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STUDIES ON POTATO BREEDING BY POLYPLOID HYBRIDS BETWEEN RELATED SPECIES.

I. The Leaf Smear Method in Identifying Polyploid Plants*

By

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The detection of chromosome doubled plant in polyploid breeding has been made by the shape and thickness of the leaf, the size and number of stomata, the size of pollen etc. But there is a wide variation in these characteristics especially in the size of stomata sometimes leads into incorrect conclusions.

Accuracy may be attained if the number of chromosomes be counted, but the general methods which have been adopted were conditioned by the time of sampling and are apt to injure plants. Particularly, most of these methods are not applicable to these breeding techniques which necessitate an early identification prior to the full maturity of plants.

The authors are now working on a method in which the young leaf is taken as a test material, so that we may identify the doubled plants earlier, and yet may not injure plants in breeding by species hybridization. The recent experiment worked out a method suitable to genus *Solanum* by adding a little modification to the Leaf Smear Method (PRAKKEN and SWAMINATHAN 1950, 1951) which was founded on TJIO's study (TJIO and LEVAM 1950, GERSTEL 1949).

MATERIAL AND METHOD

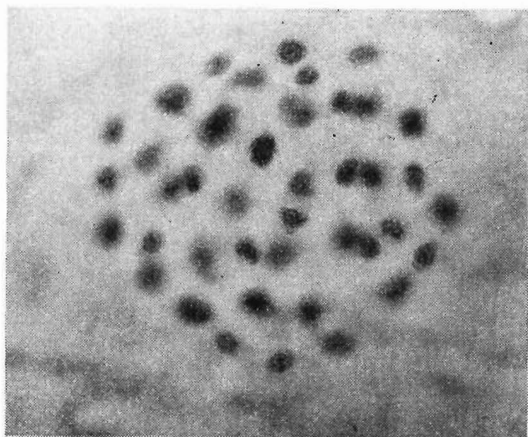
As a test material a leaf as large as approximately three mm, it was microscopically examined the parenchyma according to the following process:

1. Steeped in 8-hydroxyquinoline (0.002 M) solution.
2. Washed after fixing it in a hydrochloric acid solution (1:10).
3. Stained in aceto-carmine solution, followed by smearing.

RESULT

It was found that the effect of 8-hydroxyquinoline as a pretreatment to scattering chromosomes over a polar plate was similar to that of colchicine. Whereas, any result as to the effect on accumulating metaphases could not be noticed.

Four hours were needed for the treatment of 8-hydroxyquinoline, the room temperature (20°C) for the treatment was found appropriate. The authors also tried the treatment in 0°C (ice-water) but no effect. It has been pointed out by TJIO and others that 8-hydroxyquinoline expresses the formal characteristics of chromosomes in *Allium*, *Lactuca*, *Secale*, *Solanum* and *Vicia*, but no remarkable result could be recognized.



Leaf chromosomes treated with 8-hydroxyquinoline, fixed in hydrochloric acid and stained with aceto carmine; *Solanum tuberosum* ($2n=48$)

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Steeping in hydrochloric acid made the material softer, caused the maceration of the cell wall, and made smearing easy. Ten minutes of steeping in hydrochloric acid, and 60°C for temperature was found to be the most satisfactory.

For staining, LEVAN employs acetic orcein (1-2%), but this often resulted in over staining. In the present experiment, the authors have noticed that an aceto carmine solution was superior. The twice diluted prescription what was formerly used with acetic acid (45%) was appropriate.

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