A Procedure for Squash Preparations of Somatic Sugar Beet Tissues

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In the routine cytological examination of somatic tissues of sugar beets, a need was felt for a procedure that would give better preparations, require less handling time, and allow considerable latitude in time intervals. Attempts were made to improve the procedure and the quality of the preparations. The procedure described herein was satisfactory. It has only four steps: shrinking the chromosomes; killing, fixing, and macerating the tissues: washing out the killing, fixing, and macerating fluid; and squashing the tissue in the stain. The method is different from other methods in that the killing and fixing step is combined with the macerating step.

Materials

Tjio and Levan (1)[2] first suggested the use of 8-hydroxyquinoline as a shrinking agent. For the procedure described herein, a .002 M. (290 mg. per L.) solution of 8-hydroxyquinoline was prepared by dissolving the crystals in distilled water at 60° C. Vigorous stirring for 10 to 15 minutes brought most of the crystals into solution.

The killing, fixing, and macerating solution was a mixture of 7 parts Newcomer's fluid (2) and 3 parts 1 N. HCl. (Newcomer's killing and fixing solution is composed of 6 parts isopropyl alcohol, 3 parts propionic acid, 1 part acetone, 1 part dioxane, and 1 part petroleum ether.) For best results, the Newcomer's fluid and the acid should be mixed immediately before using.

Von Rosen (3) recommended spirit soluble nigrosin for staining beet chromosomes. The junior author of the present paper has tested several sources of nigrosin, some of which stained the chromosomes only lightly or not at all. Among the sources, however, two were found to be outstanding. The first was "Spirit Nigrosin No. 19305." made by W. S. Simpson & Co., Ltd., Old Southgate, London, N. 14. and supplied by Sidney Ellerton.[3] Dr. Ellerton also furnished information on his method for preparation of the stain, which is as follows:

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[2] Numbers in parentheses refer to literature cited.

"Take 50 cc. glacial acetic acid and 50 cc. distilled H_2O. Bring to boil and add 4 gm. of nigrosin gradually while stirring. Boil gently for 5 minutes. Cool rapidly. Filter. Dilute 1 part filtrate with 2 parts 45% acetic acid."

The second source of spirit soluble nigrosin was Arthur H. Thomas Co., Philadelphia, Pennsylvania. No appreciable difference between the two stains was noticed. In addition to Dr. Ellerton's method, the stains were also prepared by two other, slightly different methods of heating. In one method the solution was boiled in a refluxing condenser for ½ to 1 hour. In the other method the solution was placed in an autoclave for 20 minutes at 15 pounds pressure. No great differences in stains heated by the three methods were noticed.

The senior author has also had satisfactory results with synthetic orcein. One and one-half grams of dye were placed in a flask with 50 ml. of 60 percent acetic acid. The flask was placed in an autoclave at 15 pounds pressure for 20 minutes. The stain was then cooled and filtered. Ten drops of Aerosol OT (dioctyl sodium sulfosuccinate) were added. The formation of precipitate made it necessary to filter the stain at weekly or biweekly intervals.

Methods

Either leaves or root tips could be utilized in the procedure described here. When leaves were used, one of the smallest from the growing point, which was usually 3 to 8 mm. long, was taken; when roots were used, only snow-white root tips were taken. The leaves or root tips were placed in small vials with 8-hydroxyquinoline solution for 3½ to 4 hours at 55° to 65° F. The 8-hydroxyquinoline was poured out, and the killing and macerating fluid was added. The plant material was left in the fluid for ½ to 3 hours, after which the killing and macerating fluid was replaced with water. Squashes were made after the tissue had been in the water for 10 minutes to 24 hours. It was possible to make counts on tissues stored in water in the refrigerator for a week. About 1 mm. of the root tip or ½ mm. of the basal part of the beet leaf was used in making the squash. It was placed in a drop of stain on a slide, and a cover glass was put on top. Pressure applied to the cover glass spread the tissues. A slight lifting of one edge of the cover glass allowed the stain to run under and come into more intimate contact with the

[3] Dr. S. Ellerton, British Pedigree Beet Seed, Ltd., Maldon, Essex, England, tested several sources of nigrosin, found this one best, and kindly supplied samples to workers in the United States.

cells. After 30 seconds (or a few minutes if darker staining was desired), pressure was again applied to the cover glass to flatten the cells.

Discussion and Results

The greatest advantage of the procedure is the simplification of the maceration process by combining it with the killing and fixing process. Since the maceration was completed by the time the material was fixed, the time required for the preparation of each slide was reduced to a minimum. The maceration allows the cells to be spread apart and flattened rather readily without disintegration of the cell walls. The considerable time latitude for each of the steps of the process allowed the technician to use a rather flexible schedule. The flexibility, however, had certain limitations. Keeping tissues in the 8-hydroxyquinoline solution less than 3 hours resulted in preparations with the chromosomes too long and not well spread in the cell. Keeping tissues in the solution longer than 4½ hours gave preparations with few mitotic divisions and with stained cytoplasm. Increased amounts of 1 N. HCl in the killing solution resulted in less stain being absorbed by the chromosomes, while less HCl resulted in inadequate separation and inadequate flattening of cells. After 3 or 4 days in water, the chromosomes became more elongated and were not as suitable for observations.

Excellent preparations were obtained from about 90 percent of the beet plants examined by this method. Failure to obtain good preparations could usually be attributed to an unthrifty condition of the plant.

Summary

A new cytological procedure for the preparation of sugar-beet somatic tissues was developed. The outstanding feature of the procedure was combining the macerating process with the killing and fixing process.

Literature Cited

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(3) VON ROSEN, G. 1947. The rapid nigrosine-method for chromosome counts, applicable to all the growing tissues of the plant. Hereditas 33:567-570.