growers in seed-producing states has already been established and generally recognized by both the certification authorities and the growers themselves.

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FERTILIZATION AND EARLY EMBRYO DEVELOPMENT IN THE POTATO

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Many commercial varieties of the potato, *Solanum tuberosum* L., produce few, if any, fruits under ordinary field conditions. Naturally the potato breeder is seriously hampered by the difficulty in obtaining viable seed. Morphological studies of flower and seed development in the potato are of value, therefore, in furnishing a basis for the study of the effects of various genetic and environmental influences on the production of flowers and fruits.

Nannetti, (7) working with *Solanum muricatum* failed to discover a tetrad of megaspores. Young (12) concluded that in the potato, the megaspore develops directly from the archesporial cell, and no linear row of four megaspores is developed. However, Bhaduri (1), working with *S. melongena,* and Krüger (6), working with *S. nigrum, S. tubingense,* and *S. proteus,* found the normal linear tetrad of megaspores. In a more recent study Rees-Leonard (8) showed clearly that in *S. tuberosum,* a linear row of four megaspores is formed, the chalazal megaspore functioning in the development of a typical 8-nucleate embryo sac. She also found that changes involving degeneration within the ovule may occur at any time during megasporogenesis and during the development of the megagametophyte, and that degeneration of mature megagametophytes occurs frequently.

Earlier workers confined their attention chiefly to the development

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of the embryo sac prior to the time of fertilization. The present study deals more particularly with fertilization and very early embryo development.

Materials and Methods

The self-fertile variety, Earlaine, was used in this study. Material was collected in the field at Presque Isle, Maine, in the summer of 1938. During the period when collections were made, the weather was for the most part cloudy, with frequent showers. However, the fifth, seventh, eighth, and tenth days after pollination, were clear. The mean temperature during this period was approximately 64° F. with a minimum of 50° F. and a minimum of 81° F.

Material was fixed in either formalin-acetic-alcohol, consisting of 10 c.c. of formalin, 5 c.c. of glacial acetic acid, 90 c.c. of 70 per cent alcohol, or in chrom-acetic-formalin. The latter fixative was composed of equal parts of solution A, consisting of 1 gram of chromic acid and 7 c.c of acetic acid to 92 c.c of distilled water and solution B, consisting of 30 c.c. of formalin to 70 c.c of distilled water. The two solutions were mixed just before using. To facilitate the rapid penetration of the fixing agent the pistils were first dissected out, and the styles and outer ovary wall removed. Before using the chrom-acetic-formalin fixative, the material was dropped for a few seconds into Carnoy's fixative, then transferred, since it otherwise tended to float on the surface of the fixative. After 12-24 hours the material was washed, dehydrated in alcohol, cleared in chloroform and embedded in paraffin.

Transverse and longitudinal sections were cut at thicknesses varying from 8 microns to 15 microns. Slides were stained in Heidenhain's iron alum haematoxylin and in Delafield's haematoxylin. Drawings were made with the aid of a camera lucida.

Observations and Discussion

The reduction divisions in the anther precede those in the ovule, as tetrads of microspores have been observed in flowers in which the megaspore mother cells of the ovules have not yet begun to divide. Young pollen grains are already formed by the time the megagametophyte has completed its development.

The mature megagametophyte is 7-celled (Fig. 1). The three antipodal cells degenerate early, sometimes completely disappearing before the fusion of the polar nuclei, as previously pointed out by Young (12) and Rees-Leonard (8). The two synergids elongate, the micro-

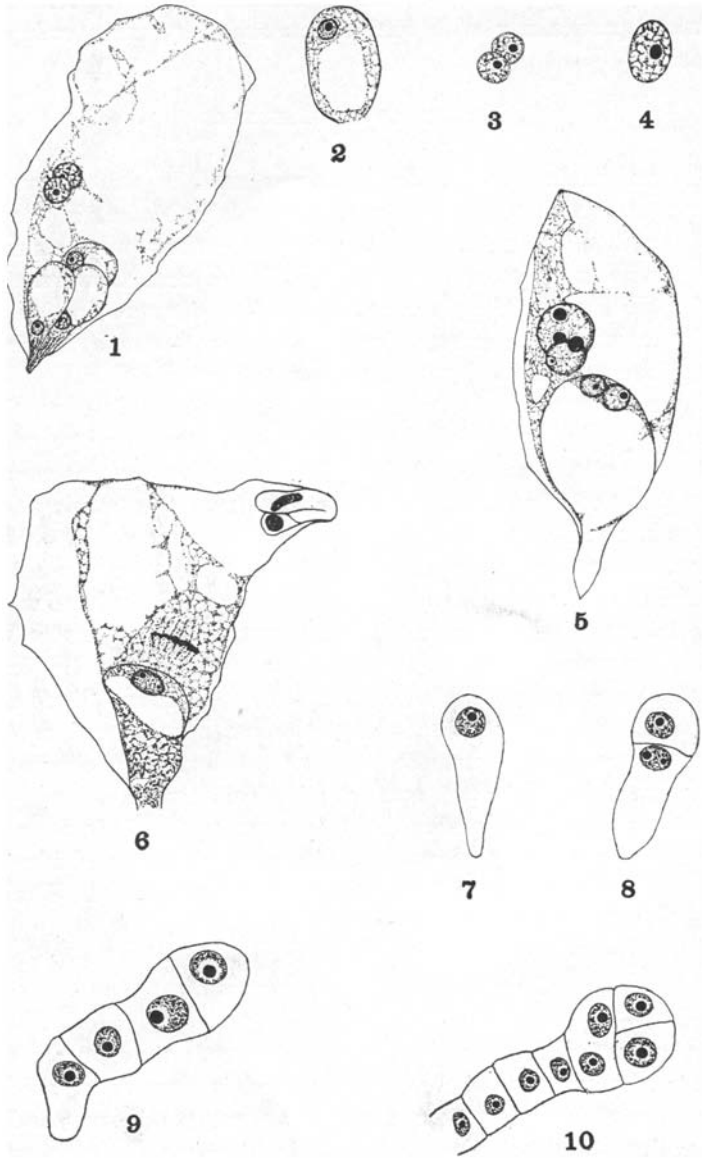

Fig. 1. Embryo sac before fertilization. Fig. 2. Egg before fertilization. Fig. 3. Polar nuclei before fusion. Fig. 4. Fusion nucleus. Fig. 5. Embryo sac during fertilization. Fig. 6. Embryo sac, showing endosperm division. Fig. 7. Zygote. Fig. 8. Two-celled embryo. Fig. 9. Four-celled embryo Fig. 10. Eight-celled embryo.

All figures at magnification of approximately 1,150 times.

pylar ends becoming somewhat narrow and pointed. The nuclei are found near the center or toward the micropylar end of the cells. As the egg enlarges it extends beyond the synergids. A large central vacuole develops (Fig. 2). The nucleus is located in the end of the cell away from the micropyle. The two polar nuclei, one from each end, move to the middle of the gametophyte and unite to form the large fusion nucleus (Figs. 3 and 4). Fusion occurs before fertilization.

In one instance fertilization in the potato ovule was observed 36 hours after pollination (Fig. 5). In this figure the large fusion nucleus appears to be about to unite with the male gamete. The egg is binucleate and one of these nuclei is presumably the male gamete just before fusion takes place. Cochran (2) reported that fertilization occurs 42 hours after pollination in *Capsicum fruitescens* L. at a temperature of 70° to 80° F. Smith and Cochran (9) found no instances of fertilization in *Lycopersicum esculentum* Mill. in less than 50 hours. Ferguson (4) reports that in Petunia the first divisions of the fusion nucleus occur before fertilization, the swollen tip of the pollen tube within the sac being sufficient stimulation to incite the development of the endosperm. However, Cooper (3) observed fertilization in the tomato and noted one case in which a male gamete nucleus was closely appressed to the nucleus of the egg, while the other male gamete was near the egg and at nearly the same level as the fusion nucleus. It would appear, therefore, that in the tomato the usual type of double fertilization occurs, a male gamete uniting with the primary endosperm nucleus before any endosperm divisions take place. The fertilization process in the potato appears to be similar to that of tomato. In any event it seems that under the environmental conditions existing at the time the experiment was conducted, the pollen tube reached the embryo sac in approximately 36 hours.

The first divisions in the endosperm take place well in advance of the division of the zygote. The dividing endosperm nucleus was observed in an ovule collected 46 hours after pollination. Another division figure (Fig. 6) was seen 58 hours after pollination, but other ovules in the same ovary had already undergone the initial steps of endosperm formation, since more than one endosperm nucleus was present. No early, free-nucleate stage in the development of the endosperm was observed. The absence of a free-nucleate stage in the Solanaceae was first reported by Guignard (5).

Rees-Leonard (8) showed that disintegration may occur at any stage during the development of the potato ovule, although it is rarely observed in very young stages. Disintegration occurring immediately

after the meiotic divisions may be the result of irregularities in chromosome distribution. In the present study it was found that in some instances the fertilized egg appeared to be degenerating. Fertilization had evidently taken place, since the formation of endosperm tissue was well advanced. Disintegration is shown by shrinkage of cytoplasm and the appearance of dark-staining masses at the micropylar end of the embryo sac. Further studies are required to determine the nature of this degeneration of ovules and its importance as a factor in the poor seed-setting capacity of many commercial varieties.

The first division of the zygote or fertilized egg was not seen, but only one-celled zygotes were found in all collections made four days or less after pollination, whereas a number of two-celled embryos were found in later collections (Figs. 7, 8). It appears certain, therefore, that under the environmental conditions occurring in Maine during the period of this test, the first division of the zygote took place between four and five days after pollination of the flower. According to Cochran (2), in the pepper, the zygote starts division 24 to 36 hours after fertilization. Smith (9) found two-celled embryos in tomato ovules about 44 hours after fertilization.

The initial division of the zygote is transverse, and the two daughter cells again divide transversely, forming a four-celled linear embryo (Fig. 9). The linear arrangement of the cells of the four-celled embryo has been reported for other members of the Solanaceae by Souèges (11) and other workers. An eight-celled embryo is shown in Fig. 10.

A knowledge of the time which elapses between pollination and division of the fertilized egg is necessary in order to determine when various treatments, such as high and low temperatures, can be applied to best advantage in attempting to double the chromosome number. It is known from work with other crops that such treatments should be applied when the fertilized egg is dividing. It is hoped that polyploids may be obtained in the potato through chromosome doubling and that some of these will show desirable commercial qualities. In order to induce a doubling of the chromosome number by such methods as the heat treatment, it will be necessary under similar environmental conditions to treat the flower between four and five days after pollination.

Summary

A study was made in *Solanum tuberosum* L. of the time elapsing between pollination and fertilization, and between pollination and the first division of the fertilized egg. A knowledge of the time that elapses

between pollination and the first division of the fertilized egg is necessary in order to determine when various treatments, such as high and low temperatures, can be applied to best advantage in attempting to induce polyploidy.

In material collected at Presque Isle, Me., during the summer of 1938, fertilization of the potato ovule was observed 36 hours after pollination. The primary endosperm nucleus was undergoing its first division in an ovule collected 46 hours after pollination. The first division of the zygote or fertilized egg was not seen, but only one-celled zygotes were found in all collections made four days or less after pollination, while two-celled zygotes were found in later collections.

In some instances the fertilized egg appeared to be degenerating after fertilization had taken place, and this may sometimes be a factor in the poor seed-setting of commercial varieties.

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