DEVELOPMENT OF THE EMBRYO SAC AND EMBRYO IN GRINDELIA SQUARROSA (PURSH) DUNAL

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Introduction

Grindelia squarrosa is found in great abundance near Lincoln, Nebraska. In the autumn of 1920, heads could be found in all stages of development. This, together with the fact that little work had been done concerning the embryo sac of any member of the section of the tribe Asterae having yellow rays, except in the genus Solidago. led the writer to undertake the present investigation. Sections from material killed the first year showed interesting features, and the study was continued the next year. In this paper the attempt is made to show the development of the megaspores, the embryo sac, and the embryo.

Materials and Methods

Flowers from plants growing near Lincoln, Nebraska, were used. The smaller heads were fixed without cutting, while the larger ones were cut into several slices.

Material was fixed in the autumn of 1920 and in the summer and autumn of 1921. Flemming's stronger and weaker solutions, acetic alcohol*, picric acid in 60% alcohol, and the solutions of Zenker, Kleinberg, Gilson, Bouin, and Henning were used. The best results were obtained with picric acid, Zenker's solution, and Henning's solution. Of the three, Henning's solution was preferred. Gilson's solution gave good results in the case of pollen mother cells and older embryos, but was almost worthless for the archesporium and early embryo sac. Safranin does not stain well after it, but acid fuchsin gave good results. In general, an alcoholic killing solution is better than an aqueous one, since the former dissolves the gum which gives difficulty in cutting. Flemming's solution did not penetrate very well to the embryo sac, and preserves the gum more than the other killing solutions. Cedar oil was preferred as a clearing agent. The material was imbedded in paraffin, and sections were cut as thin as possible,

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*Two formulas for acetic alcohol were used; two parts of 95% alcohol to one part of glacial acetic acid, and six parts of absolute alcohol to one part of glacial acetic acid.

the thickness usually being about five microns. Cutting was difficult, partly on account of the large amount of gum present and partly on account of the hardness of the seed coat. The former was overcome by the use of alcoholic killing solutions. Mercuric chloride and picric acid fixatives and the use of cedar oil softened the seed coat to some extent. It was necessary to leave the material in the melted paraffin for several days in order to secure thorough infiltration. The sections were improved by keeping the paraffin block as cold as possible while cutting.

In 1922 seeds were germinated and the root tips killed for mitosis. Flemming's stronger solution was used in this case. In dehydrating the root tips, a modification of Dudgeon's (4) method was used. Glass tubes of suitable diameter were cut short enough to go into shell vials and leave room for a cork. A piece of cloth with fine meshes was tied over one end. The specimens were then placed in the tube which was dropped into a shell vial containing the proper solution. By removing the tube it was possible to replace the solution in the vial by another without injury to the specimens. Material can thus be carried from the killing solution to paraffin without danger of injury or loss.

Haidenhain's iron alum haematoxylin and Flemming's triple stain were used. Later in the investigation gold orange was substituted for orange G in the triple stain, since its action was more rapid and it gave equally good results. Clove oil solutions of gentian violet and gold orange were used instead of aqueous ones. Gold orange was generally used with the haematoxylin to emphasize the walls.

Megaspore Formation

The ovule in its organization is similiar to that of other gamopetalae in that it is anatropous with one thick integument (fig. 13). There is only one layer of cells in the nucellus (fig. 3). According to Small (12) this is the typical form of nucellus in composites. This type is also found in Verbena (6). The archesporium was not recognizable until after the integument was fully formed (fig. 1). The divisions of the archesporium to form the megaspores were not observed. The megaspores are zonate with definite walls between them (figs. 3, 6). The tetrad resembles closely that figured by Small (12, fig. 60) and those of Lactuca and Bidens, figured by Dahlgren (3, figs. 1, 3). It is also similiar to