MINIATURE SEED—A STUDY IN THE DEVELOPMENT OF A DEFECTIVE CARYOPSIS IN MAIZE

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INTRODUCTION

THE normal product of fertilization in maize is a mature dormant seed with a well developed embryo and starchy endosperm. All the many variations from this norm have been in a minus direction with regard to embryo or endosperm development and must be regarded as defective types. These defective types may be divided superficially into two groups on the basis of comparison of their growth to that of plants of similar genetic constitution but carrying the normal allele of the gene in question. The first group would comprise those defectives in which the homozygous defective plants are approximately equal in vigor, size, and maturity to the normal, although they may be somewhat smaller in early growth stages. The second group would include all the lethals, semi-lethals, and stocks in which the factor in question finds expression throughout the sporophytic generation and makes the plants shorter, lighter in color, more slender, and generally less vigorous.

Defectives of the first class are waxy, sugary, shrunken, and miniature germ. All these genes approach normal very closely in seed development as judged by weight. Waxy and sugary are products of upsets in carbohydrate metabolism—waxy corn forming exclusively amylopectin instead of a mixture of both amylose and amylopectin as in ordinary maize (SPRAGUE, BRIMHALL and HIXON 1943), while sugary cannot synthesize glucose into maize starch (EAST and HAYES 1911). WENTZ (1924) reported that miniature germ with a very small embryo has low germination and is weak in early growth stages but recovers to make a normal, mature plant.

The second group of defectives includes many genes, most of which form seeds with only two to 30 percent of normal development. Many workers ascribed such deficiency to faulty pollination or arrested development due to competition and dominance. However, JONES (1920) showed a heritable basis for some of these defects—stocks where development of the embryo and endosperm stopped completely shortly after fertilization. Since that time many deficiencies have been shown to be heritable. MANGELSDORF (1926) made a comprehensive study of 14 defective seed characters. These stocks ranged from those in which development was about 57 percent of that in normal seeds, and homozygous plants although stunted could be grown (*de2*), to one where development is about three percent of normal and viability is nil (*de14*). MANGELSDORF found a good deal of correlation between endosperm and embryo development in all his stocks. There was no satisfactory development of one without the other.

Other investigators have not always confirmed this in other defectives.

EYSTER (1931) found in the reduced endosperm stocks that the embryo showed almost normal development while the endosperm was nearly lacking. DEMEREC (1923) has reported a germless condition where kernels possessing a well-developed endosperm lacked an embryo. His genetic studies have shown that at least four genes are concerned in this situation.

Some of the defectives in this group approach normal closely in weight. Disintegrating endosperm (ROBERTS 1938) is about 98 percent by weight of the normal. This character shows a complete breakdown of carbohydrate metabolism when in the late milk stage. By an autolytic process a cavity is

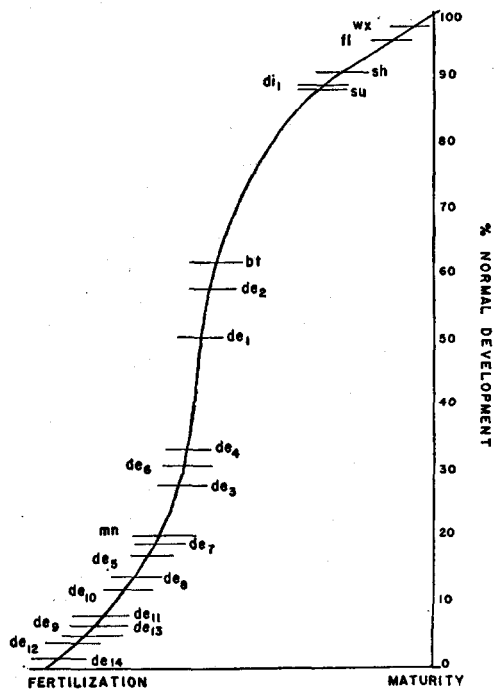


FIGURE 1.—Percentage of normal development of defective seed characters.

formed in the middle of the endosperm. Associated with this self-digestion is a swollen and distorted embryo.

MINIATURE SEED

The defective in this study, designated as miniature seed (*mn*), on the basis of growth, belongs to the first group, since plants from the miniature seeds are equal in final growth to those from normal seeds. However, it is the only member of this group which falls below 85 percent of the weight of the normal seeds of similar constitution. Miniature seed is only 20 percent by weight of normal seeds and, as may be seen by reference to MANGELSDORF's table of defectives (fig. 1), it occupies a position on the scale of normal development well below many of the defectives whose viability is very poor or almost nil.

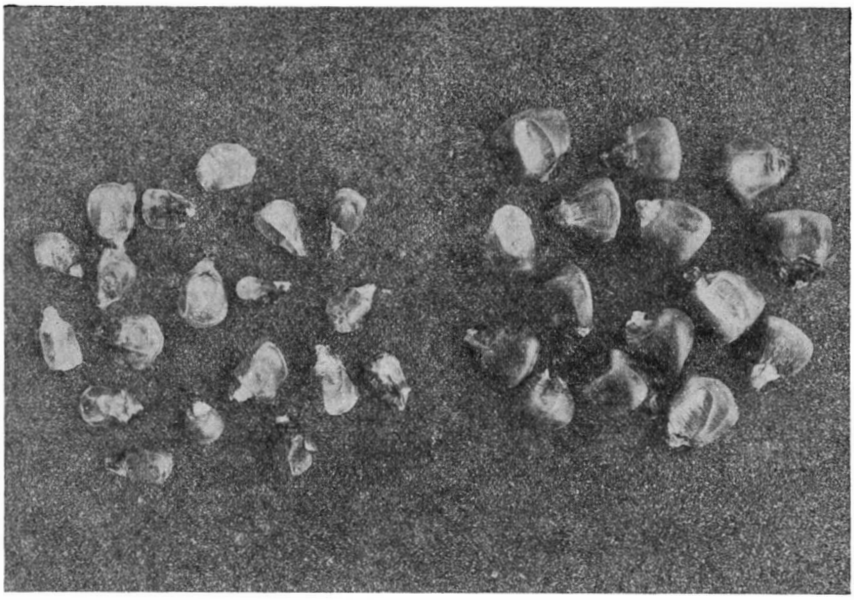


FIGURE 2.—Photograph of miniature seeds (left) and normal seeds (right).

Miniature seed which is 20 percent by weight of normal seeds represents an end product in which both embryo and endosperm are much reduced yet are present in about the same ratio as in normal seeds (table 1). The pericarp stimulated by fertilization outstrips the new tissue so that an easily-broken, papery covering characterizes the seed (fig. 2). The starch in the miniature is identical with that in normal seeds. A normal aleurone layer is developed, and corneous starch is present as in normal seeds. Genetically, miniature seed is a single factor difference, recessive to normal but epistatic to brittle and sugary.

TABLE 1

	65 NORMAL SEEDS		65 MINIATURE SEEDS	
	WEIGHT IN GRAMS	% TOTAL WEIGHT	WEIGHT IN GRAMS	% TOTAL WEIGHT
Embryo	3.3	12.34	0.75	13.74
Endosperm	23.6	87.66	4.71	86.26
Total	26.9		5.46	

In spite of small size, miniature seed germinates well. In one test seeds were taken from an ear segregating for normal, miniature, and brittle kernels. The normal seeds germinated 100 percent, miniature 98 percent, and brittle only 68 percent. Due probably to lack of food, homozygous miniature plants start more slowly than either heterozygous or normal plants. This difference per-

sists until flowering time when the miniature begins to approach normal in height. When mature, there is no significant difference in height, or in diameter of stalk (fig. 3). There is a significant difference in weight of ear and total plant weight due to the much smaller weight of the miniature seeds on the ears of recessive plants.

Further, it is possible to show that the tubes of pollen grains carrying the miniature allele are competitively equal to those carrying the normal allele.

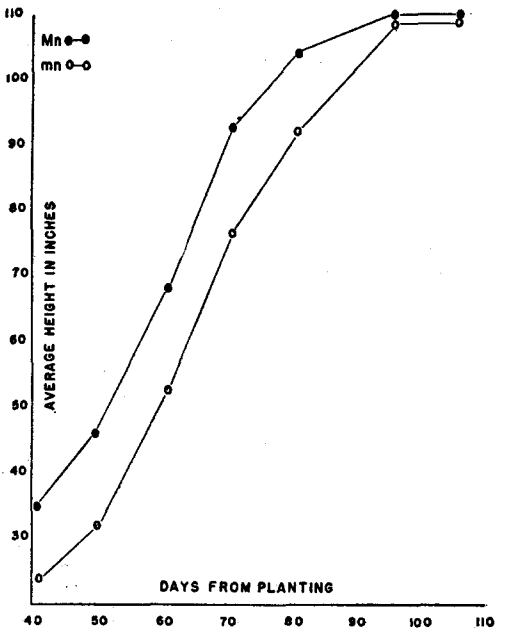
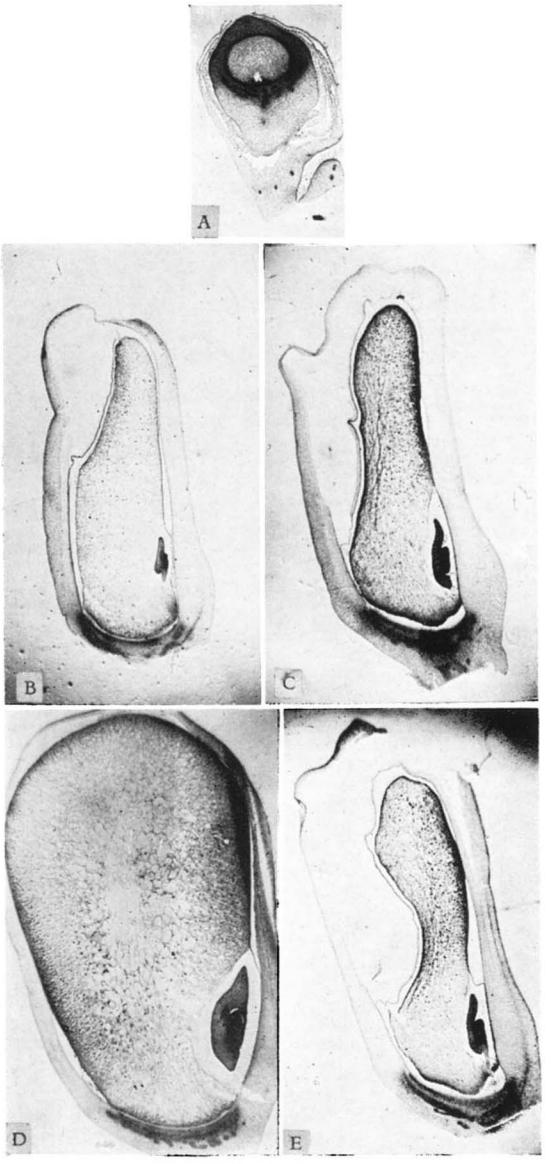


FIGURE 3.—Height of plants from normal and from miniature seeds plotted against days from planting.

Nineteen ears on plants of *mn*/+ genetic constitution were self-pollinated the day after the silks had been cut back almost to the end of the cob. After harvest the ears were divided into equal halves by the aid of a ruler, and the miniature and normal seeds in both the upper and lower halves counted. The total number of all the seeds was 8,929, of which 2,296 or 25.65 percent were miniature. The total number of seeds in the lower halves was 4,419, of which miniature numbered 1,148 or 25.97 percent. In the upper halves the total number of seeds was 4,510, with miniature numbering 1,148 or 25.46 percent.

Had this gene miniature extended its effects to the pollen tube, a significant difference between the ratios of recessive (*mn*) to dominant (++ and +*mn*) in the upper and lower halves of the ears would be expected. The chi squares for the upper and lower halves respectively are .496 and 2.25, showing that the small discrepancies obtained might easily be due to chance alone.



EXPLANATION OF PLATE I

All magnifications 12×.

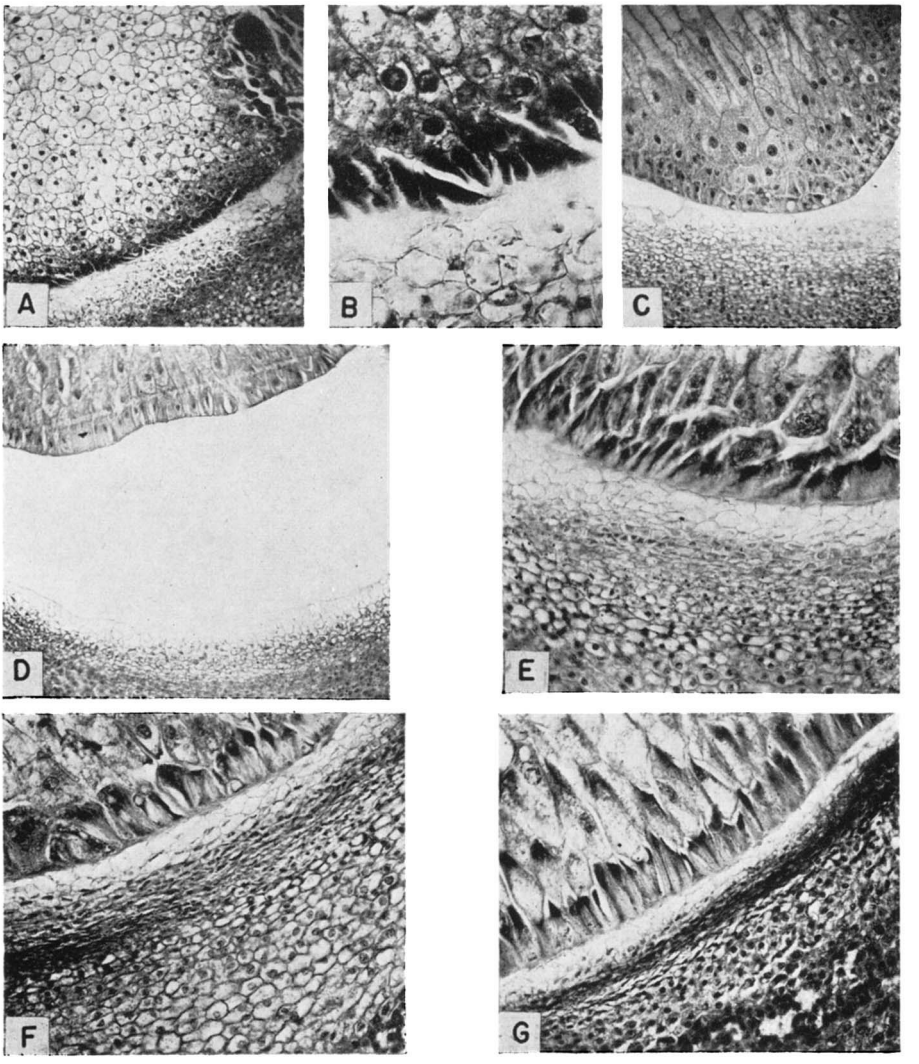
FIGURE A.—Cross section of a seed of corn two days after pollination, showing ovary wall, nucellus, and embryo sac.

FIGURE B.—Cross section of a normal seed 12 days after pollination.

FIGURE C.—Cross section of a miniature seed 12 days after pollination.

FIGURE D.—Cross section of a normal seed 19 days after pollination.

FIGURE E.—Cross section of miniature seed 19 days after pollination.



EXPLANATION OF PLATE II

In all the figures the endosperm of the seed is at the top, and the maternal tissue is at the bottom of the photograph.

FIGURE A.—Cross section of a miniature seed nine days after pollination. The endosperm is well developed, and its differentiated absorptive basal cells are in contact with the chalazal layer which serves as a bridge for nutrient supply between the conductive elements in the maternal tissue and the endosperm. However, the photograph shows that the chalazal cells are beginning to break down. 99×.

FIGURE B.—Same section as in figure A, but at a higher magnification to show more clearly the disintegration of the chalazal cells. 490×.

FIGURE C.—Cross section of a miniature seed 14 days after pollination showing the slight contact between the endosperm and the few remaining chalazal cells. 99×.

FIGURE D.—Cross section of a miniature seed 17 days after pollination showing the complete gap between the endosperm and the maternal tissue. 99×.

FIGURE E.—Cross section of a normal seed 12 days after pollination, showing a complete connection between the endosperm and the maternal tissue by the intact chalazal tissue. 200×.

FIGURE F.—Cross section of a normal seed 14 days after pollination, showing the intact layer of chalazal cells between the endosperm and the maternal tissue. 200×.

FIGURE G.—Cross section of a normal seed 19 days after pollination, showing that the chalazal layer is still completely intact. 200×.

HISTOLOGY

Materials and Methods

Kernels were collected from ears segregating *Mn* and *mn* seeds in a 1:1 ratio at intervals of two, five, nine, 12, 14, 17, and 19 days following pollinations; also from homozygous *mn* ears seven and nine days after selfing. The material was fixed in CRAF and dehydrated in an ethyl-butyl alcohol series according to the method of RANDOLPH (1935), embedded in paraffin, sectioned at 12 micra, and stained with Delafield's haematoxylin. Photographs of entire kernels were taken with the aid of a Bausch and Lomb dissecting microscope; close-up views of internal structures were made with a Bausch and Lomb compound microscope.

General Development of the Normal Seed

The mature ovule, which almost completely fills the ovarian cavity, consists of the many-celled nucellus, with its inner and outer integuments, and the embryo-sac embedded in and occupying a relatively small portion of the nucellar tissue. At the time of pollination this sac contains two synergid nuclei near the micropylar end, the egg cell, the two polar nuclei not yet fused, and a number of antipodal cells at the end opposite the micropyle. From 23–28 hours after pollination the pollen tube reaches the micropylar end of the ovule, enters the embryo sac, and discharges the two sperm nuclei, one of which unites with the egg cell to form the diploid zygote; the other with the two polar nuclei to form the triploid primary endosperm nucleus. Within two to four hours after fusion, this nucleus starts dividing so that by the time of the first division of the zygote, 10–12 hours following fertilization, there are usually four or eight free endosperm nuclei in the embryo sac. The endosperm expands rapidly, digesting away the surrounding nucellar tissue until only a thin layer of cells remains at the periphery. Both embryo and endosperm continue to grow and differentiate until the seed is mature, about 45 days after pollination (WEATHERWAX 1923; MILLER 1920; RANDOLPH 1936). See Plate I, fig. A, B, D.

Development of Miniature Seed

Early stages in the development of *mn* kernels appear perfectly normal. However, microscopic study of sections from seed nine days after pollination show that the cells of the chalazal pocket of the ovule are becoming empty and that their cell walls are breaking down. This layer of chalazal tissue, in the normally growing caryopsis, serves as a bridge for the passage of nutrients from the vascular bundles in the ovary wall to the well differentiated, densely-cytoplasmic, absorptive cells at the basal region of the endosperm. Sections of later stages of *mn* show a progressive disintegration of the chalazal cells. At 14 days after pollination there is only a very slight connection between the basal endosperm cells and the chalazal pocket, and at 17 and 19 days, the gap is complete. An arrest in growth in endosperm and embryo accompanies this

break-down of chalazal tissue. Sections from *Mn* kernels at comparable stages show that the chalazal tissue completely bridges the area between the vascular elements in the ovary and the absorptive cells at the base of the endosperm, and that the endosperm and embryo continue to enlarge (Plate I, fig. B–E; Plate II, all figures).

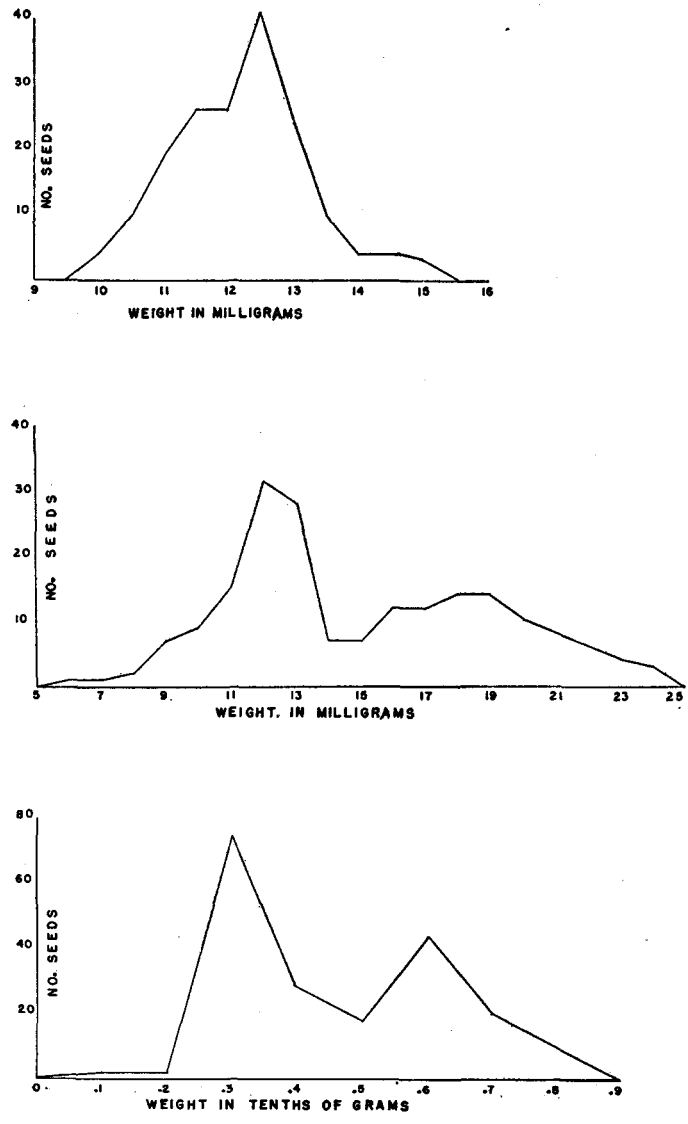
DISCUSSION

Through the recent studies of COOPER and BRINK (1940) and BRINK and COOPER (1941) on seed failure both in interspecific crosses in tobacco and in self-fertilization in the normally cross-pollinated alfalfa, more and more has it been realized that normal development of the endosperm is extremely important for the normal development of the embryo and the entire seed. Sections from seeds in the completely incompatible cross *Nicotiana rustica*×*Nicotiana glutinosa* and from the highly sterile cross *Nicotiana rustica*×*Nicotiana tabacum* reveal a retarded growth of the endosperm; failure of formation of conducting tissue between the vascular bundle of the integument and the chalazal pocket; prevention of contact between the basal endosperm cells and the chalazal pocket by the hyperplastic activity of the nucellus; and death of the embryo following disintegration of the endosperm. A similar situation occurs in self-fertilized alfalfa. Endosperm breakdown accompanied by proliferation of the surrounding maternal tissue leads to the collapse of 34.4 percent of the fertile ovules; after cross pollination only 7.1 percent of the fertile ovules abort. These investigators believe that the endosperm must be so constituted genetically that it can develop fast enough to keep an upper hand over the surrounding maternal tissue; otherwise, this tissue utilizes an abnormal proportion of the available food supply, thereby starving the endosperm and its nursling, the embryo, to death.

In tetraploid-diploid, and in incompatible species crosses in Datura, the situation is somewhat different in that sometimes it is the proembryo and sometimes the endosperm which collapses first, as maternal tissue proliferates (SANSOME, SATINA, and BLAKESLEE 1942).

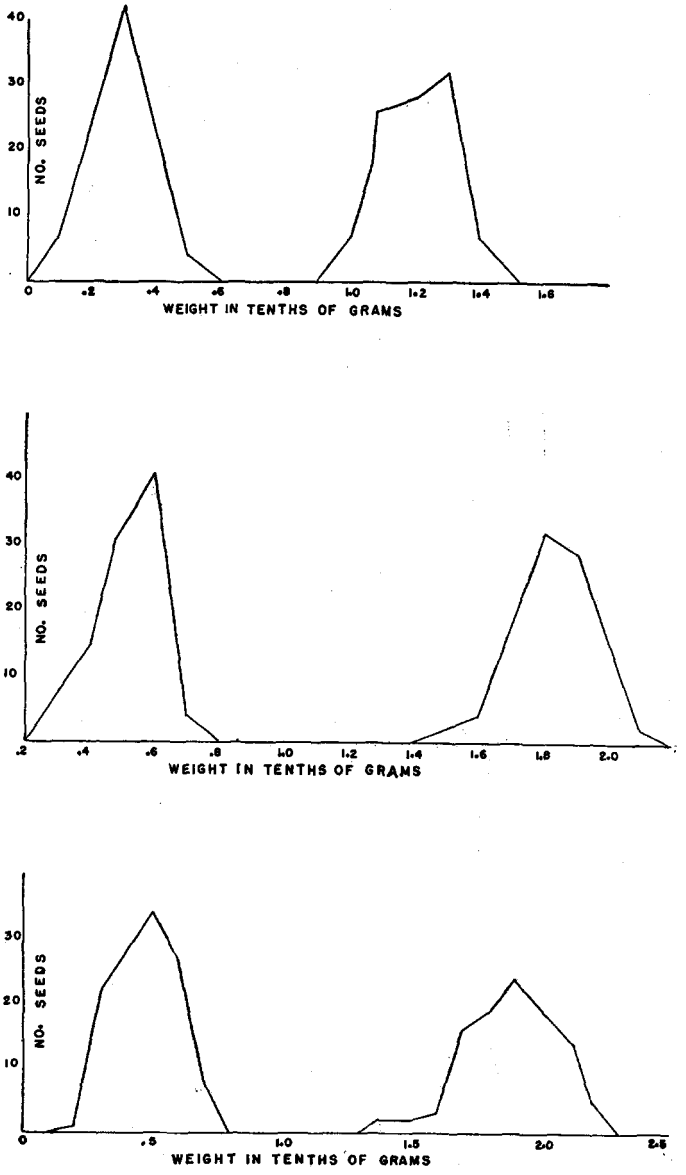
In the present investigation of miniature seed, histological evidence shows that development of the young caryopsis is normal till the ninth day after pollination. Apparently this regular initial growth of both endosperm and embryo prevents excessive proliferation of maternal tissues, as occurs in sterile tobacco and Datura crosses. However, at the ninth day, the chalazal layer of cells connecting the vascular elements of the ovary and the absorptive cells of the endosperm begins to disintegrate. This breakdown of chalazal tissue continues until by the seventeenth day the gap between the conducting cells of the maternal tissue and the endosperm is complete. In normal seeds the chalazal bridge remains intact. Therefore, the small size of the mature miniature seed is presumably caused by partial starvation of the endosperm and embryo. Nevertheless, before the chalazal gap is complete, either enough nutrients have already been absorbed by the endosperm to allow it and the embryo to mature with little further growth, after the 14th day, or else the endosperm can still obtain a limited amount of food from the side walls of the ovary (Plate I, fig. B–G).

The situation suggests that the normal endosperm tissue produces some substance which either promotes longevity of the chalazal layer or inhibits a deleterious effect of the maternal tissue. The miniature endosperm, on the other hand, may lack this substance either partially or entirely, or may produce a different substance which promotes dissolution of the chalazal layer. In either case the breakdown of the connecting cells, and the resulting gap in the nutrient supply between ovary and endosperm causes miniature seed.



FIGURES 4–6.—Frequency graphs of weight of each kernel plotted against the number of kernels in each weight class for ears segregating miniature and normal, harvested at one week (fig. 4 above), two weeks (fig. 5 center), and three weeks (fig. 6 below).

These histological investigations correlate well with frequency graphs made by plotting the weight of each kernel against the number of kernels in each weight class for ears segregating miniature and normal seeds harvested at stated intervals from pollination to maturity (fig. 4–9). The first graph shows a unimodal curve typical of a rather homogeneous weight population. However, the graph representing the kernels taken from an ear harvested 14 days



FIGURES 7–9.—Frequency graphs of weight of each kernel plotted against the number of kernels in each weight class for ears segregating miniature and normal, harvested at four weeks (fig. 7 above), five weeks (fig. 8 center), and six weeks (fig. 9 below).

after pollination shows a distinct bimodal phase in the weight population. This bimodality is enhanced with time as the kernels develop into the normal, and into the thin, papery, miniature seeds.

SUMMARY

Miniature seed is unique among defective seed characters in that it combines a high degree of defectiveness (the weight is only one-fifth that of normal seeds from the same ear) with normal growth in both the gametophytic and sporophytic generations. The character is recessive to normal, epistatic to sugary and brittle, and is a single factor difference. The locus of the gene is not known. The miniature endosperm apparently is lacking a substance or substances found in normal endosperm which are necessary to continuity of the chalazal layer, or else it produces a substance which is deleterious to the connecting chalazal cells, for they begin to break down about nine days after pollination. Up to this point growth and development are apparently normal. After this point growth slows down and stops almost entirely by 14 days, whereas development continues. Embryo and endosperm are present in the same ratio as in normal kernels. An aleurone layer and corneous starch are present. The discontinuity of the chalazal layer and resulting interruption in nutrient supply to endosperm and embryo are responsible for the defective condition.

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