

A Rapid Squash Method for Grass Embryology¹

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INTRODUCTION

The aceto-carminine squash technique of ovules provides a rapid and efficient method to study embryo sac development in apomictic grasses. In facultative apomicts where sexual and asexual embryo sacs develop simultaneously and ultimately one dominates the other, it is essential to study each ovule in a spike individually for a full understanding of the mechanism involved in the embryo sac development and embryogeny. An aceto-carminine squash technique for mature embryo sacs which involves maceration and squashing of many ovules simultaneously was described by Bradley (1948). This technique, however, is good only for plants with many small ovules in a single ovary, more commonly found in dicot than in monocot families, and cannot be used with success where each floret has a single ovule and a serial scoring is required. A modified Bradley squash technique used by Brooks (1958) for embryological investigations in grasses has the same drawback.

The present paper outlines a very simple aceto-carminine technique which was employed with success in the *Andropogoneae* genera *Bothriochloa*, *Capillipedium* and *Dichanthium*.

MATERIALS AND METHODS

Spikes at different stages of development were fixed in freshly prepared Carnoy's fluid; 6 parts 95% ethanol, 3 parts glacial acetic acid and 1 part chloroform. Fixatives with other combinations of these chemicals were also tried but they caused inferior staining of the embryo sac nuclei.

After approximately one hour of fixation 25 drops of a saturated aqueous solution of ferric chloride were added for every 50 ml of fixative. The ovules were ready for squashing after 48 hours of fixation in this mixture. Inflorescences could be stored in this fluid for several months without any evident deterioration.

The fixed inflorescence was placed in a petri dish and each floret studied beginning with the youngest raceme. With the help of a pair of fine needles the pistil was removed and placed on the slide in a drop of aceto-carminine. The styles were cut off at their bases and the ovary was tapped gently with the needle until the ovule popped out. With a little practice it was easy to take out the ovule without the help of a dissecting microscope. While viewing the slide on the microscope under low power, the top of the cover glass was pressed carefully with the needle to spread the ovule. Gentle heating of the slide helped in further spreading apart of the cells. With the desired pressing and tapping of the cover glass, the intact embryo sac was separated from the ovule (Figs. 1-4).

Temporary preparations could be preserved for 4-5 days by sealing the cover glass with a mixture of gum mastic and paraffin. Permanent preparations could also be made by any of the usual procedures.

DISCUSSION

The advantages of aceto-carminine squash technique over paraffin sectioning in embryological studies have been emphasized by Bradley (1948). The use of this technique in the embryological investigations of facultative

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apomicts is especially suitable since it facilitates tracing the pathway of both sexual and asexual embryo sac development. Knox and Heslop-Harrison (1963) have shown in a tetraploid race of *Dichanthium aristatum* (Poir) C. E. Hubbard, a facultative apomict, that different day-length conditions have a significant effect on the incidence of sexuality and apomixis. For an investigation of this type, where it is necessary to study each and every ovule in the inflorescence serially, the aceto-carmin squash technique is much more convenient and reliable than paraffin sectioning, particularly when the plant has small ovules.

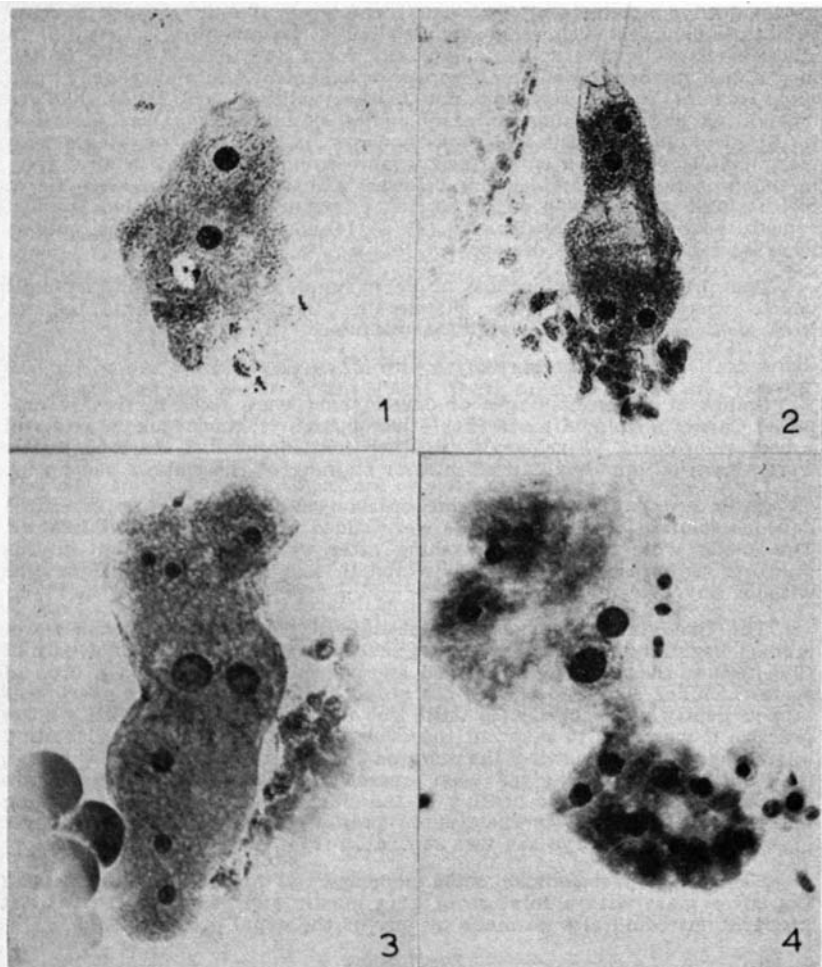


Fig. 1-4. Stages showing development of sexual embryo sac in *Boerhaavia grisea*. 1. two-nucleate sac 2. four-nucleate sac 3. eight-nucleate sac 4. mature sac with a characteristic group of antipodals. All figures $\times 450$.

Bradley's aceto-carminc squash technique which involves maceration and squashing of several ovules at one time is not suited for this type of investigation.

The technique outlined in this paper makes the embryo sac squash technique as simple as anther squashing. The fact that ferric chloride is used, in this method, directly with the fixative makes it much more convenient as compared to other compounds of iron such as iron alum or iron acetate. Ferric chloride, as a mordant, provides excellent nuclear differentiation and helps to keep the embryo sac intact. Using this technique, it has been possible to study and trace the various stages in the development of embryo sacs, sexual as well as asexual, and their ultimate fate. Early stages of embryogeny have also been studied.

Although this technique has been employed only in *Bothriochloa*, *Capillipedium* and *Dichanthium*, it is being extended to other members of Andropogoneae. It is expected that this technique will prove to be a rapid method for embryological studies in other plants also, especially grasses with small ovules.

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