The Development of the Megaspore in the Sweet Potato

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THE DEVELOPMENT OF THE HEGACAMOTOPHYTE IN THE SWEET POTATO

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in The Department of Horticulture

by

Jesse Peterson
B.S., Southwestern Louisiana Institute, 1943
May, 1945
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This investigation was designed to study the normal development of the gametophytic generation up to and including fertilization in the sweet potato.

The material used in this experiment consisted of four seedling clonal lines of sweet potatoes, 16-3, 98, 14-5, and 15-7, produced at the Louisiana State University Agricultural Experiment Station. Young buds and unpollinated flowers were killed, embedded in paraffin, cut at 12-30 microns, and stained. Mature flowers were pollinated, removed at one, four, eight, and twelve-hour intervals following pollination, treated as the above, and cut at 30 microns. Smears of pollinated flowers were used to show the germinating pollen grains and tubes.

The development of the embryo sac was normal. The functional megaspore underwent three divisions to form eight nuclei arranged in linear order. The mature embryo sac, as found in this study, was ordinarily composed of five nuclei; two synergids, egg, and two polar nuclei. The three antipodals, as a rule, degenerated soon after they were formed, but occasionally remained until after fertilization.

It was observed, in flowers pollinated with normal pollen, that the pollen grains had germinated and entered the embryo sac within three and one-half hours after pollination. Four hours elapsed between the time the pollen tube entered the embryo sac and fertilization. The fusion of the sperm and egg and sperm and one polar nucleus occurred simultaneously. The product of the latter
fusion then united with the other polar nucleus to form the endosperm.

There were indications that self-incompatibility factors existed in the sweet potato.
INTRODUCTION

The sweet potato, Ipomoea batatas, Poir, a member of the morning glory family, has been in cultivation in America for hundreds of years. In fact, the Indians were growing it extensively at the time of the discovery of America (10). The sweet potato ranks third in value among the cultivated vegetable crops of this country and is exceeded only by the Irish potato and tomato. In 1942 the United States produced 65,380,000 bushels of sweet potatoes which were valued at approximately $73,000,000 (1).

The sweet potato does not usually bloom in the United States without special treatment. Investigations concerning inducement of flowering in the sweet potato were first reported by Dr. Julian C. Miller in 1937, and again in 1939, at the Louisiana State University (8)(9). He suggested the following methods to promote flowering:

1. Overwintering plants in the greenhouse, in order to force flowering early in the season. 2. Staking and trellising, in order to give the plants space for light. 3. Girdling, in order to accumulate food products in the vine, which would tend to encourage flowering.

The physiology of blooming is not yet well enough understood to make possible an unlimited production of flowers whenever desired. The purpose of this paper, therefore, is to show chronologically the normal development of the embryo sac, megagametophyte, and the process of fertilization. It is hoped that, with a definite standard for reference, identification of abnormalities may lead to a solution...
of the problem of flowering and seed setting. This can only be possible when the normal course of development is fully understood.
and vacuolated. On the periphery of the egg is located a small nucleus embedded in a thin layer of cytoplasm. The round polar nuclei generally lie close together near the vicinity of the egg and fuse prior to fertilization.

According to Frances Marion Greene Kenyan (6), working with Ipomoea trifida, the eight-nucleate embryo sac is formed in the usual manner by three successive divisions of the functional megaspore. The nuclei extend from one end of the sac to the other, and tend to be in pairs. She also observed that the nuclei undergo a rearrangement, the antipodal nuclei being pushed to the extreme upper end of the sac, and the polar nuclei in the center of the sac becoming large and prominent. The former do not persist long and soon degenerate. The synergid s and egg are pear-shaped and are located near the micropylar end of the sac. The polar nuclei do not fuse until after fertilization.

Gertrude Elizabeth MacPherson (7), working with Dodder and Morning Glory, has found that the development of the embryo sac up to and including the eight-celled stage follows the usual pattern. In the two-celled stage of the embryo sac there is a dense mass of protoplasm lying between and surrounding the two nuclei which are located at the opposite ends of the sac. In the eight-celled stage the polar nuclei usually stand out more prominently than the others. She found that a long, narrow, curved embryo sac was characteristic of both genera studied.

Arthur I. Weinstein (12) and Mabel M. Brown (2) in separate studies on Phaseolus Vulgaris, report early degeneration of the antipodals with only an occasional trace of them in the mature embryo sac. The
synergids in some varieties persist until after fertilisation. The male nuclei are much smaller than the egg and polars and retain their spherical shape during fusion. One polar fuses with the male nucleus, and this product in turn unites with the remaining polar.
MATERIALS AND METHODS

The source of material used in this experiment consisted of four seedling clonal lines of sweet potatoes, 16-3, 98, L4-5, and 15-7, produced at the Louisiana State University Agricultural Experiment Station. They were chosen for their ability to produce flowers and seed under a variety of environmental conditions. Individual plants of the three clonal lines were secured by the use of stem cuttings.

Buds of various sizes and unpollinated flowers were killed and fixed in Belling's Modification of Navashin's Fluid, dehydrated with normal butyl alcohol, transferred to paraffin with Johansen's Paraffin Oil Method (4), embedded in paraffin, and cut at 12-30 microns. Mature flowers were pollinated with pollen known to be compatible and removed at one, four, eight, and twelve-hour intervals following pollination. Corolla, sepals, and other extraneous parts were removed, and the flowers then prepared as above, and cut at 30 microns. All sectioned material was stained in Heidenhain's iron hematoxylin.

Smears were used to show germinating pollen grains and pollen tubes. Suitable material was removed from the plants and placed in Carnoy's fluid (4). The Carnoy seemed to have a toughening effect which enabled the style to be separated longitudinally into two halves without breaking. Other fixatives left the styles too soft and fragile to be dissected in this manner. The half-styles were then stained with cotton blue lactophenol and placed with the cut
surface up on a slide, pressed and mounted in clear lactophenol (11).
Development of Embryo Sac. Meiosis, as observed in the material used in this work, appears to be entirely normal. The fact that no meiotic divisions were observed in the hundreds of sections studied would indicate that this phenomenon takes place in a relatively short space of time. The tetrad stage has been observed, but unfortunately was not confined to any one section, and thus made a photomicrograph of it impractical.

The four nuclei comprising the tetrad are located at the chalazal end of the ovule. Apparently, any one of the four may become the functional megagametophyte, while the other three degenerate. Plate I shows the young megagametophyte prior to the formation of the nuclear membrane. This membrane is the first tissue of the embryo sac to differentiate, is relatively thick, and envelopes the nuclei completely, as shown in Plate II. Shortly after the formation of the sac-like membrane around the nucleus, the first division of the megagametophyte occurs. In most instances this division takes place at the chalazal end of the ovule. The two nuclei separate and are found near the center of the ovule, as illustrated in Plate III. The nuclear membrane has, in the meantime, stretched out to the point where it is almost invisible.

Following second division the two pair of nuclei are found at the opposite ends of the ovule, connected by a thin cytoplasmic strand as shown in Plate IV. There is now no surrounding membrane visible, and the immature embryo sac is encompassed by a large
vacuolated area. The sac has elongated considerably during the first and second divisions, and occupies about three-fourths of the length of the ovule. Physical pressure, created by its own rapid growth against the unyielding cells of the ovary, has apparently forced a longitudinal growth.

The third division, with the resulting eight nuclei in linear arrangement, was not observed. One slide, however, contained a section in which four nuclei were found in linear arrangement, while the adjoining section contained a fifth (Plate V). No trace of the antipodals could be found. It is possible that they were removed from the other nuclei in the process of cutting, or they may have already degenerated. The synergids have begun to acquire their characteristic pyriform shape and are nearest to the micropylar end of the ovule. Just above the synergids is a much smaller nucleus, which is destined to become the egg, but has not yet completed its transformation into the form observed in the mature embryo sac. The two remaining nuclei located above the egg are the polars, and are much larger than any of the others. Identification of the above nuclei was made possible by comparing their shape and position to those of a mature embryo sac.

It has been clearly evidenced by observations that the division of the nuclei to form the typical eight-nucleate stage of the embryo sac does not occur at the chalazal end of the ovule, which is contradictory to the work of Juliano. Instead, the nuclei follow a regular progressive pattern which places them in definite positions within the sac following each division. This pattern approximates
the ultimate location the nuclei will take in the mature embryo sac.

The usual position of the nuclei in the mature embryo sac following the third division are shown in Plate VI. The embryo sac is long, narrow, curved, and has a definite membrane which embraces five nuclei located at the micropylar end. The two synergids are large pyriform, dark staining bodies located at the base of the sac and overlap one another. The egg lies just above the synergids, and is a large, highly vacuolated nucleus, while the two polars are found near the center of the sac just above the egg. The antipodals at the opposite end are not present. The above pattern of the embryo sac is usually obtained at least three days prior to the opening of the flower.

A mature embryo sac observed eight hours following pollination was found which showed the normal complement of eight nuclei (Plate VII). At the micropylar end of the ovule were located the five nuclei comprising the egg apparatus, while at the other end of the ovule were three small antipodals. The difficulty encountered in finding an embryo sac complete with the antipodals would suggest that these bodies usually disintegrate almost immediately after they are formed. They may, however, occasionally persist up to fertilization and possibly even beyond.

**Pollen Germination, Pollen Tube Growth, and Fertilization.**

Pollen grains of self fertile clones of the sweet potato germinate rapidly. Plates VIII and IX show a surface and longitudinal view respectively of the grains one hour after the pollen was placed on the stigma. It is interesting to note that the pollen tubes have
already covered a third of the distance from the stigma to the ovary. Continuing this rapid growth, they reach and enter the ovules as early as three and one-half hours following pollination. Plate X, taken shortly after entrance of the pollen tube into the ovary, shows the disintegrating tube and the enlarged apical end containing the functional nuclei.

There is apparently a considerable lag following the entrance of the pollen tube into the embryo sac and actual fertilization of the sperm and egg. The two sperm nuclei appear in a resting state lying next to the egg and one of the polar nuclei, as shown in Plates XI and XII, respectively. This period of quiescence may last from one to four hours following the entrance of the sperm into the embryo sac.

The actual process of double fertilization was not observed, but no sections were found in which the sperm and egg had fused and not the sperm and polar, nor were any sections found in which the reverse was true. This would indicate that fertilization of the egg with the sperm and fusion of the sperm with one of the polars apparently occurs simultaneously.

The 3N condition of the endosperm is obtained through the fusion of the sperm and one of the polars, and this product in turn fuses with the remaining polar. This is in accord with Kenyan (6) and Weinstein (12) but is contradictory to the findings of Juliano (5).

Sterility Study. It was observed in two seedlings known as 15-7 and L4-5 that when self-pollinated, the pollen grains failed to germinate. However, when 15-7 and L4-5 were crossed reciprocally with 98, a self fertile seedling, some seed was obtained. A
cytological examination of the behavior of pollen grains of 15-7 and L4-5 in combination with 98 disclosed some interesting information. Some of the pollen grains germinated normally and grew down the stylar tissue, while others germinated, but immediately formed a large swelling on the tip of the tube and failed to grow further. An example of such an abnormality is shown in Plate XIII.

Although there is nothing in the literature to indicate the presence of incompatibility factors in the sweet potato, the above observations would suggest the possibility that they may be present in this plant.

The occurrence of sexual incompatibility is widespread throughout the plant kingdom. Crane and Lawrence, in summarizing the works of others, indicate that such sterility is due to the failure of the pollen, although functional, to grow down the style and effect fertilization (3). Experiments have shown that incompatibility is determined by genes arranged in a multiple allelomorphic series. Thus, a cross involving the factors $S_1 S_2 X S_1 S_2$ will be completely sterile, "like repels like", while a cross between $S_1 S_2 X S_3 S_4$ will be completely fertile. Likewise, a cross between $S_1 S_2 X S_1 S_3$ will give rise to two intra-sterile and inter-fertile groups.

It is conceivable that 15-7 and L4-5 may contain sterility factors $S_1 S_2$, and 98 may contain $S_1 S_2$, and in addition, $S_3 S_4$. Such an arrangement would account for the self-incompatibility observed in 15-7 and L4-5, and at the same time, would indicate why abnormalities were observed in crosses between these three strains. Further work along this line, however, is needed before any definite statement can
be made concerning the number and disposition of sterility factors in the sweet potato.
SUMMARY

The object of this study was to trace the development of the gametophytic generation to fertilization. The formation of the embryo sac in the sweet potato was normal in the material studied in this experiment. The functional megaspore located at the chalazal end of the ovule underwent three divisions to form eight nuclei in linear arrangement. The mature embryo sac was found to be a long, curved structure, containing two synergids located near the micropylar end, two polars near the center of the sac, and an egg which lay between the above two pairs of nuclei. The three antipodals, as a rule, usually degenerated soon after they were formed, but occasionally remained until fertilization.

It was found that, in normal pollen, the pollen tube had grown down the style and entered the embryo sac in about three and one-half hours following pollination. There was a lapse of about four hours following the time of the entrance of the pollen tube into the embryo sac and fertilization. Double fertilization of the egg and polar nuclei occurred simultaneously. The sperm united with one polar nucleus, and this product in turn united with the remaining polar nucleus to form the endosperm. Fertilization was completed in about eight hours following pollination.

The behavior of crosses involving 15-7, 14-5, and 98 suggested the possibility that incompatibility factors may be present in the sweet potato.
Plate I. The functional megaspore of the immature ovule of the sweet potato. Magnification approximately 625.

Plate II. The megaspore after the development of a nuclear membrane which is the first trace of the embryo sac. Magnification about 500.
Plate III. The two nuclei resulting from the first division of the functional megaspore. The nuclear membrane is becoming invisible. Magnification about 450.

Plate IV. The four nuclei, resulting from the second division, are located in pairs at the opposite ends of the ovule and connected by a thin cytoplasmic strand. The membrane is invisible. Magnification about 110.
Plate V. Four of the eight nuclei resulting from the third division in linear arrangement. The lowermost one of the group is a synergid. The small nucleus, located just above the synergid, is the egg, while the remaining two nuclei at the top are the polar nuclei. Magnification approximately 400.

Plate VI. Mature embryo sac containing five nuclei. The two large, dark staining bodies at the base are the synergids. In the center is located a large, vacuolated egg, and the two polar nuclei are the uppermost of the group. Magnification approximately 650.
Plate VII. The photomicrographs A. and B. represent a mature embryo sac containing three antipodals which persisted until eight hours following pollination.

View A. Three small antipodals at the chalazal end of the ovule. Magnification about 60.

View B. Five nuclei located at the micropylar end of the ovule. Magnification about 100.
Plate VIII. A surface view of germinated pollen grains on the stigma one hour following pollination. Magnification approximately 90.

Plate IX. Three pollen tubes growing down stylar tissue one hour following pollination. Magnification approximately 280.
Plate X. Pollen tube in the embryo sac four hours after pollination. Magnification approximately 110.

Plate XI. The egg and sperm just prior to fusion eight hours following pollination. The egg is the larger of the two nuclei. Magnification approximately 535.
Plate XII. Sperm prior to fusing with one of the polar nuclei six hours following pollination. The sperm is the smallest of the three nuclei. Magnification approximately 570.

Plate XIII. Two germinated pollen grains.
A. is normal; the tube penetrated the stigma and grew down the style.
B. is abnormal and has formed a large swelling at the tip of the tube and failed to grow further. The time, four hours following pollination. Magnification approximately 180.
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