POLLEN VIABILITY ASSESSMENT

CENTRO INTERNACIONAL DE LA PAPA
CIP
A member of the CGIAR Consortium

STC
STC-INIA/CIP

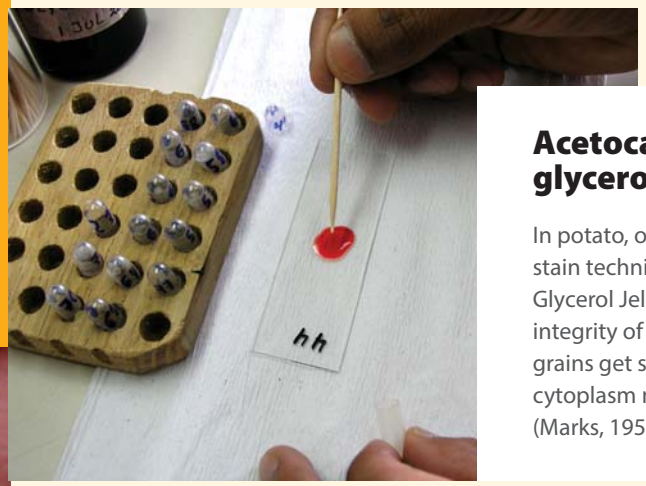
In potato breeding programs, determining pollen viability is crucial to start crossing plans, achieve successful hybridizations and obtain hybrid seeds.

There are different methods to evaluate pollen viability; some noteworthy tests are dyes and *in vitro* germination. Dye tests have advantages as indicators of pollen viability because they are faster and easier compared with pollen germination, but they do tend to overestimate the viability and real germination of pollen grains. On the other hand, *in vitro* germination depends on the genotype, environmental conditions, pollen maturity, composition and pH of the medium; thus it is necessary to determine optimum conditions for pollen germination.
It is recommended simultaneously use several tests to reflect pollen performance.

Sample collection

Collect completely open flowers, with the anthers near dehiscence or completely dehiscent. Then, extract the anthers and place them in an envelope and leave to dry. Finally, hold a vibrator against the envelope to help release the pollen (Fig. 1).

Fig. 1. Extract the pollen, holding a vibrator against the envelope to help release the pollen.



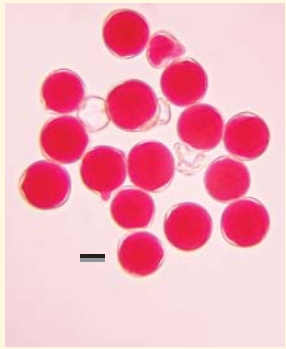
Acetocarmine glycerol jelly test

In potato, one of the most well-known stain techniques is the Acetocarmine Glycerol Jelly. This test measures the integrity of the cytoplasm; the pollen grains get stained red when the cytoplasm membrane is integral (Marks, 1954).

Fig. 2. On the drop of dye, spread the pollen with light circular movements on a slide.

Procedure

Place one or two drops of 2 percent acetocarmine glycerol jelly in the center of a slide, previously identified with the progenitor's code (Annex 1a). Using a wooden toothpick, take a small amount of pollen out of the capsule, and place it on the drop of dye, spreading the pollen with light circular movements (Fig. 2). Let it stand for a minute, and then put the slide cover on. Mounted slides should lie flat for one or two days. If it is required that the samples be stored, place them in boxes designed for this purpose in a cold room or refrigerator at 4 °C.



Afterwards, observe them under a light microscope at 200 or 400x magnification. A bright red staining of the cytoplasm of a pollen grain is indicative of viable pollen, while cytoplasm not red or pink indicates non-viable pollen (Fig 3). Compared with the viable pollen grains, the sterile ones are deformed; with a granular cytoplasm and/or an unstained gap which is usually situated at one side of the pollen grains giving them an eclipse-like appearance (eclipse sterility).

Fig. 3. Observation of pollen sample stains using Acetocarmine Glycerol Jelly at 200x magnification. Bar = 20µm

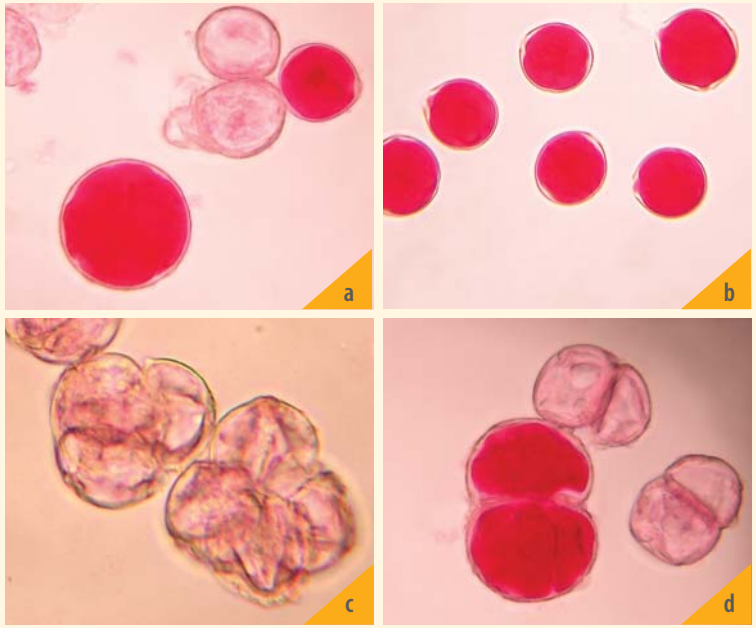


Fig. 4. Observation of pollen grain abnormalities at 200x magnification a) 2n pollen; b) viable pollen, c) Sterile tetrads), d) Sterile dyads. Bar=20um.

The observation of abnormalities such as tetrads or pollen grains with four cores indicates that the sample is infertile (Fig 4 c, d). In samples of pollen from diploid potatoes, pollen grains may be larger than normal grains, in which case they correspond to pollen with a non-reduced genetic load. This phenomenon of unreduced gametes is rarely observed in tetraploid potatoes (Fig 4a).

We recommend the evaluation of 250 pollen grains in the slide, expressing the results as a percentage of the viable pollen.

Besides, we propose a scale based on the range of pollen viability of promising genotypes in order to determine whether they can be used as male parents in breeding programs (Table 1).

Tabla 1. Scale of ranges of pollen viability in potato.

Scale	Range	Description (viability)
1	0	Sterile
3	<50	Low
5	>50-80	Moderate
7	>80-100	High

In vitro pollen germination

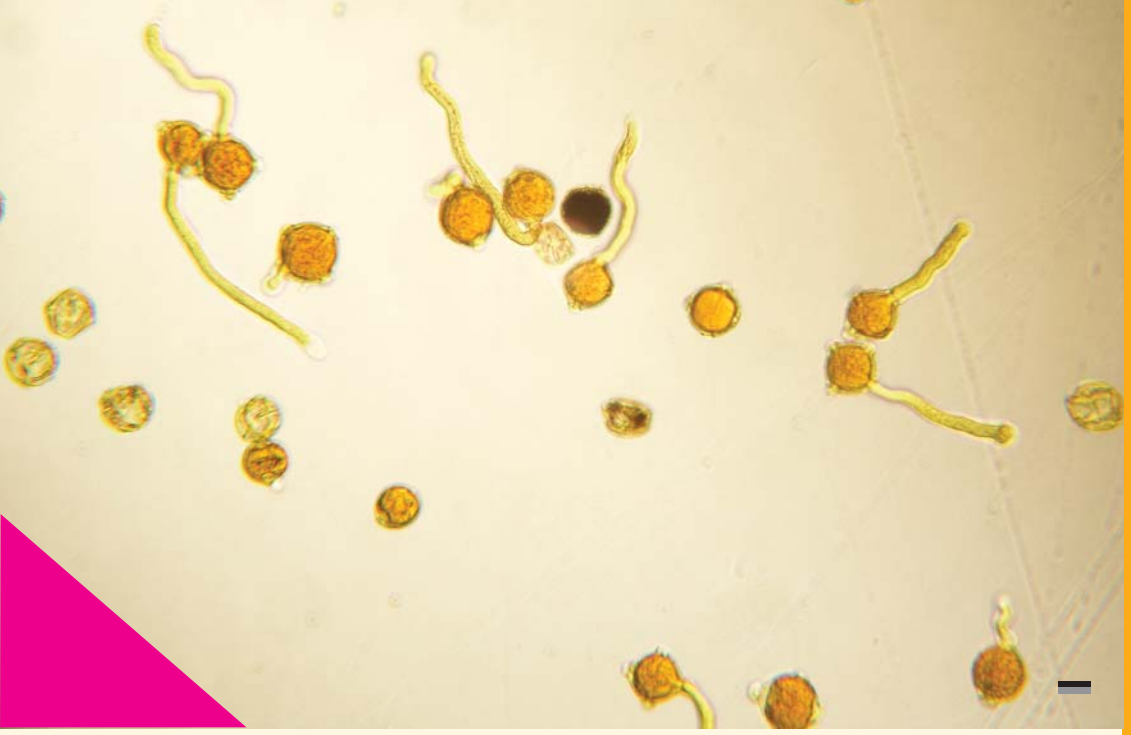
In vitro pollen germination is a test that simulates the growth of the pollinic tubes in the stylar tissue, since the culture medium used in its composition resembles the stigma mucilage (Rodriguez-Riano & Dafni, 2000; Van Marrewijk, 1993).

The culture medium used for germination and growing of the pollinic tubes in potato contains sucrose, boric acid and Polysorbate 20 (Tween 20) (Annex 1b).

Procedure

Place 4 drops on the base of a 5.5-centimeter Petri dish, distributing them in the vertices of an imaginary square. Then, with the tip of a wooden toothpick, add small amounts of pollen from the same sample on each drop, spreading the pollen with light circular movements (Fig 5). Prepare a humidity chamber, by placing a filter paper moistened with distilled water covering the bottom

Fig. 5. Place the pollen on the drops of culture medium.



the lid. Cover the bases of the Petri dishes with the lids and leave them overnight at a temperature of 20 to 24 degrees Celsius. The next day, place a drop of iodine solution (iodine-potassium iodide) on the sample, previously laid on the base of the Petri dish, and cover it with a 22x40-millimeter slide. After that, observe it under a light microscope with 100x magnification.

A pollen grain is considered to have successfully germinated when the pollinic tube length reaches a size equal to or greater than the diameter of the pollen grain (Fig. 6). The germinated and ungerminated pollen grains count must be performed in a minimum of 10 fields. Results are expressed as the percentage of the total number of pollen grains per field. An average rate of 80 percent indicates fertile pollen.

Fig. 6. Observation of pollen germinating in the culture medium at 100x magnification. Bar=10um.

References

- Marks, G.E. 1954. An aceto-carmine glycerol jelly for use in pollen fertility counts. Stain Technol. 29:277.
- Van Marrewijk, G. A. Flowering biology and hybrid varieties. Hybrid varieties.- En: International Course on Applied Plant Breeding. The Netherlands. IAC. 80 p., 1993. 66(1):61-71.
- Rodriguez-Riano & Dafni, 2000: A new procedure to asses pollen viability. Sex. Plant. Reprod. 12: 241-244.

Annex

1a) **Preparation of Acetocarmine Glycerol Jelly stain**
Acetocarmine Glycerol Jelly stain is prepared using a solution of 100 ml of 45% acetic acid; boil this solution until boiling point, then add 2 g of carmine until the carmine is completely dissolved (approximately when the solution has a volume of 60 ml). Leave to cool, and filter; finally, add the same volume of glycerol as the final solution (approximately 60 ml). The whole process must be performed in a laminar flow chamber and under constant agitation.

1b) **Culture medium used for *in vitro* germination and growth of pollinic tube**
In a beaker of 100 milliliters, add 5 milliliters of a stock solution of boric acid, 20 grams of sucrose and 0.2 milliliters of Polysorbate 20, commercially known as Tween 20. Complete with 100 milliliters of distilled water, and take to a magnetic stirrer to homogenize the solution, which should be at pH 5.5.

1c) **Iodine solution (iodine-potassium iodide)**
Dissolve 1 g of iodine (I2) and 1 g of potassium iodide (KI) in 100 ml 70% ethanol and store in an amber bottle at room temperature.

International Potato Center
P.O. Box 1558, Lima 12, Peru
www.cipotato.org

Citation:
Ordoñez B. 2014. Brochure: Pollen Viability Assessment. International Potato Center (CIP), Lima, Peru. 8p.

Contributors: M. Gastelo / C. Bastos