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A METHOD FOR OBTAINING MITOTIC FIGURES IN SEEDLINGS OF *ALISMA* [DILL.] L.

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Introduction

Root-tips have been most widely used as a source of mitotic figures because they are ordinarily quite accessible. For example, in classroom study of mitosis, *Allium* root-tips, grown from bulbs, are easy to prepare. However, the number of root-tips that can be obtained from seedlings is often limited. This is especially true in many aquatic plants which are difficult to grow in the laboratory. Other meristematic tissue may be used for taxonomic work because it is only necessary to have a few cells in phases that show the chromosomes distinctly.

While making rapid-smear preparations of root-tips from seedlings of *Alisma* (water-plantain) for another project, the author found few mitotic figures in root-tips. This dearth of proper mitotic figures led to the conjecture that perhaps meristematic tissue other than root-tips in seedlings from monocotyledonous paludal plants might be used. Because shoots and adventitious roots emanate from the epicotyl-hypocotyl axis, various regions of the axis were checked. The presence of good mitotic figures indicated that more detailed examinations of these regions might be profitable, to determine if portions of the epicotyl-hypocotyl axis of *Alisma* plants showed mitotic figures suitable for taxonomic study.

Information from the literature (Maheshwari, 1950; Priestley, 1928; Foster, 1936) indicated that the entire epicotyl-hypocotyl axis of the newly-emerged seedling in some monocotyledons might show excellent figures. Despite this fact, entire seedlings have not been used extensively by systematists as a source of mitotic figures.

Maheshwari (1950) described in some detail the embryogeny of *Alisma* and *Saggittaria*. He included a series of drawings by Hanstein (1870) showing the ontogeny of the embryo in *Alisma*. Hanstein's drawings indicated that the shoot apex (plumule) and true leaf-primordium (bud) develop laterally from the embryo axis, with the leaf-primordium forming a collar of meristematic tissues around the shoot's apex. This collar then functions as an intercalary meristem building up the leaf from below. This fact indicates that the apical leaf meristem is not as active as the basal portion of the leaf.

Materials and Methods

Species used in this work were: *Alisma plantago-aquatica* L. var. *michalettii* (Aschers and Graebner) Buchenau; *A. plantago-aquatica* L. var. *parviflorum* (Pursh) Torr.; *A. canaliculatum* A. Br. and Bouche; and *A. gramineum* Gmelin var. *graminifolia* (Wahlenberg) Hendricks. Seeds were collected in various localities in consecutive years and were

germinated by a modification of a technique for cracking seedcoats, devised by Crocker and Davis (1914). The cracked seeds were placed in a petri dish containing approximately 25 ml. of tap water to which 3 drops of 0.25% sodium hydroxide had been added to soften the seed coats. Approximately 50% of the water was replaced with fresh water every other day. The seedlings were kept under artificial light from 7 a.m. to 9 p.m. daily. Their growth patterns were closely followed. Killings were made in Carnoy's solution at various times of day.

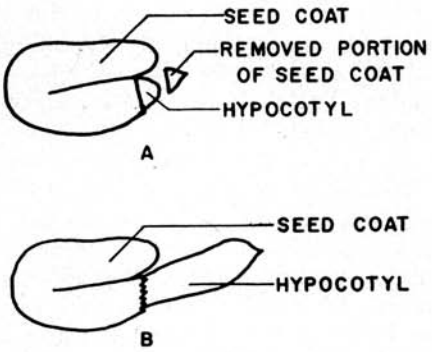

FIG. 1.—*A*, *Alisma* seed with terminal portion of seed coat newly removed; *B*. seed from *A* showing increase in length of hypocotyl occurring in less than 12 hours.

Seedlings were macerated by using a modification of Stewart's method (1950). The tissue was left in the macerating fluid for ten minutes. Some of the seedlings were left in Carnoy's solution as long as seven days, and most of these were not put in macerating fluid. The material was put in proprionic carmine stain for three to five minutes or until the chromatin material stained. Differentiated tissue absorbed very little stain even after immersion in the stain for about 20 minutes. Slides of tissues were made permanent by removing cover slips with 45% acetic acid, dehydrating in alcohol, and mounting in euparol. It was found that the specimen remained on the slide or cover slip better if the stain had started to dry out beneath the cover slip but had not dried as far as the edge of the material. In some seedlings smears were made from only leaf-tips and root-tips; in others, smears were also obtained from leaf-bases, shoot-apices, and the hypocotyl region immediately below shoot-apices.

RESULTS

Growth patterns of seedlings emerging from the seed were as follows: normally, hypocotyls extended from the seed coats first because of their increase in length (Fig. 1). This occurred in practically all of the seeds and did not necessarily indicate that actual growth would follow. In fact, a few showed no further change. Subsequent to this rapid increase in size, the viable seeds appeared to enter a somewhat dormant period for one or two days and, in seeds in one collection, this period lasted about three weeks. The single cotyledon was the last to escape from the seed coat, and it became green in a short time. Next, root-hairs appeared around a collar at the end of the hypocotyl. Between the third and seventh days after the seed coats were cracked, leaves and adventitious roots began to develop in all seeds except those in the collection mentioned above. In the one exception, the seedlings did not foliate for about three weeks. Development in the epicotyl region

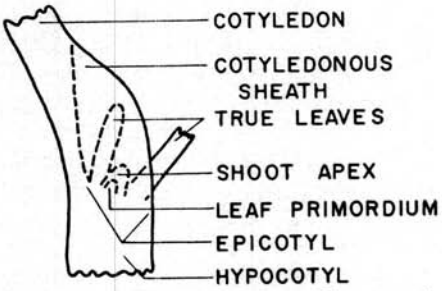

FIG. 2.—Section of young *Alisma* seedling showing epicotyl region with lateral emergence of true leaves from base of shoot-apex.

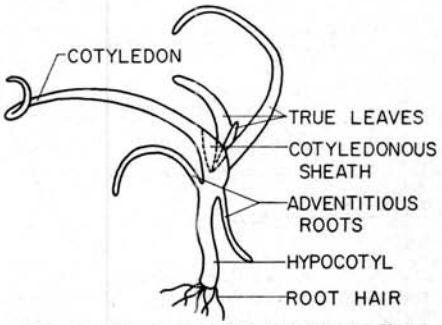

FIG. 3.—Seven-day-old *Alisma* seedling.

occurred before adventitious roots appeared (Fig. 2). True leaves developed from the base of the apical shoot which was covered by a sheath formed by the cotyledon and, shortly, the slender leaves became green. After leaves started to appear, adventitious roots emerged from around the hypocotyl a short distance from the epicotyl (Fig. 3). Sometimes a root would grow out from the end of the hypocotyl.

Seedlings which were in Carnoy's solution from a half-hour to two days and then placed in macerating solution for ten minutes broke down into separated, but complete cells. Plasmolysis of the cells indicated excessive maceration, when seedlings were left in Carnoy's solution longer than two days and then placed in macerating fluid for ten minutes. When seedlings in Carnoy's for six or seven days were transferred directly to 80% alcohol, the cells would separate but were not damaged.

Well-stained meristematic tissue was found in root-tips, leaf-tips, leaf-bases, and leaf-buds, and in the area between the shoot-apices and adventitious roots. In that part of the study in which smears were made solely of root-tips and leaf-tips, mitotic figures were found in only 25% of the seedlings. Figures found in root-tips were usually good; however, when all meristematic portions of the seedlings were examined, mitotic figures were found in 80%. These results were obtained from killings at various hours. Smears made for leaf-primordia, root-tips, and shoot-apices contained more mitotic figures than other areas.

Discussion and Summary

It is known that plants vary in their growth and developmental patterns due to inherited and environmental differences. Patterns of growth in *Alisma* seedlings, with rather slow development of roots and early development of leaves, indicated that the epicotyl and immediately adjacent hypocotyl might provide mitotic figures for systematic work. This was found to be the case; good mitotic figures were found with relative ease in these regions. Inasmuch as there were only two or three roots per seedling, it was more expedient to use other meristematic tissues along with root-tips.

The epicotyl region seemed to be especially meristematic in these aquatic seedlings. Somewhat slow development of the root system, as

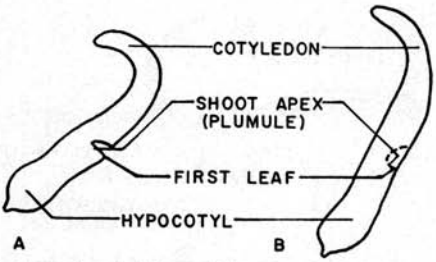

FIG. 4.—*A*, Hanstein's sketch of *Alisma* seedling, adapted from Maheshwari (1950); *B*, *Alisma* seedling, approximately same stage of development as *A*, illustrating cotyledonous sheath.

compared to the foliage, might possibly be the normal growth-pattern of seedlings of other aquatic taxa. *Alisma* seedlings often float for some time before reaching shore.

In searching the literature it was found that root-tips, shoot-apices, and leaf-bases in monocotyledonous plants could be expected to possess meristematic tissue. These views have been verified in *Alisma*.

As a side issue, it is interesting to note that tissue which has been in Carnoy's solution for six to ten days without the usual maceration has good cell separation. Evidently, in about a week the hydrochloric acid in Carnoy's solution macerates sufficiently the small amount of tissue present in plants of *Alisma*. Workers who kill material one week and make smears the following week may find it useful to eliminate one step in the macerating process.

The sheath of the cotyledon that surrounds the shoot-apex seems to be somewhat different from what might be inferred from Hanstein's (1868) diagrammatic sketch of an *Alisma* seedling in Maheshwari's book (1950). His sketch does not show any evidence of the shoot-apex being enclosed by the cotyledon (Fig. 4). The author's findings seem to be similar to Holm's (1890-1891). Holm mentioned "an incipient swelling at the place where the plumule [shoot-apex] has to penetrate".

The initial rapid increase in length of the hypocotyl of newly-exposed embryos did not necessarily mean that all those embryos were viable. In fact, a few of these grew no more and were evidently dead. It may be possible that increase in length of the hypocotyl was due to imbibition.

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