A TECHNIQUE FOR MAKING PREPARATIONS OF MITOTIC AND MEIOTIC CHROMOSOMES FROM *Ipomoea* SPECIES AND HYBRIDS

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ABSTRACT

A combination of Feulgen and acetocarmine squash method of preparing mitotic and meiotic chromosomes of sweetpotato and related *Ipomoea* species and hybrids is presented. The method includes pretreatment in 0.002 mol 8-hydroxyquinoline, fixation in modified Newcomer's solution, hydrolysis, staining of DNA by the Feulgen reaction, further chromosome staining with acetocarmine, and slide preparation by the standard squash technique. Some notes in preparing solutions for specific steps are also included.

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KEY WORDS: Chromosomes. Cytological technique. *Ipomoea*. Sweetpotato.

INTRODUCTION

Carmine dye, in combination with acetic or propionic acid, has been reported to be the stain used in studies involving chromosomes of sweetpotato and its relatives (Ting and Kher, 1953; Ting *et al.*, 1957; Jones 1964, 1970; Jones and Deonier, 1965; Magoon *et al.*, 1970; Hrishi and Bai, 1977; Orjeda *et al.*, 1990). However, there has been no reported detailed technique on the preparation of mitotic and meiotic chromosomes from *Ipomoea* species. The purpose of this report is to describe in detail a tested technique for making preparations of mitotic and meiotic chromosomes from species and hybrids belonging to the genus *Ipomoea*. The technique was originally designed for sweetpotato (*I. batatas*) chromosomes (Dr. I. Shiotani, personal communication). However, it was also proven very effective when applied to other *Ipomoea* species and hybrids. Since 1987,

this technique has been routinely used in cytological studies involving sweetpotato and its relatives (Oracion and Saladaga, 1988; Oracion and Shiotani, 1989, 1992; Oracion *et al.*, 1990). The method produces darkly stained, swollen and easily spread chromosomes than when either only Feulgen staining or carmine staining is used, the latter being the more commonly used stain for sweetpotato chromosomes and its *Ipomoea* relatives. In addition, the cytoplasm appears clearer than when only carmine staining is used. With the present method, a good contrast is achieved between the dark chromosomes and the cytoplasmic background. These results gave a better advantage especially in studying sweetpotato chromosomes which are so minute and numerous being a hexaploid with 2n = ca. 90.

MATERIALS AND METHODS

Mitotic chromosome preparation

The general procedure for mitotic chromosome preparation is presented in Fig. 1.

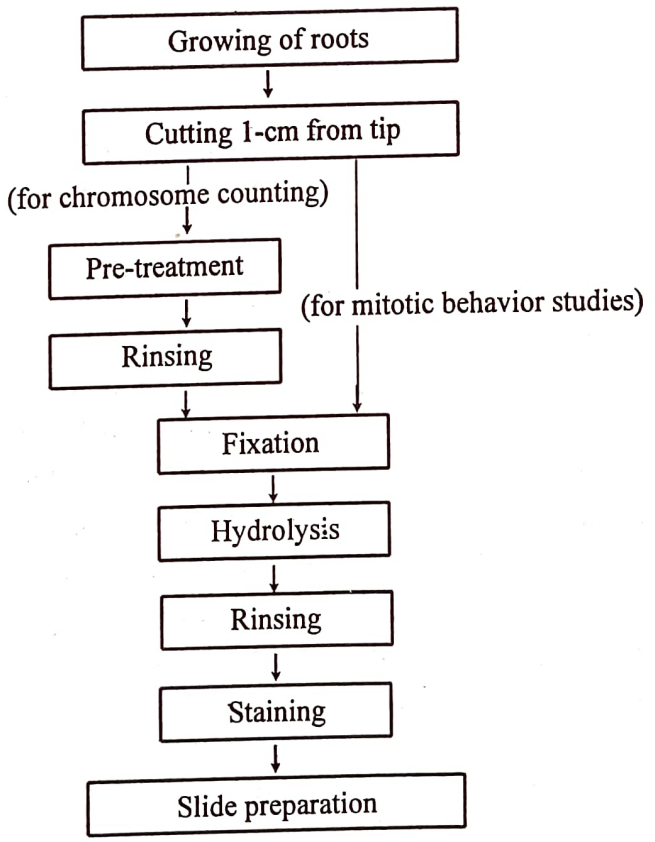

Figure 1. General procedure for mitotic chromosome preparation.

Root tip preparation. Roots easily grow from nodes of most *Ipomoea* forms. Several nodes of healthy cuttings immersed for a few days in running or aerated water produce actively growing root tips. The roots are gently rinsed well in distilled water then cut about 1 cm long from the tip. The roots are cut immediately before the succeeding step.

Pretreatment. To determine the somatic chromosome number of individuals, the 1-cm long root tips are first immersed in an aqueous solution of 0.002 mol 8-hydroxyquinoline to bring about scattering of chromosomes thereby making them easy to count. This solution is prepared by dissolving 0.145 g of 8-hydroxyquinoline powder in 1 L of distilled water in a clean glassware. (Note: The chemical reacts with metal). The roots are immersed in this solution at 12°C for 3-6 hrs then rinsed well in distilled water. If the pretreatment was appropriately done, cells with shortened chromosomes, some in ski-configuration, spreading on the equatorial plate will be observed in the later steps. This pretreatment is skipped if mitotic behavior is to be studied.

Fixation. A mixture of Newcomer's Fluid and glacial acetic acid in 12:5 volume ratio is used as the fixing solution. Newcomer's Fluid is a mixture of six parts isopropyl alcohol, 3 parts propionic acid, one part acetone, one part dioxane, and one part petroleum ether. Fixing time is 10-30 min. The plant material can be stored in this fixative for up to 3 months under refrigeration.

Hydrolysis. In preparation for the Feulgen reaction, the chromosome DNA is hydrolyzed in a mixture of the fixative and hydrochloric acid (HCl) solution, 17:2 volume ratio. The HCl solution is prepared by mixing 69 ml of commercial HCl (35.0 - 37.0%; Sp.gr. 1.18 - 1.19) with 31 ml distilled water. The root tips are hydrolyzed at 40°C for 1 hr and immediately rinsed in distilled water to stop hydrolysis.

Staining. The plant material is transferred to Schiff's reagent for Feulgen staining in the dark for 1/2 to 2 hrs. To prepare Schiff's reagent, 0.5 g of Merck's basic fuchsia is added to 100 ml of boiling distilled water. The boiling is continued for 5 min with continuous agitation. The solution is then cooled down to 56°C, filtered and added with 10 ml of 1 N HCl and 1.5 g potassium (or sodium) metabisulphite, shaken well inside a covered glass bottle then kept in the dark for 12-18 hrs (overnight). If the color of reagent is still yellowish, 0.5 g active carbon powder is added and the reagent is shaken well and filtered twice. The filtrate will become colorless and is ready for use. It will last up to one year when kept refrigerated in a dark stoppered bottle.

After treatment of the roots with Schiff's reagent, a root tip is transferred to a clean slide under a dissecting microscope, then a small piece of tissue is cut quickly and carefully from the purple-colored group of cells just behind the root cap. This tissue is placed on a clean area of the slide and stained with a drop of 2% acetocarmine. A cover slip is placed on the stained tissue still bathed in acetocarmine. To prepare 2% acetocarmine, 2 g carmine dye, 45 ml glacial acetic acid, and 55 ml distilled water are needed. Glacial acetic acid is added to the water to form 45% acid solution which is heated to boiling in a conical flask. The dye is added slowly to the solution stirred with a glass rod, and boiled gently until completely dissolved. The solution is cooled down to room temperature, filtered then stored in a dark bottle with a glass stopper.

Slide preparation. The cells are spread to a one-cell layer thickness by tapping the cover slip with the rubber end of a pencil. The spreading of cells is checked under low microscope magnification. The slide is warmed gently by passing its undersurface over a lighted alcohol lamp. The coverslip is tapped again until the cells are evenly spread. Finally, the cells are squashed flat by applying even pressure on the slide under a piece of blotting paper before temporarily sealing the sides of the cover slip. Chromosomes prepared by this method of Feulgen-acetocarmine squashes retain their best condition at least 1-2 days after preparation. It would be advantageous to make the detailed observations or take photomicrographs soon after the chromosome preparations are made.

Meiotic chromosome preparation

The general procedure for meiotic chromosome preparation is presented in Fig. 2. The flower buds for meiotic studies in pollen mother cells (PMC) are gathered from the nursery and brought to the laboratory for preliminary determination of meiotic stages. Generally, flower buds of right stages collected between 0730-1200 hr on a sunny day from regularly watered plants produce PMCs with active meiosis. Because *Ipomoea* species and cultivars vary in bud sizes, depending on the species or strain and on environmental conditions, all sizes of flower buds from smallest to the largest per species or strain per plant are collected first and brought to the laboratory for determination of optimum bud size (per plant) with meiotic stages. Once the optimum bud size is determined, further sampling of buds is done. Those with the desired stages are given further treatments as discussed in the following paragraphs.

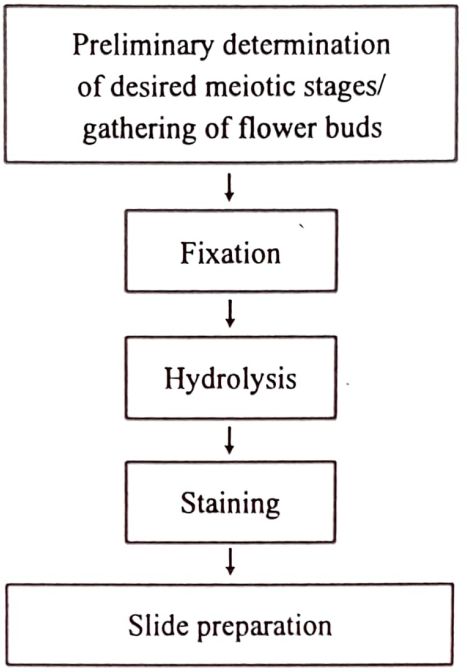

Figure 2. General procedure for meiotic chromosome preparation.

Fixation. The same procedure or method of fixation used in root tips is applied.

Hydrolysis. The same solution and method used in mitotic cells is applied but the buds are hydrolyzed at 42°C for 10 min.

Staining. The same procedure used for mitotic cells is applied.

Slide preparation. A similar procedure used in mitotic cells can be applied but the PMCs are squeezed out of one anther into a drop of acetocarmine on a slide.

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LITERATURE CITED

HRISHI, N. and BAI, V.K. 1977. Cytology of diploid and triploid *Ipomoea obscura* (L). Ker-Gawl. J. Root Crops. 3(1):13-16.

JONES, A. 1964. Cytological observations and fertility measurements of sweetpotato (*Ipomoea batatas* (L.) Lam.). Proc. Am. Soc. Hortic. Sci. 86:527-537.

JONES, A. 1970. Asynapsis in *Ipomoea gracilis*. J. Heredity. 6(4):151-152.

JONES, A. and DEONIER, M. T. 1965. Interspecific crosses among *Ipomoea lacunosa, I. ramoni, I. trichocarpa*, and *I. triloba*. Bot. Gazette 126(3):226-232.

MAGOON, M. L., KRISHNAN, R. and BAI, V.K. 1970. Cytological evidence on the origin of sweetpotato. Theor. Appl. Gene. 40:360-366.

ORACION, M. Z. and SALADAGA, F. A. 1988. Somatic chromosome number in *Ipomoea* species from the Philippines and Japan. Philipp. J. Crop Sci. 10:S11.

ORACION, M.Z. and SHIOTANI, I. 1989. Cytology and fertility of sweetpotato x diploid *Ipomoea trifida* F_1 hybrids and their potential in analytic breeding of sweetpotato. Philipp. J. Crop Sci. 14:S20.

ORACION, M.Z. and SHIOTANI, I. 1992. Sweetpotato Technology for the 21st Century. Hill, Bonsi and Loretan (Eds.), Tuskegee University, Alabama, USA. 607 pp.

ORACION, M.Z., NIWA, K. and SHIOTANI, I. 1990. Cytological analysis of tetraploid hybrids between sweetpotato and diploid *I. trifida* (H.B.K.) Don. Theor. Appl. Gene. 80:617-624.

ORJEDA, G., FREYRE, R. and IWANAGA, M. 1990. Production of 2n pollen in diploid *Ipomoea trifida,* a putative wild ancestor of sweetpotato. J. Heredity 81:462-667.

TING, Y. C. and KEHR, A. E. 1953. Meiotic studies in the sweetpotato (*Ipomoea batatas* (L.) Lam.). J. Heredity 34:207-211.

TING, Y.C., KEHR, A. E. and MILLER, J. C. 1957. A cytological study of the sweetpotato plant *Ipomoea batatas* (L.) Lam. and its related species. Am. Naturalist 91:197-203.