Chromosome count on banana root tip squashes

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Abstract — Introduction. This protocol makes it possible to determine the chromosome number of banana clones in root tip squashes. The principle, key advantages, starting plant material, time required and expected results are presented. **Materials and methods**. This part describes the required laboratory materials, and the four steps of the protocol used for the chromosome counts (root sampling and treatment, slide preparation, observation of useful cells and chromosome counts on useful cells). Possible troubleshooting is discussed. **Results**. The protocol usually results in condensed and intensely stained chromosomes.

France (Guadeloupe) / *Musa* sp. / methods / chromosome number

Comptage de chromosome sur des pointes racinaires de bananier.

Résumé — Introduction. Ce protocole permet de déterminer le nombre de chromosomes des clones de bananier sur des pointes de racines écrasées. Le principe, les principaux avantages de la méthode, le matériel végétal de départ, le temps nécessaire et les résultats attendus sont présentés. **Matériel et méthodes**. Cette partie décrit le matériel de laboratoire nécessaire et les quatre étapes du protocole utilisé pour le comptage des chromosomes (prélèvement et traitement des racines, préparation des lames, repérage des cellules adéquates, comptage des chromosomes sur ces cellules). Des problèmes éventuels sont évoqués. **Résultats**. Le protocole permet habituellement d'obtenir des chromosomes condensés et intensément colorés.

France (Guadeloupe) / *Musa* sp. / méthode / nombre chromosomique

1. Introduction

Application

This protocol makes it possible to determine the chromosome number of banana clones by chromosome counts in root tip squashes. It is based on a simplified method developed in Jamaica in the 1960s [1].

Principle

Root tips are harvested from greenhouse plants maintained in pots. Collected root tips are pretreated by 8-hydroxyquinoline, fixed overnight, then squash-stained in an orcein solution.

Key advantages

The method is easy and rapid, with no requirement for acid hydrolysis or for embedding in paraffin or a resin. It gives more accurate results for ploidy determination than chloroplast counts in stomata guard cells on leaves.

It is cheaper than flow cytometry and thus saves investments and consumable costs.

Starting material

Root tips (1 cm long) are collected from healthy plants grown in rigid plastic pots of 2–3 L capacity under optimal conditions in a greenhouse. Plants can be generated from

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in vitro plants or from small suckers taken from mother plants in the field.

Note: roots must be white with a diameter of about 1 mm, firmly turgescent but not hard, with no secondary ramification and showing a yellowish dome-shaped cap. Old roots with necrotic regions must be avoided. Roots should be harvested in the morning (1 to 2) h after the sunrise because of the high mitotic activity.

Time required

One hour is necessary for the harvest of about 10 root tips and their treatment with 8-hydroxyquinoline; 15 min, to transfer root tips for overnight maceration-fixation; 20 min, for each root tip squash; 60 min, for observation and chromosome counts on each slide with repetitions on five good cells.

Note: a maximum of eight to ten clones can be characterized daily.

Expected results

An average of one to two good cells with well-spread chromosomes and two to five cells with minor defects for each squash can be expected.

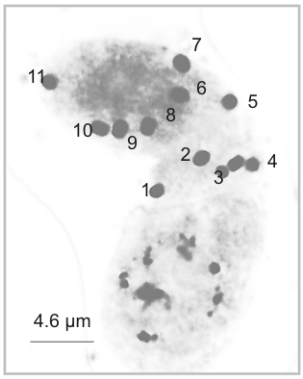

Figure 1.
Haploid cell of *Musa acuminata malaccensis* (clone Pahang) with 11 chromosomes. The chromosomes are not very contracted and well spread around the cell in the same plane with no overlaps. The average length of chromosomes is about 1.8 µm. Chromosome no. 3 has a satellite. No minichromosome is detected.

2. Materials and methods

Laboratory materials

The protocol requires:

– a standard microscope with objectives of ×20, ×40 and ×100 magnification (with oil immersion),
– 0.03% (w/v) aqueous solution of 8-hydroxyquinoline prepared with distilled water,
– a maceration-fixation solution containing acetic acid (4 vol.), distilled water (5 vol.) and 95% ethanol (1 vol.),
– a staining solution containing 2% (w/v) orcein in lactophenol [solution of lactic acid (1 vol.), phenol (1 vol.), glycerol (1 vol.) and distilled water (1 vol.)],
– stainless steel forceps and scalpels,
– tubes (10 mL) with caps,
– microscope slides and clean extra-fine cover glass,
– wood pencil with eraser,
– filter paper,
– rubber gloves.

Chromosome counts

• Step 1. Root sampling and treatment

– Collect root tips (1 cm long) about (1 to 2) h after the sunrise because of the high mitotic activity.
– Immerse root tips directly in 1 mL of 0.03% 8-hydroxyquinoline solution at 20–22 °C for (4 to 9) h.

Note: keep the tubes protected from direct light during the 8-hydroxyquinoline treatments; do not seal the tubes to facilitate root respiration.

– Transfer root tips into the maceration-fixation solution in sealed tubes (5–10 mL) for about 12 h (overnight).

• Step 2. Slide preparation

– Use rubber gloves to avoid skin contact with the staining solution. Pull out a root tip from the maceration-fixation solution and briefly blot dry on filter paper with the forceps.
– Cut off the apical root portion of about 1.5 mm length.
– Place the apical portion in the center of a microscope slide in a drop of the staining solution. Put the cover glass on and firmly squash the preparation with a finger without horizontal moving. Wipe the excess stain off the slide with filter paper.
– Flatten the preparation with radial vertical pressures from the center to the exterior of the slide with the eraser of the pencil. *Caution:* pressure must be exercised to remove air bubbles from the preparation with enough force to spread chromosomes within the cells but not to burst cell walls.
– Wipe the excess stain off the slide again with filter paper.
– Wait (6 to 8) h before starting observations.

Note: the stain should take about 2 days to intensify and provide good contrast. The preparations should also be sealed with nail polish for later microscopic observations.

• Step 3. Observation of useful cells

– Make a mark on the top right side of the slide.

– Put the slide on the platform of the microscope with the mark on the right.
– Scan the slide in forward and backward directions of about 0.4–0.5 mm width (less than the microscope field) at ×200 to ×400 magnifications.
– Locate the position of all good cells with well-identified chromosomes on the slide by reading both graduate scales of the platform.
Note: this precaution will prevent accidental removal of the preparation from the record and allows observations by different persons.
– Complete the scan of the slide and position the previously identified good cells by the noted coordinates.
– Pass the microscope to the ×100 objective and use a drop of immersion oil.

• Step 4. Chromosome counts on useful cells
– Select only the cells with the chromosomes well separated and little overlap, in which chromosomes can be unequivocally defined and counted.
– Make a drawing and a photograph or an electronic image of the cell showing all the chromosomes.
– Count chromosomes from five or six independent cells from the same plant material.
Note: as an added precaution, counts have to be agreed by two (or more) observers. Some chromosomes of reduced size (called 'minichromosomes') are observed, which could be interpreted as products of breakage of microsatellites. The 'minichromosomes' are typically rectangular in outline and sometimes relatively weakly stained.

Troubleshooting

Three main problems can occur:

(a) No cells with condensed chromosomes are observed, which can result from:
– Inadequate root tips.
Solution: improve the plant growth conditions in good drained soil in the greenhouse to promote active cell division in healthy roots. Collect roots again and change the collection time.

– Variations due to the clones.
Solution: quality of the cells also depends on the genotype [2]. Try another hour for collection.

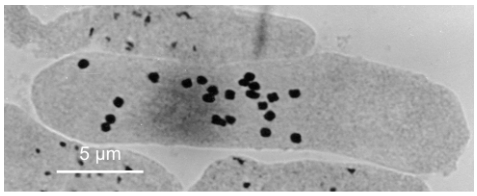

Figure 2.
Diploid cell of *Musa acuminata* hybrid (cross: Tjau Lagada × Calcuta) with 22 chromosomes. The chromosomes are more contracted and spread around the cell with a small vertical dispersion. The average length of chromosomes is about 1 µm.

– Inefficient pretreatment.
Solution: the quality of 8-hydroxyquinoline can be variable. Try other sources of the product. Keep the samples protected from direct sunshine.

(b) No or few cells with well spread chromosomes are observed. The cause could be an inadequate flatness of the cells.
Solution: carefully increase the vertical pressure on the pencil with the eraser to spread chromosomes without bursting the cells.

(c) Staining of chromosomes is not intense. This is due to the quality of orcein, whose solubility is low in lactophenol.
Solution: obtain orcein from another source. Gurr's Natural Orcein is recommended.

3. Typical results obtained

The protocol usually results in condensed and intensely stained chromosomes, which does not permit the observation of centromeres except on rare occasions. The length of chromosomes may vary between 0.7 and 1.5 times the average. One chromosome with a satellite can be recognized as the longest one in a haploid set (*figures 1* to *3*).

In memorandum

The first author dedicates this publication to the memory of the second author, Kenneth Shepherd, who taught him, in the '80s, this method that he still uses today.

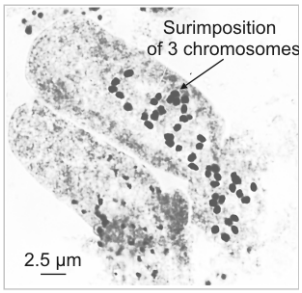

Figure 3.
Tetraploid cell of *Musa* hybrid (cross: Popoulou CAM × Calcuta) with
44 chromosomes. The chromosomes are more contracted and spread around the cell with a small vertical dispersion. The average length of chromosomes is about 0.85 µm. The precise chromosome count is uncertain because of burst in the lower cell.

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