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STORAGE EFFECTS ON *ACTINIDIA DELICIOSA* POLLEN

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RESUMEN: Se estudia la influencia de la temperatura del periodo de almacenamiento en la calidad del polen de *Actinidia deliciosa* cv. *Tomuri*. El polen cogido se almacenó a –20ºC (51% RH) y se han tomado los valores de germinación *in vitro* y de viabilidad regularmente. Después de 36 semanas, el polen presentaba una capacidad de germinación de 75% (r_g = 0,983, p_g < 0,0) y viabilidad de 82% (r_s = 0,855, p_s < 0,030). Los coeficientes de determinación (r^2) germinación *vs.* viabilidad se ordenaron desde 0,83 haste 0,99. En este año, el polen mejor preservado se usó en la polinización artificial para evaluar la capacidad de crecimiento de la semilla. Treinta días depués, todos los frutos estaban perfectamente desarrollados, indicando una buena polinización.
PALABRAS CLAVE: *Actinidia deliciosa* cv. *Tomuri*, germinación *in vitro*, viabilidad, polinización artificial.

SUMMARY: The influence of storage temperature and storage period on pollen quality was studied in *Actinidia deliciosa* cv. *Tomuri*. Collected pollen was stored at -20ºC, 51%RH, and *in vitro* germination and viability were scored at regular periods. After 36 storage weeks, pollen presented 75% germinability (r_g = 0,983, p_g < 0.0) and 82% viability (r_s = 0,855, p_s < 0,030). Coefficients of determination (r^2) for germination *vs.* viability ranged from 0,83 to 0,99. In 2002 blossom season, best-preserved pollen was used in artificial pollination to evaluate seed set ability. Thirty days after fruit-set all fruits were long and well developed indicating a successful pollination.
KEY WORDS: *Actinidia deliciosa* cv. *Tomuri*, *in vitro* germination, viability, artificial polination.

INTRODUCTION

Pollen storage is one important aspect in plant breeding programs when the species or individuals to be crossed present different blossoming seasons or are geographically isolated (Maguire & Sedgley, 1997 and Sato *et al.*, 1998).

Fruit tree pollen preservation is one of the methods for long-term plant germplasm storage. In crops that require artificial pollination pollen storage is also beneficial for crop stabilization (Akihama & Omura, 1986 and Ganeshan, 1986).

Actinidia deliciosa, usually known as kiwifruit, is a dioecious species, meaning that pollen needs to be transported from male to female flowers. Although, in nature, *A. deliciosa* pollen is transported by wind, honeybees also play a minor role on pollen transport. Insufficient pollination has been found to be responsible for the production of fruit not

commercially acceptable. To overcome this problem, growers resort to artificial pollination using either pollen collected from their own orchards or bought in the market.

Although pollen viability can be tested using either staining methods, like FDA (fluoresceine diacetate), or *in vitro* germination, it was been considered that the ability to effect seed set is the most accurate viability test (Maguire & Sedgley, 1997 and Sato *et al.*, 1998). For this reason, in our work, the data collected from germinability and viability tests was complemented with data collected from artificial pollination tests.

In several species the incapacity to effect seed set is due not to pollen non-germination but to the failure in the migration of the generative cell and the vegetative cell into the pollen tube. To follow the migration of these structures, DAPI (4,6-diamino-2-phenylindole) can be used.

The aim of this work is to settle a reliable method for kiwifruit pollen preservation and to determine for how long this pollen maintains its germinability and viability in sufficient high levels to assure satisfactory pollination.

MATERIAL AND METHODS

POLLEN COLLECTION

Several male flowers were harvested and brought to the laboratory where the undehisced anthers were removed. Anthers were dehydrated at 27ºC and 35% relative humidity (RH) for 12 hours.

Experiments were performed with pollen collected during the 2001 blossoming season using plants of *A. deliciosa* cv. *Tomuri* from the Northwest of Portugal (Minho Litoral region).

POLLEN QUALITY TESTS

Pollen longevity differs among species so it is necessary to assess pollen viability before use in practice. Thus, *in vitro* germination and viability percentages of pollen were scored at the beginning and during storage time at regular periods.

Stored samples were germinated for 2, 4 and 6 hours in a thermo-controlled dryer, at 37ºC, with agitation. The germination medium used in these experiments comprised sucrose, boric acid and calcium nitrate (Abreu *et al.*, 1997).

In vitro germination was scored using a Leica DM LB light microscope at a magnification of 200x or 400x. At least 300 randomly selected pollens were scored for germination in three different fields (100/field). The criterion for pollen germination was a pollen tube length that exceeded the pollen diameter.

In vitro germination test was compared with a fluorochromatic test (FCR) using 2 mg/ml FDA. Pollen was placed on a slide with two drops of FDA (Heslop-Harrison *et al.*, 1984). An interval of 30 minutes allowed penetration of the fluorochrome into the pollen grains before observation. Pollen fluorescence was determined with the microscope referred above with blue excitation provided by a mercury lamp at a magnification of 400x. Pollen that fluoresced with a bright yellow color was scored as viable. Viability percentage was determined using three replicates of more than 100 pollen grains each.

The migration of the nuclei of vegetative and the generative cells were visualized by

DAPI specific fluorochrome (5 mg/ml in phosphate buffer at pH 7,0). The observations were made under a Leica DM LB light microscope using an ultraviolet light provided by a mercury lamp at a magnification of 200x.

STATISTICAL ANALYSIS

The *in vitro* germination and viability results were analyzed using an analysis of variance program. Correlation coefficients (r) and coefficients of determination (r^2) were calculated by plotting a line of best fit.

ARTIFICIAL POLLINATION

After one year of preservation at -20ºC, pollen was used to perform artificial pollination. Pollen was dusted to receptive stigmas and the branches containing artificially pollinated flowers were isolated. Thirty days after fruit set, the fruit were evaluated in terms of size. Control plants were not pollinated and were also isolated.

RESULTS

POLLEN QUALITY TESTS

In vitro germination of fresh and preserved pollen was assessed after 2, 4 and 6 hours. No significative differences were recorded between 4 and 6 hours so all results presented refer to *in vitro* germination after 4 hours (Tab. 1).

Pollen grains of *Actinidia deliciosa* cv. *Tomuri* are bicellular, presenting a vegetative and a central generative cell. In the generative cell, the nucleus occupies almost the entire cell and presents plastids. Pollen grains usually germinated 30 minutes after sowing under our experimental conditions and are rapidly developed pollen tubes.

Fresh pollen presented *in vitro* germination of 87±3,5% (Figs 1a and 1b) and a viability of 98±1,25% (Fig. 1c). During 36 weeks, this pollen maintained high *in vitro* germination and high viability percentages. After the storage period, in a freezer (-20ºC, 51%RH), our results point to constant germinability of 75% (r_g=0,983, p_g<0,0) and viability of 82% (r_v=0,855, p_v<0,030) (Fig. 2).

High coefficients of determination (r^2) for germination *vs.* viability were found, ranging between 0,83 and 0,99 (Tab. 2).

In *A. deliciosa* pollen preserved at -20ºC for 36 weeks it was possible to visualize the migration of the vegetative and generative nucleus to the growing pollen tube (Fig. 3).

ARTIFICIAL POLLINATION

As control, we used non pollinated flowers. These flowers soon aborted and did not form fruits.

All flowers artificial pollinated with pollen preserve during this work (Figs 4a to 4c) were able to effect fruit-set. Thirty days after fruit-set we observed long and well-shaped fruits (Fig. 4d).

	2 hours	4 hours	6 hours
In vitro germination	55,6 ± 1,71	82,9 ± 4,26	91,9± 2.14

TABLE 1. Evolution of pollen *in vitro* germination percentage.

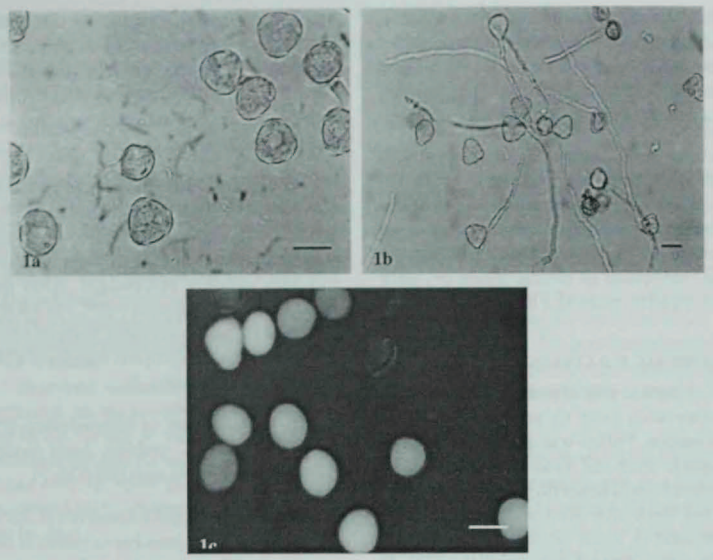

FIGURE 1. Micrographs of germinated pollen after 2 hours (a) and after 4 hours (b) and determination of pollen viability, using FDA (c) (bar 20 mm).

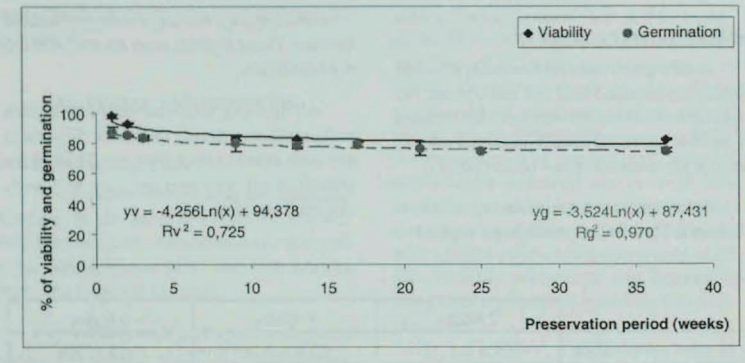

FIGURE 2. Evolution of viability and germination percentages during pollen preservation at -20°C (36 weeks).

DISCUSSION

POLLEN QUALITY TESTS

Other *in vitro* germination media have been proposed for *Actinidia* species (Bomben *et al.*, 1999 and González & Coque, 1994) but in previous works (Abreu *et al.*, 1997) our *in vitro* germination medium showed higher *in vitro* germination percentage in pollen collected in the northern region of Portugal.

A little decrease in pollen germinability and viability was recorded in *A. deliciosa* pollen stored at –20°C during 36 weeks.

Although there is little information on pollen preservation of this species but a previous work on *A. deliciosa* cv. *Matua* reports that pollen stored at -18°C for 32 weeks maintained 80% germination but pollen viability was totally lost at the 96th week (Bomben *et al.*, 1999).

		Germination			Viability		
		Pollen A	Pollen B	Pollen C	Pollen A	Pollen B	Pollen C
Germination	Pollen A	1.00					
	Pollen B	0.99	1.00				
	Pollen C	0.97	0.97	1.00			
Viability	Pollen A	0.83	0.90	0.85	1.00		
	Pollen B	0.99	0.99	0.99	0.83	1.00	
	Pollen C	0.98	0.96	0.91	0.78	0.96	1.00

TABLE 2. Coefficients of determination (r^2) for *in vitro* germination *vs.* viability of different pollen samples (A, B and C) stored at -20°C for 36 weeks.

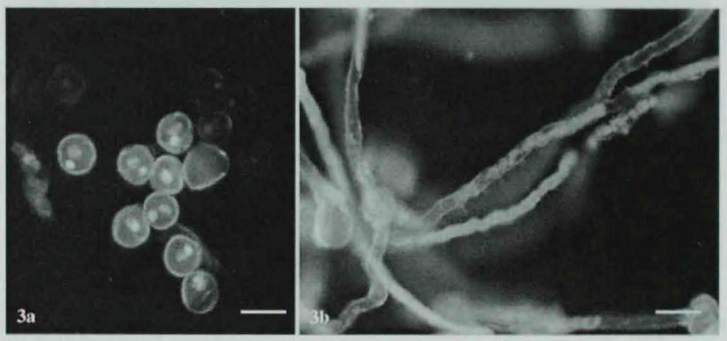

FIGURE 3. Micrographs of mature (a) and germinated pollen grains (b), using DAPI, for nuclei localization (bar 20 mm).

Another aspect of pollen germination is pollen tube length. A previous work in *A. deliciosa* pollen recorded that during storage at -20ºC pollen tube length was not significantly affected (ABDUL-BAKI, 1992).

In *A. deliciosa* pollen preserved at -20ºC was possible to observe the vegetative and the generative nucleus in the growing pollen tube. This indicates that, probably, this pollen will be capable to perform ovule fertilization.

ARTIFICIAL POLLINATION

Thirty days after fruit-set, all fruits resulting from pollination with pollen preserved in our lab, were long and well developed. This indicates a successful pollination.

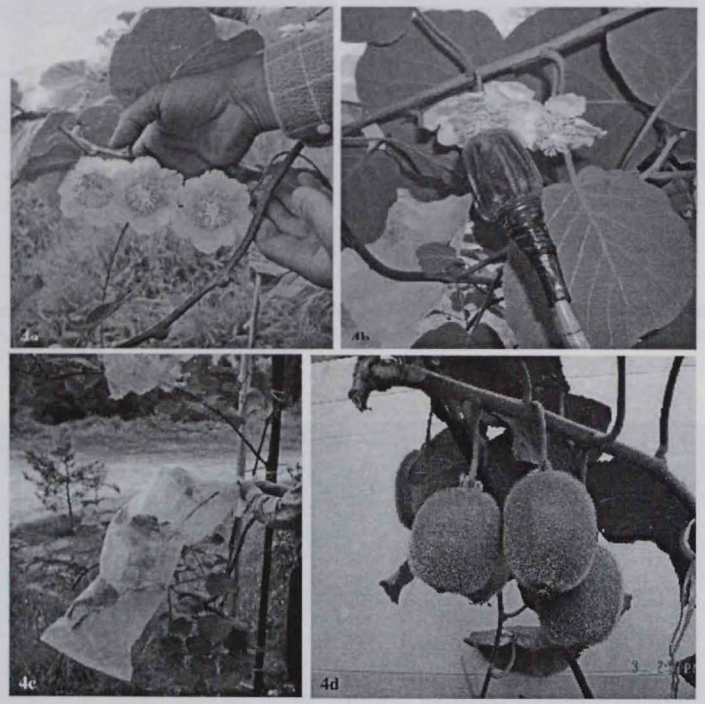

FIGURE 4. Phases of artificial pollination; several flowers were chosen (a) and hand pollinations were performed (b). Then the control flowers (non-pollinated) and test flowers (hand pollinated) were isolated using fabrics with a pore inferior to pollen diameter (c). Thirty days after fruit set, the fruits from both type of flowers (D) were compared (d).

CONCLUSION

After 36 weeks of pollen preservation at -20ºC, pollen maintained high levels of germinability and viability.

These results were confirmed when pollen was used in artificial pollination, since obtained fruits presented all characteristic features.

All results indicate that our process of pollen preservation might be a simple and reliable method to conserve and distribute pollen to the farmers.

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