SEM observations. The exine is composed of a reticulated tectum cross linked by 20-24 heterobrocatated circular germination pores (Fig. 2). According to these observations, the average diameter of the pollen grains oscillates between 107.04  $\mu m$  according to the X axis and 106.45  $\mu m$  according the axis of Y. Our structural and ultrastructural description of *Opuntia ficus-indica* (L.) pollen grain is in accord with what was reported by Garalla and Cuadrado (2006) for *Opuntia* spp.

### **Pollen Viability**

In addition to its morphological features, the pollen is characterized by some biological parameters reflecting its reproductive performances such as viability defined

by Heslop-Harrison (1992) as the capability of the pollen grain to deliver two male gametes to the embryo sac. Due to a fluorochromatic reaction between a pollen grain cytoplasm enzyme and the FDA, the viable pollen grains appeared fluorescent and brighter than those non viable which were extinguished and did not present any fluorescence (Fig. 3A).

Cultivar 'Bianca' presented the highest viability 91.28% ( $\pm 4.6$ ) which was quite similar to cultivar 'Rossa' value 88.1% ( $\pm 3.94$ ). Meanwhile, cultivar 'Gialla' presented the lowest average rate 76.7% ( $\pm 11.21$ ).

### **In Vitro Pollen Germination**

The cultivars 'Bianca', 'Rossa' and 'Gialla' showed quite the same values of in vitro germinability: 28.76% ( $\pm 6.6$ ), 27.6% ( $\pm 3.1$ ) and 28.15% ( $\pm 7.36$ ) respectively. Our results are quite consistent with previous studies carried out on *Opuntia ficus-indica* (L.) by Weiss et al. (1993) (Fig. 3B).

### **Pollen Tube Growth**

Cultivars 'Gialla', 'Rossa' and 'Bianca' showed a rapid progamic phase in which pollen grains germinated normally on stigma within the first hours following pollination (Fig. 4A) and the pollen tubes did not present any incompatibility symptoms as described by (Safavian and Shore, 2010). The presumed seedless cultivar 'Bianca' presented higher in vivo germination rate (75,77%  $\pm 5,32$ ) than the seedly ones ('Gialla' and 'Rossa') which had an average value of 53,5%  $\pm 6,51$ .

Pollen tubes grew along the style (Fig. 4B) and reached, in all analyzed flowers, the base of the style. The cultivars 'Gialla' and 'Rossa' presented a percentage of pollen tubes presence (79,51%  $\pm 11,8$ ) higher than that registered for 'Bianca' cultivar (44,54%  $\pm 12,46$ ). We noticed also that such trend was conserved in proximity of ovule specially in cultivar 'Rossa', and in proximity of micropyle (Fig. 4C) in cultivar 'Gialla' and 'Rossa' (Table 1).

This pattern of increase in the number of pollen tubes in the style and near the ovules in 'Gialla' and 'Rossa' is typical of seedly cultivars and the lower number of pollen tubes in 'Bianca' cultivar suggests that it might have a particular fruit development behaviour. For cultivar 'Bianca' we can hypothesise that its seedlessness could be based on a partial stenospermocarpy as reported for grape and *Citrus* (Vardi et al., 2008).

## **CONCLUSION**

As outlined in this study, we were able to provide a detailed description of the structure and ultrastructure of *Opuntia ficus-indica* (L.) pollen grain. Moreover, the study evidenced that the microgametophyte of the cultivars 'Gialla', 'Rossa' and 'Bianca' characterizing the Sicilian germoplasm has good reproductive performances registering high levels of viability, in vitro and in vivo germination. Our investigations on the pollen tube growth behaviour allowed to verify the seedly compartment of cultivar 'Gialla' and 'Rossa', and to individuate an atypical fruit development model for the 'Bianca' cultivar which should be confirmed by ulterior post fertilization analysis.

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## **Tables**

Table 1. In vivo pollen germination and pollen tube growth in pistils of *Opuntia ficus-indica* (L.) cultivars ‘Gialla’, ‘Rossa’ and ‘Bianca’ 60 h after anthesis.

Cultivar	Mean	Mean (%) (Gialla + Rossa) <sup>1</sup>
	In vivo germinability (%)	
Gialla	50,68 ± 7,36 b <sup>2</sup>	53,5 b
Rossa	57,74 ± 1,16 b	
Bianca	75,77 ± 5,32 a	
	Pollen tube in style (%)	
Gialla	85,42 ± 16,44 a	79,51 a
Rossa	73,61 ± 2,81 ab	
Bianca	44,54 ± 12,46 b	
	Pollen tube in proximity of ovule (%)	
Gialla	14,58 ± 4,52 b	20,44 a
Rossa	29,23 ± 6,94 a	
Bianca	13,63 ± 6,23 b	
	Pollen tube in proximity of micropyle (%)	
Gialla	14,28 ± 0 a	13,96 a
Rossa	13,80 ± 8,06 a	
Bianca	6,13 ± 0,42 b	

<sup>1</sup> Means of cultivars ‘Gialla’ and ‘Rossa’ compared with cultivar ‘Bianca’.

<sup>2</sup> Means with different letters within a column indicate significant differences using Duncan’s test ( $P \leq 0.05$ ).

## **Figures**



Fig. 1. *Opuntia ficus-indica* (L.) cladodes.

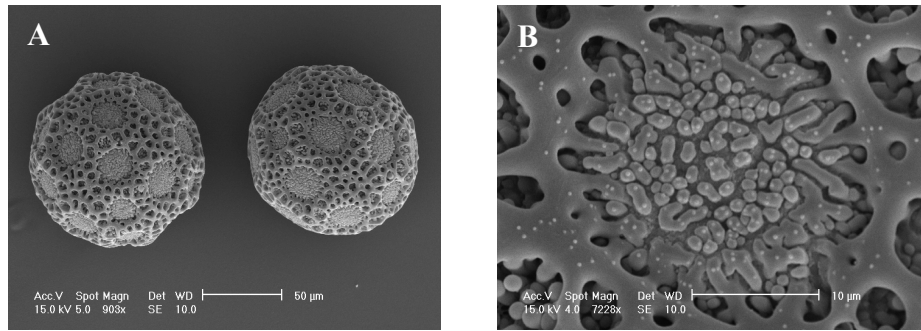


Fig. 2. *Opuntia ficus-indica* (L.) pollen grain ultrastructure. A: germination pores ( $\times 900$ ), B: tectum structure ( $\times 7000$ ).

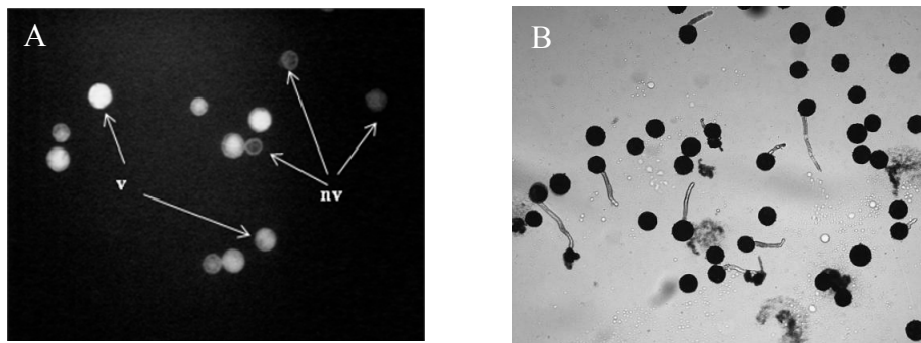


Fig. 3. *Opuntia ficus-indica* (L.) A: pollen grains stained by fluorescein diacetate  $\times 20$  (v: viable, nv: non viable), B: germinating pollen grains of *Opuntia ficus-indica* (L.)  $\times 20$ .

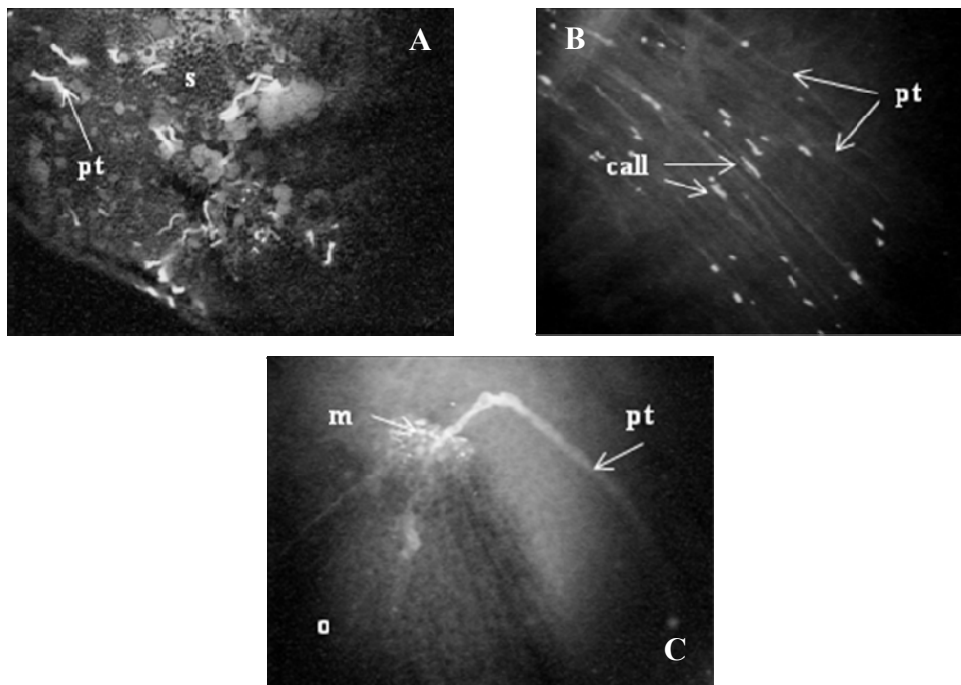


Fig. 4. Pollen tube growth on pistil. A: pollen grain germinating on stigma, B: pollen tubes along the style, C: pollen tube entering the micropyle (call: callose plugs, m: micropyle, pt: pollen tube, s: stigma, o: ovule).



