ASSESSMENT OF PLOIDY BY CHLOROPLAST COUNT IN STOMATAL GUARD CELLS

Measuring ploidy level is an essential technique in the process of developing new hybrid genotypes in potato breeding.

Methods to determine ploidy level can be direct (chromosome counting) or indirect (chloroplast number of the guard cells, size of pollen grains, flow cytometry, morphological observations, among others).

Determination of ploidy level using chloroplast number in stomatal guard cells from potato leaf epidermis is simple to use and less labor intensive, and can be considered a practical alternative because it makes it possible to distinguish diploids from other ploidies (Huamán, 1995). It is recommended to use this technique as a preliminary screening to select diploids, because there is a correlation between the mean number of chloroplasts and the number of chromosomes.

Sample collection

Collect 1 to 5 folioles of the apical part of the plant, previously identified with the code assigned to the plant. The samples are placed in a Petri dish containing a filter paper moistened with distilled water covering the bottom of the lid.

Observation

Measurement in each of the two guard cells of stomata was scored in ten stomata per sample. (Fig. 2). The average indicates the ploidy level (Table 1).

Tabla 1. Scale to determine the ploidy of a potato genotype.

Chloroplast number in stomatal guard cells	Ploidy
6 - 8	Diploid (2n=2x=24)
9 - 11	Triploid (2n=3x=36)
12 - 14	Tetraploid (2n=4x=48)

CHLOROPLASTS

GUARD CELLS
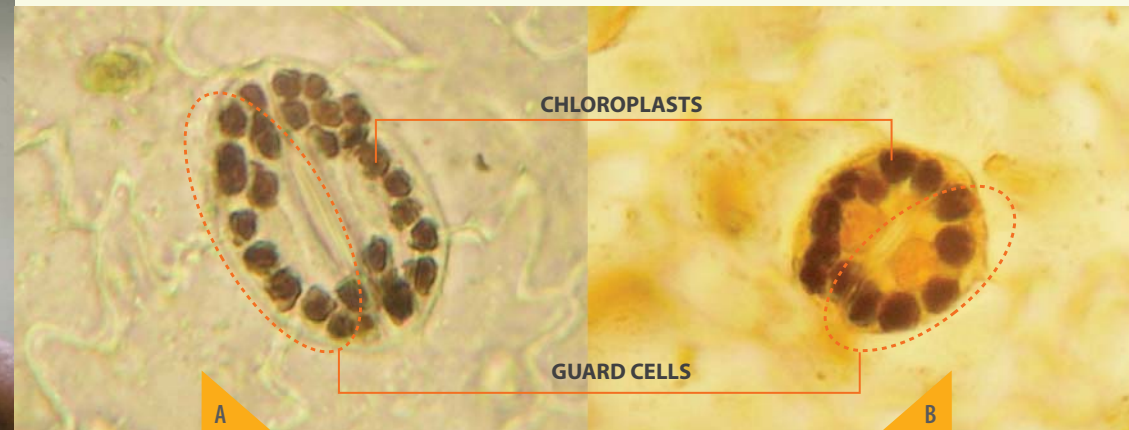
Fig. 2. A) Probable tetraploid genotype, B) Diploid genotype

Procedure

One or two drops of iodine solution (iodine-potassium iodide) were placed in the center of a slide. (Annex 1a). Then, epidermal peels were taken from the abaxial side near the vein structure using a pair of fine tweezers and immediately put on the slide. (Fig. 1). After 2 min, a cover slip was mounted and observations were made using a light microscope at 100, 200 or 400x magnification.

Fig. 1. Peel off the surface cell layer from the undersurface of the leaf.

References

- Huamán, Z. 1995. Técnicas citológicas para determinar el número cromosómico y la fertilidad de las papas. Guía de Investigación CIP 10. Centro Internacional de la Papa, Lima, Perú. 18p.

Annex

1a) 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.