EFFECT OF THE DE17 ALLELE ON DEVELOPMENT OF THE MAIZE CARYOPSIS

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MOST of the defective seed types which come to light in maize populations subjected to self-fertilization are lethal. Search discloses an occasional one, however, which may be propagated in homozygous condition. The present study deals with the developmental effects of a gene of the latter kind. A pronounced deleterious influence is exerted on the seed but not upon the ensuing plant. The investigation was undertaken to determine the manner in which the gene acts to impair seed development.

MATERIAL

The parent ear from which the defective stock was derived was borne upon a hybrid plant in a population obtained by pollinating the variety Western Plowman by an inbred strain known as Indiana Kd 104. We are indebted to DR. N. P. NEAL for the material. The recessive defective allele may have originated as a recent mutation in the inbred line but it is much more probable that it was present in the Western Plowman parent. The parent ear had been self-fertilized and bore 441 normal and 147 defective seeds. This is an exact three to one ratio. Subsequent tests disclosed the presence of the same, or a similar, defective allele in an unrelated family derived from a self-pollinated plant in the Minnesota 13 variety. The original self-pollinated ear in this case bore 199 normal and 120 defective seeds. This ratio departs widely from expectation on the simple Mendelian basis, the normal class being low. Since these defective seeds were relatively thin and papery, the Minnesota 13 stock was not used further. It is proposed to designate this allele for defective seed *de17* in accordance with established usage (EMERSON, BEADLE and FRASER 1935).

The simple recessive behavior of the defective character is borne out by the data in tables 1 and 2. Pollination of four heterozygous plants with pollen from homozygous defectives gave equal numbers of normal and defective kernels within the limits of random sampling (table 1). Self-pollination of heterozygotes gave a close approximation to the expected 25 percent of defectives (table 2). The kernels at the tips of these ears were discarded before the counts were made in order to reduce the likelihood of including parthenocarpic seeds in the defective class. It may be concluded from the breeding data that the defective allele is transmitted through the male and female gametophytes in normal Mendelian proportions.

An important advantage of using a defective which can be propagated in

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homozygous condition in a histological study is that material of known genetic composition can be examined at the stages of development through which the seed passes before it is classifiable on the cob as defective or non-defective. It is clear from observations on seed development following interspecific hy-

TABLE 1

Backcross ratios from De17de17 ♀ ×de17de17 ♂ matings.

PED. NO.	NUMBER OF SEEDS	PERCENT DEFECTIVE
T10-7×*de17*	477	54.5
-10×*de17*	530	50.4
R10×R11A	457	50.1
R11×R10A	703	51.8
Total and Average	2,167	51.7

TABLE 2

F_2 ratios from De17de17 plants selfed.

PED. NO.	NUMBER OF SEEDS	PERCENT DEFECTIVE
R1-1	699	28.3
-2	684	25.1
-3	645	26.0
R5-1	444	22.1
-2	452	24.7
-3	291	26.1
R6-1	295	28.5
R8-1	443	24.8
-2	725	23.2
-3	579	23.0
Total and Average	5,257	25.05

bridization that the changes which occur during the early post-fertilization period are critically important for the subsequent course of growth (compare BRINK and COOPER 1947). The defective used in the present investigation cannot be recognized on segregating ears with certainty until about ten days after pollination. All the material for histological study was collected, therefore, from ears borne by homozygous defective plants. The two classes of seeds were obtained on different ears through the application of pollen from defective and homozygous normal individuals, respectively.

No departure from the regular type of seed development can be detected in kernels whose endosperms and embryos carry the normal allele of *de17* in single dose. Weights of the normal kernels borne on the ears from three *De17 de17* plants crossed with *de17de17* were compared with those of normal kernels obtained by pollinating three closely related *de17de17* individuals with

pollen from a *De17de17* stock. The average weights of the two groups of *De17* kernels were almost identical. That is to say, kernels of normal phenotype develop as well on *de17* as on *De17* plants.

MATURE WEIGHT OF THE DEFECTIVE KERNELS

The weight of the mature defective caryopsis in the line in which the allele was first discovered was approximately 25 percent, on the average, of that of the normal kernels on the same ears. This is shown by the data in table 3. The

TABLE 3

Weight in grams of normal and defective seeds on segregating ears.

EAR NO.	NORMAL		DEFECTIVE	
	NO. SEEDS	AVGE. WEIGHT	NO. SEEDS	AVGE. WEIGHT
7787	100	.327	26	.103
7787	100	.321	21	.149
7788	101	.315	24	.011
7910	100	.347	39	.089
7912	101	.332	29	.042
7915	101	.302	36	.124
7915	100	.278	32	.072
7920	101	.322	41	.098
7923	100	.352	30	.069
7924	100	.332	37	.028
7926	101	.332	39	.112
7929	100	.300	38	.150
7951	100	.366	28	.031
7957	100	.337	28	.161
7965	100	.338	23	.037
7979	100	.349	33	.029
Total		5.250		1.305
Average		.328		.082

defectives when extracted from outcrosses subsequently made to various unrelated lines, however, regularly proved to be relatively lighter in weight than in the original stock. The weights on several such ears were only 12 to 15 percent of the values for the respective normal sib kernels. The decreased development of the extracted defective seeds is doubtless a result of changes in the residual inheritance. Although few outcrosses were tested it appears reasonably certain that this defective lies relatively low on the scale which MANGELSDORF (1926) used in classifying heritable maize variations of this kind.

THE STARCH GRAINS IN DEFECTIVE SEEDS

The amount of starch stored in the endosperm of defective seeds is much less, of course, than in non-defective kernels. The starch grains present in

defective seeds, however, are nearly normal in size and form, and give the usual reaction with iodine.

GERMINATION AND SEEDLING ESTABLISHMENT

Seed from five segregating ears, free of disease, on which the defective kernels were relatively well developed were planted in sand in the green house. Counts made 15 days later showed that the percentages of seedlings emerging

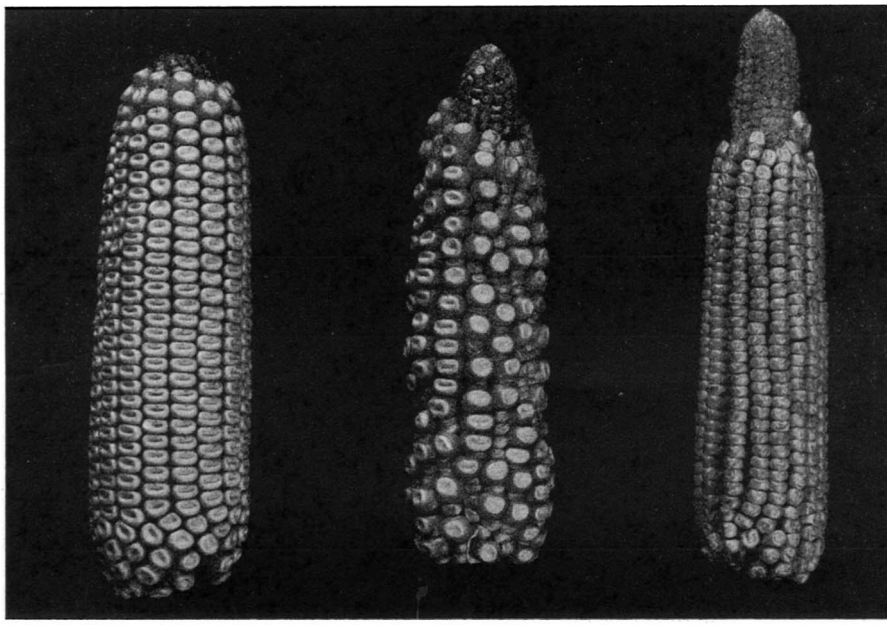


FIGURE 1.—An ear from a homozygous defective plant selfed is shown at the right. The middle ear is the result of backcrossing a heterozygous defective to the defective type. The ear at the left is normal.

from the normal seeds were 96, 99, 98, 100 and 99, respectively. The corresponding values for the defective kernels were 52, 50, 36, 58 and 31 percent. Many of the seedlings from the defective kernels were very small and abnormal in form. Most of these weak individuals die even under favorable conditions for growth. A small proportion of the kernels from many ears, however, give rise to thrifty but undersized seedlings.

The more vigorous homozygous defective seedlings after transplanting to the field grow into vigorous plants. When full grown the latter are about one foot shorter than their normal sibs. Ear shoots and tassels are well developed on the defectives. Pollen is formed abundantly and full complements of defective seed are readily obtained on selfing. Representative normal, defective and segregating ears of the original stock in which the defective character was found are shown in figure 1.

GROWTH OF DEFECTIVE AND NORMAL KERNELS

Data on the growth of defective and normal kernels were obtained from a single family of *de17* plants. The two classes of kernels were obtained by pollinating alternate individuals in a row with *De17* and *de17* pollen, respectively. Collections were made at 0, 6, 9, 12, 16, 24 and 32 days after pollination. The fresh kernels were removed from the cobs and the glumes and projecting vascular tissue were cut off with a sharp scalpel. The caryopses were then dried in a current of warm air. The kernels were allowed to stand for two days at room temperature before counting and weighing. The data are summarized in table 4 and shown graphically in figure 2.

TABLE 4

Average weight in grams of normal and defective kernels up to 32 days after pollination.

DAYS	NORMAL KERNELS			DEFECTIVE KERNELS		
	NO. OF EARS	NO. OF KERNELS	AVGE. WT.	NO. OF EARS	NO. OF KERNELS	AVGE. WT.
0	—	—	—	4	251	.0017
6	6	414	.0045	6	390	.0044
9	8	532	.0083	8	544	.0086
12	12	789	.0172	12	818	.0132
16	8	539	.0461	8	533	.0241
24	8	548	.1107	8	538	.0385
32	8	543	.1774	8	549	.0402

No difference is observable in the dry weights of defective and normal kernels at six and nine days after pollination although both classes of caryopses increase their substance about five-fold over this period. The normal kernels, on the average, are about 30 percent heavier than the defectives at 12 days. The difference increases to 90 percent at 16 days. The average weights for the eight respective samples of defective kernels no longer overlap those of the normal kernels at this time. The defective caryopses show a further increase in dry weight at 24 days, but little change occurs beyond this time. As the defective kernels approach their maximum weight at 24-32 days the normal kernels are three to four times as heavy. Weights were not taken beyond 32 days, although the normal kernels continue growth.

CHANGES IN THE ENDOSPERM, EMBRYO AND PLACENTAL REGION AT SIX TO TEN DAYS

The above data on dry weight indicate that the defective and normal caryopses begin to differentiate from each other in total growth prior to 12 days. In searching for the basis of this differentiation an attempt was made to estimate the size of the endosperm and embryo at six, eight and ten days, and to

characterize the placenta at these times in the two classes of kernels. The term "placenta," as here used, is intended to apply to the saucer-shaped region extending from the nucellus and basal margin of the endosperm to the terminus of the main vascular element of the spikelet. Two groups of four well-developed plants, bearing all defective and all heterozygous normal seeds, respectively, were chosen in the family from which the weight data presented

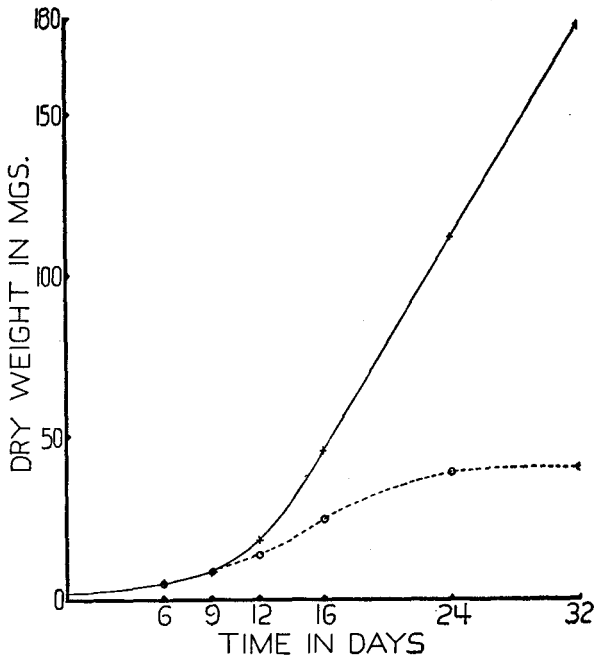


FIGURE 2.—The dry weight of normal (solid line) and defective (broken line) caryopses up to 32 days after pollination.

in table 4 were taken. Successive collections of portions of the ear were made from these plants at six, eight and ten days. The distal one-third of each ear was discarded at the time the first samples were taken, and the basal parts of the ears were not used. The kernels taken for examination, therefore, came from the relatively homogeneous mid-sections of the ears.

The material was fixed in Carnoy's and Karpechenko's solutions and preserved in 70 percent alcohol. The caryopses were dehydrated, imbedded in paraffin, mounted and cut on the microtome so as to obtain median longitudinal sections passing through the silk attachment region and the embryo, bisecting the latter symmetrically. After staining in Delafield's haematoxylin the numbers of cells in endosperm and embryo in these median sections were estimated by counting nuclei under a microscope fitted with a 10×ocular, an 8 mm objective, and a net micrometer.

During early growth of the maize caryopsis nearly all of the nucellus is

digested, the contents of the cells presumably being absorbed by the rapidly expanding endosperm. Attempts were made to measure the rate at which the nucellus disintegrates in normal and defective seeds. A satisfactory way of doing this was not found since the structure, including the cell walls breaks

TABLE 5

Average number of nucleated cells in endosperm, embryo and the placental region in median sections of the caryopsis at 6, 8 and 10 days.

NORMAL					DEFECTIVE				
PLANT NO.	NO. OF CARY-OPSES	AVGE. NO. OF NUCLEATED CELLS			PLANT NO.	NO. OF CARY-OPSES	AVGE. NO. OF NUCLEATED CELLS		
		ENDOSP.	EMBRYO	PLA-CENTAL REGION			ENDOSP.	EMBRYO	PLA-CENTAL REGION
				6-days					
A10	8	335	50	265	A9	2	321	44	272
A12	4	384	93	154	A11	2	368	75	202
A14	7	429	99	243	A13	6	375	105	275
A16	7	372	66	161	A15	6	371	71	258
Total & Avge.	26	380	77	206	Total & Avge.	16	359	74	252
				8-days					
A10	9	889	228	162	A9	5	834	172	131
A12	4	815	433	76	A11	10	1,027	490	133
A14	7	977	387	144	A13	10	842	408	217
A16	0	—	—	—	A15	7	936	290	203
Total & Avge.	20	894	349	127	Total & Avge.	32	910	340	171
				10-days					
A10	7	1,679	719	86	A9	8	1,181	524	143
A12	6	1,591	824	36	A11	10	1,442	1,085	121
A14	7	1,710	1,104	92	A13	9	1,415	928	160
A16	8	1,432	717	52	A15	8	1,521	985	192
Total & Avge.	28	1,603	841	66	Total & Avge.	35	1,390	880	154

down in a rather diffuse manner. The visual evidence is clear, however, that the nucellus in a defective seed is somewhat more persistent than in a normal seed.

The cellular changes in the placental region of the kernel can be measured in the median sections. As in the nucellus, the contents of the cells of that part of the placenta adjacent to the endosperm are progressively absorbed by the

actively growing endosperm. The cell walls tend to persist, however, leaving the framework of the tissue more or less intact after nucleus and cytoplasm have disappeared. This makes it possible to estimate the number of nucleated and non-nucleated cells in this region as regression of the tissue proceeds. The number of stainable nuclei was counted in a band of the placenta .225 mm (five micrometer divisions) wide bounded on one end by the endosperm and at the other by the massive disc-shaped structure in which the principal vascular elements of the caryopsis terminates. The area chosen for the counting lay in the uniform mid-portion of the placenta. All the stained nuclei were recorded in the area thus circumscribed.

The data on number of endosperm and embryo nuclei and on number of nucleated cells in the designated portion of the nucellus in median sections of normal and defective seeds are summarized in table 5. It is apparent from these data that the period from six to ten days is one of rapid growth of the endosperm and embryo. The latter increases in size by about the same amount in both normal and defective seeds. This relation appears to hold for the endosperm also at six and eight days. The estimated average number of cells in median section of the normal seed at ten days is 1603. The corresponding value for the defective seed is 1390. The data are too few to establish the significance of this difference but they suggest that the defective endosperm has begun to lag behind the normal. Two other facts point to the conclusion that at ten days the endosperms in the two classes of seeds are different. The fresh seeds collected at this time are unlike in texture, the normals being firmer. The difference is less perceptible in fixed material due to hardening of the pericarp. The endosperm in the ten day old defective seed is also more subject to shrinkage on fixation than that of the normal.

The evidence shows that the cells in the placental region are depleted of their contents more rapidly in normal than in defective seeds. At six days there are about 22 percent more cells containing stainable nuclei in the designated placental area in defective than in normal seeds. The difference increases to 35 percent and 133 percent, respectively, at eight and ten days. It appears probable that better development of the endosperm in normal seeds and the more rapid depletion of the adjacent placenta are associated phenomena.

DEVELOPMENT OF THE CARYOPSIS

A detailed histological study of the normal and defective kernels was undertaken using material collected at intervals of 18 and 28 hours, two, four, six, eight, nine, ten, 12, 14 and 18 days after pollination. The two classes of seeds again were obtained by applying pollen from homozygous normal and defective plants to ear shoots on *de17* individuals. The kernels were fixed in Carnoy's and Karpechenko's solutions, imbedded in paraffin, sectioned at 12 micra and, for the most part, stained in Delafield's haematoxylin. Various combinations of stains were used on material collected at the critical stages of development, namely, six, eight and ten days, in order to bring out the relationships existing between the different parts of the developing caryopsis.

THE NORMAL KERNEL

The ovary is nearly spherical at the time of fertilization, being somewhat flattened on its upper surface immediately below the region of the silk attachment (figure 3). The ovule is oriented within the ovary in such a manner as to bring the female gametophyte (F) immediately beneath this flattened face The vascular tissue which enters the pistillate spikelet is profusely branched a short distance below the ovule. The branches, barring three, are entwined and terminate in the ovary wall opposite the base of the ovule so as to form a disk of vascular tissue (VD) across the stalk of the spikelet. Two branches on the upper side extend the full length of the ovary and out into the silk. A third branch on the lower side extends through the ovary wall to the region of the stylar canal.

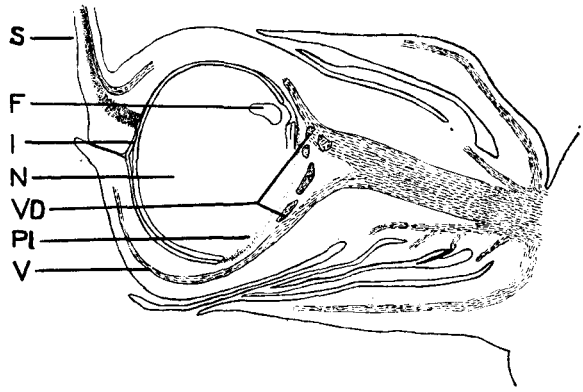


FIGURE 3.—Longitudinal section of mature ovary. S, silk; F, female gametophyte; I, integuments; N, nucellus, VD, vascular disk; Pl, placenta; V, vascular bundle. ×24

No vascular elements enter the ovule. Conduction from the vascular tissue is by means of the storage and parenchymatous tissue present in the placental region (Pl). The cells of the stalk just beyond the vascular disk are small and densely cytoplasmic and later (four to five days after fertilization) become packed with storage materials. The thin-walled parenchymatous cells distal to the storage region are large and highly vacuolate with prominent nuclei. These cells are similar in size and shape to those of the nucellus (N) with which they merge. There is no definite line of demarcation between the cells of the two regions.

The amphianatropous ovule virtually fills the ovarian cavity. It is unlike any of the standard forms being intermediate between the amphitropous and anatropous types. The female gametophyte occupies a relatively small portion of the nucellus. It is oriented so that its longitudinal axis coincides with a line drawn from the base of the silk (S) to the micropyle.

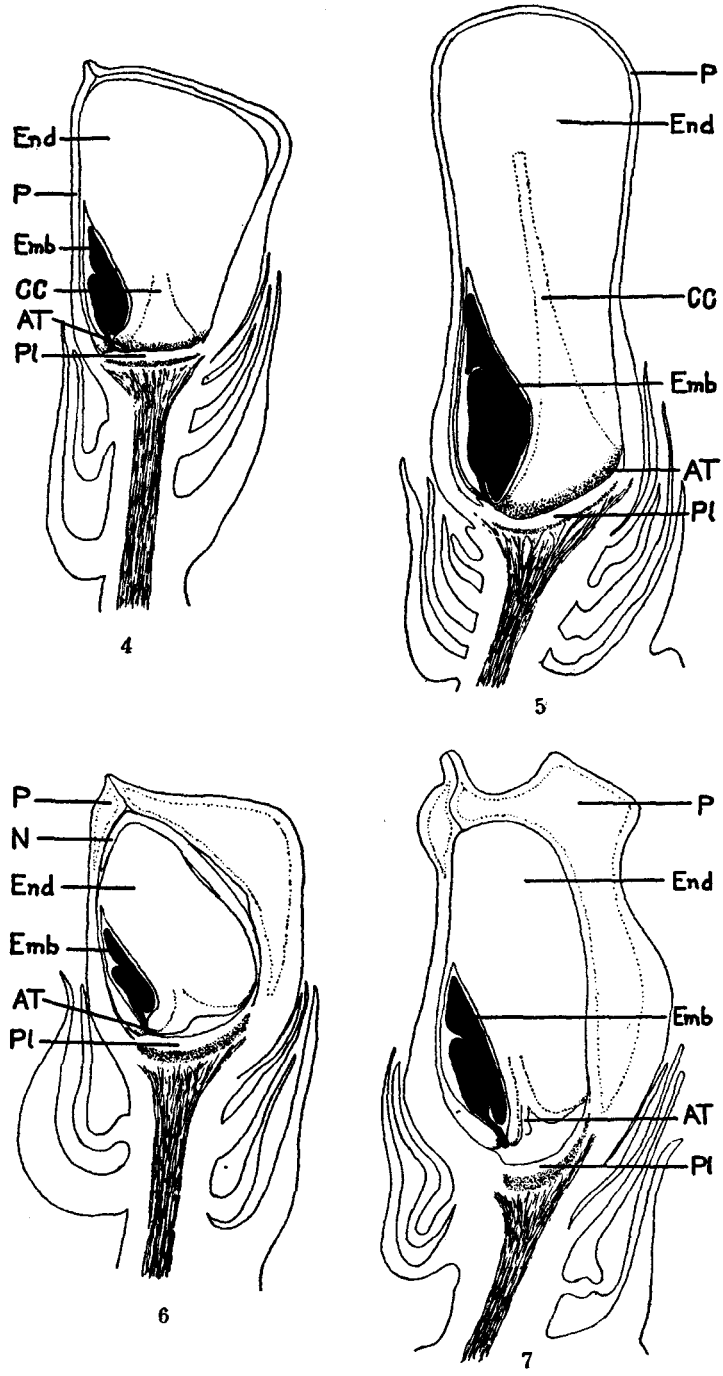
Fertilization was found to occur between 16 and 18 hours after pollination and the entire ovary is then stimulated to rapid growth. The primary endosperm nucleus divides shortly after fertilization and mitoses occur in rapid

sequence so that endosperms with eight and 16 nuclei accompanied by a zygote or two-celled proembryo are present in material collected ten hours later. The endosperm grows first at the expense of the enlarging cells of the nucellus. Later it absorbs nutrients from the placental region.

The rate of growth of the endosperm is such that the space occupied by it and the accompanying embryo in material collected four days after pollination is at least 20 times the size of that occupied by the mature female gametophyte. The growth of the endosperm is toward the antipodals; and at the base it expands into the nucellus at right angles to the longitudinal axis of the ovary so that an inverted, irregularly cone-shaped structure is formed which is flattened on the germinal face. This flattened face is approximately at right angles to the base of the endosperm. The endosperm continues to grow at least partly at the expense of the nucellus and maintains the same general shape for the first eight or nine days of development. The base of the endosperm has, by that time, spread out so that its periphery has reached the outer limits of the nucellus. Thereafter the endosperm quickly consumes the remaining nucellus and has assumed the shape of the ovule in material collected at the 12-day period (figure 4). During these early stages of development the cells are generally rather than locally meristematic. Later the peripheral layers remain meristematic and the central cells enlarge and elongate. Storage of starch begins about 12 days after pollination. The initial storage is in the large cells near the apex of the kernel.

The epidermal cells of the endosperm remain relatively undifferentiated during the first four or five days of development. Thereafter the cells on the basal surface of the endosperm facing the placental region become transformed into absorbing cells which function in the transfer of nutrients from the maternal tissue to the growing endosperm. This transformation is initiated in the cells immediately adjacent to the base of the embryo and gradually spreads from that point so that in material collected 12 days after pollination all of the basal epidermal cells have become converted into an absorbing tissue (AT). In the course of this transformation each cell becomes much elongated and its nucleus moves to the end of the cell toward the main body of the endosperm. The walls on the basal surfaces of these cells become much thickened and such thickening extends along their lateral faces. This thickening of the lateral walls gradually diminishes toward the apices so that the upper portions and the upper faces are thin walled (figure 9). The fibrillar appearance of the cytoplasm in fixed material is due to the fact that longitudinal folds of the cytoplasm extend into these thickened walls to the primary wall of the cell (figure 10). The basal layer remains in close association with the cells of the placenta even in poorly fixed kernels. If shrinkage occurs there is a break across the endosperm above the differentiated cells of the absorbing layer.

The transformation of the basal epidermal cells into an absorbing tissue has an immediate effect on the adjacent layers (ten to 12) of parenchymatous cells in the placenta. The protoplasm in the superficial placental cells becomes disorganized and the nuclei disappear even though the cells remain turgid. The breakdown of the protoplasm in the cells of this tissue advances across the



FIGURES 4 to 7.—Longitudinal sections of developing kernels. Fig. 4. Normal kernel, 12 days after pollination. Fig. 5. Same, 18 days after pollination. Fig. 6. Defective kernel, 12 days after pollination. Fig. 7. Same, 18 days after pollination. P, pericarp; End, endosperm; CC, conducting cells of endosperm; Emb, embryo; AT, absorbing tissue of endosperm; Pl, placenta. ×8

placenta in conjunction with the transformation of the adjacent epidermal cells of the endosperm into a specialized tissue. The breakdown extends eventually into the middle layers of placental tissue so that a cup of cells with disorganized contents comes to lie below the endosperm and opposite the apex of the vascular bundles although not extending to the latter.

The first evidences of the disorganization of the protoplasm in the parenchymatous cells of the placenta are to be found in young kernels collected six days following pollination. A group of cells with disorganized protoplasm is present immediately opposite the region of the embryo. It extends into the placenta. The surface cells of the latter tissue show evidences of a loss of some of the materials stored therein indicating a movement of these materials toward the actively absorbing region of the endosperm. As growth proceeds these storage materials continue to move toward the endosperm and thereafter the proximal portion of the placenta serves as a region for temporary storage and transfer of nutrients from the vascular system to the endosperm. The parenchymatous cells in the placental region and even the deeper lying storage cells of the placenta become more or less flattened as the endosperm continues to grow and expand.

Further cells at the base of the endosperm become transformed into elongate absorbing cells so that three or four layers of such cells are present in 18-day old kernels (figures 5 and 8a). A central core (CC) of large, elongate cells extends from the base of the kernel toward the apex. This core acts as a conducting tissue and transfers nutrients from the absorbing region to the area of storage and later also serves as a place of storage (WEATHERWAX 1930, LAMPE 1931).

The peripheral layers of cells other than those in the absorbing region remain in a meristematic condition until some 20 or 25 days after pollination (FISK 1927). The epidermal layer consists of cells that are small and densely cytoplasmic. This epidermal layer becomes the aleurone layer in the mature kernel.

THE DEFECTIVE KERNEL

All of the defective kernels have badly shrunken endosperms and poorly developed embryos at maturity. The usual interval (16 to 18 hours) elapses between pollination and fertilization and early development of the kernel proceeds at the normal rate. Zygotes and two-celled proembryos accompanied by endosperms with eight or 16 nuclei are present in samples collected 28 hours after pollination.

The development continues at a normal rate up to six days after pollination at which time a cone-shaped endosperm is present. Thereafter growth slows down so that the base of the endosperm does not reach the periphery of the ovule until 12 days after pollination (figure 6). A large mass of the nucellus (N) is still present near the apex of the ovule at this interval. This nucellar tissue is consumed during the next few days so that little, if any, remains at 18 days (figure 7).

The cells of the basal epidermal layer of the endosperm are densely cytoplasmic in the material collected at the six day interval. These cells do not

elongate and become thick walled and as a result they do not acquire the fibrillar appearance characteristic of this tissue in the normal kernels at later stages of development. Rather they enlarge and become highly vacuolate (figures 11 and 12). There is a close relationship between them and the parenchymatous tissue of the placenta in well-fixed material. When differential shrinkage occurs due to fixation, the endosperm pulls away from the surrounding maternal tissue leaving a wide gap between the basal portion of the endosperm and the placenta.

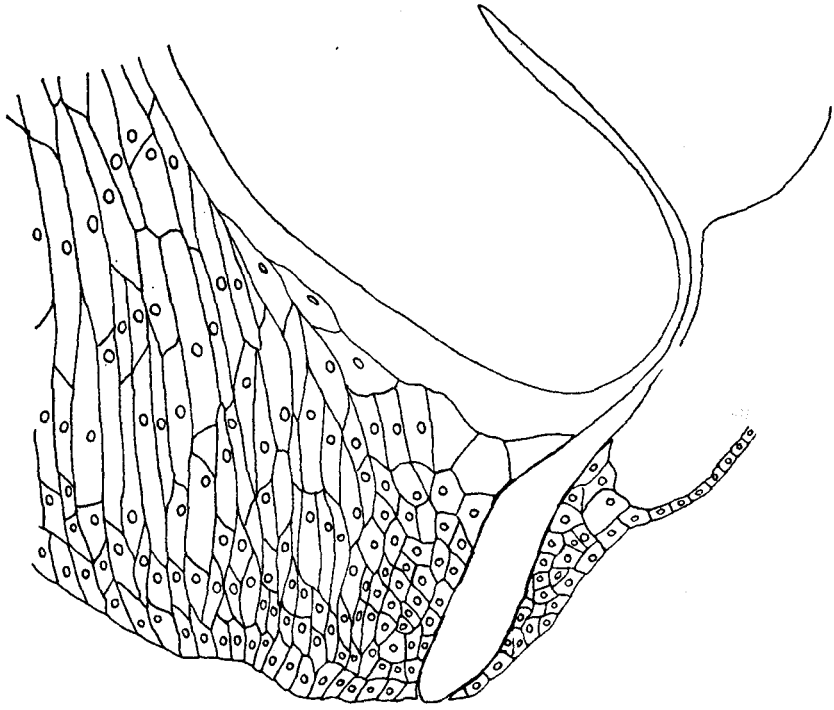
The protoplasm of the cells of the central portion of the basal epidermal layer which acts as an absorbing tissue in the normal kernels begins to break down in the defective kernel at ten to 12 days after pollination (figure 11). The breakdown spreads to the adjoining cells both of epidermal layer and the adjacent endosperm so that the basal layers of cells of 18-day endosperms are, for the most part, empty and badly shrunken (figures 7 and 8b). A collapse of the central core of the endosperm accompanies this breakdown and the endosperm is unable to obtain the requisite supply of nutrients for normal development.

The effect of the basal epidermal cells on the placenta is quite different from that in the normally developing kernel. The adjacent parenchymatous cells increase in size and in many the cytoplasmic content becomes shrunken about stainable nuclei in 12-day material. Other cells lose their entire content. They continue to expand and in 18-day material many of the cells immediately adjacent to the endosperm have ruptured. The deeper lying cells of the placenta tend to retain their storage materials.

The embryos develop at the normal rate for the first six days after pollination and then growth appears to slow down slightly. The lag in embryo development was noted in the samples collected at each interval thereafter. The embryo continues to grow at the expense of an undernourished endosperm so that much of the endosperm is empty and badly collapsed at 18 days.

DISCUSSION

The differentials conditioning normal and defective seed development in the present study are the *De17* and *de17* alleles, respectively. The effect of the recessive allele in homozygous condition is to reduce kernel weight to 25 percent or less of the standard value. Homozygous defective embryos are potentially capable of growing into thrifty, fertile plants only a little shorter than their normal sibs. Special care must be taken, however, in propagating *de17* *de17* plants at the early stages because even the most vigorous seedlings of this class initially are weak. Homozygous defective plants to which normal pollen is applied develop normal seeds. This makes it possible to obtain phenotypically normal and defective kernels at will by controlling the pollination of *de17* plants. It is evident from this fact, also, that the difference in seed development is attributable to the composition of the endosperm or embryo, or both and is independent of the genetic make-up of the pistillate parent in



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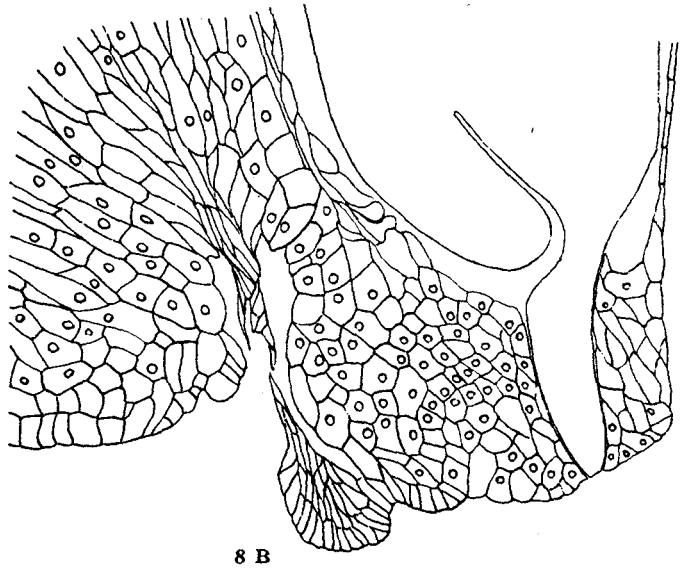
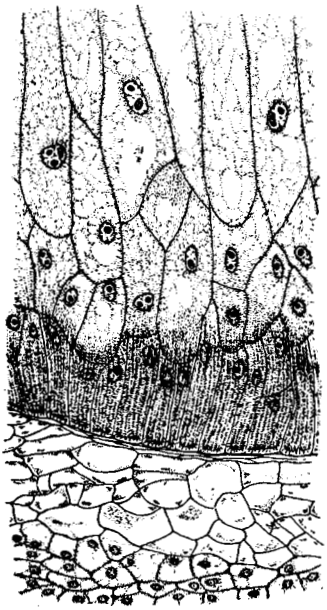


8 B

FIGURE 8.—Basal portion of endosperms, 18 days after pollination. A, Normal. B. Defective. ×80



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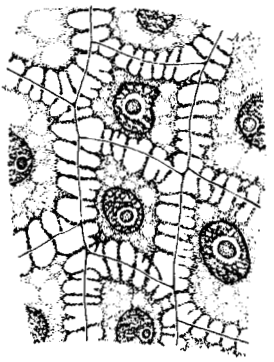

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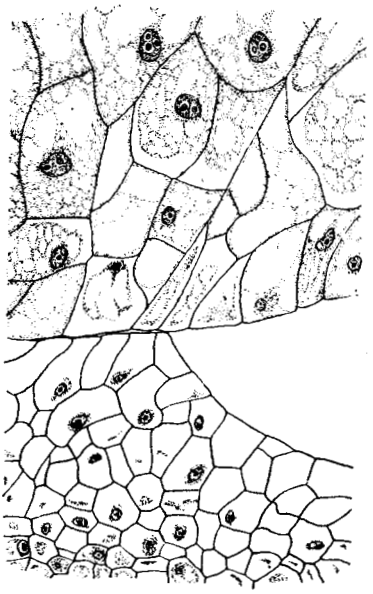
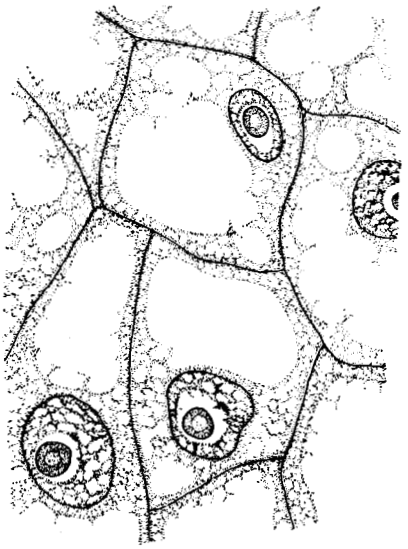


11



12

FIGURES 9–12

respect to the *De17* and *de17* alleles. Both endosperm and embryo are markedly underdeveloped in the mature defective caryopsis.

Both normal and defective caryopses increase in weight approximately fivefold during the first nine days after pollination. The distribution of the dry weight increases as between endosperm and embryo and the pericarp was not determined. Much of it probably occurs in the pericarp. The embryo remains a comparatively small structure during this period; and endosperm growth involves displacement of the nucellus, the substance of the latter tissue being rapidly consumed in the process. At 12 days the defective kernels have fallen slightly behind the normals in dry weight. The difference is much larger at 16 days, and continues to increase rapidly up to 24 days beyond which time the defectives make little growth.

The histological studies reveal a relationship between the initial divergence in weight of the two classes of kernels and the differentiation of an absorbing region in the endosperm. During the interval between six and 12 days the cells on the basal surface of the endosperm facing the placental region in normal kernels become elongated, the nuclei move to the inner end of the cells, and the cytoplasm assumes a dense, fibrillar appearance. These structural changes indicate that the basal cells become highly active at this time in absorbing nutritive materials from the placental region and transmitting them to the more distal portions of the endosperm. The basal cells of the endosperm in defective seeds do not become similarly transformed into absorbing elements. Rather they enlarge about equally in all dimensions and become highly vacuolate. Failure of the defective endosperm to differentiate a basal absorbing tissue during the interval between six and 12 days explains the limited growth which these kernels subsequently make. A few days later the cells in this region of the defective endosperm begin to break down. Eventually many cells in the basal area and in the adjoining central region of the endosperm collapse thus becoming entirely nonfunctional in the absorption of nutrients into the seed.

There is some evidence of a lag in growth of the defective endosperm paralleling the formation of the absorbing layer in the endosperm of normal seeds. The data in table 5 relating to eight- and ten-day endosperms suggest this. Furthermore, the expanding base of the young endosperm reaches the outer limit of the nucellus about three days earlier in normal than in defective seeds.

The changes which appear during the first 12 days in the placental tissues of the two classes of caryopses are in conformity with the behavior of the re-

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FIGURE 9.—Longitudinal section through portion of absorbing tissue of endosperm of normal kernel showing fibrillar appearance of cytoplasm and thick walls of epidermal layer, 12 days after pollination. ×210

FIGURE 10.—Transverse section of same showing folds of cytoplasm in the thickened walls of the epidermal cells. ×920

FIGURE 11.—Longitudinal section through portion of absorbing tissue of endosperm of defective kernel showing thin-walled epidermal layer, 12 days after pollination. ×210

FIGURE 12.—Transverse section of same showing large, highly-vacuolate, thin-walled cells of the epidermal layer. ×920

spective associated endosperms. The parenchymatous cells of the placenta are quickly and extensively depleted of their total contents by the regularly differentiating normal endosperm. The placental cells in kernels possessing defective endosperms are more slowly and less completely depleted. The difference appears to be a direct function of the absorptive capacities of the normal and defective endosperms.

Miniature seed (*mn*) in maize which is similar to *de*17 in that it gives vigorous and fertile adult plants even though the caryopsis is greatly reduced in size has been studied recently by LOWE and NELSON (1946). These investigators conclude that the principal factor operating to restrict development of the miniature seed is a break in cellular contact along the interface between the endosperm and the placenta. In sectioned preparations the two tissues at 14 days are loosely connected and at 17 days are completely separated in miniature kernels. We have made the same observation repeatedly in *de*17 caryopses. The phenomenon appears to us to be due mainly, however, to the pronounced shrinkage of the defective endosperms on fixation. It is evident that with the eventual disorganization and breakdown of the absorbing layer in the endosperm of defective kernels physical continuity between the endosperm and the chalazal region will be interrupted. We interpret this rupture as a direct consequence of the collapse of the outer or basal endosperm cells rather than as a result of a shrinkage of the chalazal tissue due to some prior influence of the defective endosperm on the cells in this region as LOWE and NELSON suggest.

It seems important to point out in this connection that the outer layers of cells in the chalazal region of both normal and defective seeds become moribund very quickly as the young endosperm expands. The protoplasts are digested and their products are presumably absorbed by the endosperm. The cell walls are more persistent than those of the nucellus, however, where a similar process occurs in the juvenile seed. Data in table 5 show that depletion of the chalazal cells occurs more rather than less rapidly in the normal caryopses. The significant fact, however, is that in both normal and defective kernels this region of the chalaza becomes physiologically inactive due to destruction of the protoplasts. The two classes of caryopses are alike in showing a break in the continuity of living cells.

Functionally, this region in the maize caryopsis becomes a chalazal pocket (compare COOPER and BRINK 1940) into which nutrient materials are moved from the vascular tissue below and from which they are absorbed by the endosperm. It is probable that the nutrients are present in relatively soluble form and that they are ordinarily lost in the preparation of stained sections. Occasionally, however, we have seen massive amounts of dense material, homogeneous in appearance, lying between the absorbing cells of the young endosperm and the adjacent framework of cell walls in the placenta. Such deposits have been observed only in normal kernels. It is likely that they are accumulated less abundantly in association with defective endosperms and more completely dissipated in processing the tissue.

The available data do not show whether the *de17* gene exerts a direct parallel influence on the endosperm and embryo or acts directly on the endosperm only. The severely restricted development of the defective endosperm in itself is sufficient to account for the failure of many of the associated embryos to reach a viable condition and for the others to yield weak seedlings. The somewhat shorter stature of adult *de17de17* plants, as compared with their normal sibs may be due either to the handicap incurred at the seedling stage because of poor seed development or to this factor plus a continuing but only mildly deleterious effect of the *de17* allele on later growth.

SUMMARY

The study is concerned with the effect on development of the maize caryopsis of the recessive defective -17 (*de17*) allele.

The *de17* allele in homozygous condition reduces the weight of the mature kernel to 25 percent or less of the normal value. The embryos in many *de17* seeds are viable, however, and produce seedlings which, on overcoming the initial handicap of small size, may grow into vigorous, fertile plants. Mature *de17* plants are about one foot shorter than their *De17* sibs.

De17 kernels develop as well on homozygous *de17* plants as on normal individuals. Comparable samples of the two classes of caryopses, *De17* and *de17*, may be obtained at all stages of development, therefore, following the appropriate pollinations on *de17* plants.

Defective and normal kernels increase in weight at the same rate up to nine days after pollination. Thereafter the *de17* kernels lag markedly behind the normals and cease growth prematurely at 24–30 days.

The cells on the basal surface of the endosperm facing the placental region in normal kernels differentiate into an absorbing tissue at six to 12 days. This differentiation does not occur in the defective caryopsis. Failure to form this absorbing tissue appears from the evidence to be the basic histological manifestation of the action of the *de17* allele.

Many cells in the basal region of the defective endosperm show evidences of collapse at ten days. The breakdown spreads to adjacent cells and to the adjoining central core during the succeeding eight or ten days. The collapse of these cells interrupts the absorption and transfer of nutrients necessary for growth of the seed and contributes directly to the limited development of the latter.

The parenchymatous cells of the placenta are extensively depleted of their total contents by the regularly differentiating normal endosperm. These cells are more slowly and less completely depleted in defective kernels. The difference appears to be a consequence of the unlike absorptive capacities of the normal and defective endosperms.

The somewhat shorter stature of adult *de17* plants, as compared with their normal sibs, may be due either to the handicap incurred at the seedling stage because of poor kernel development or to this factor plus a continuing but only mildly deleterious effect of the *de17* allele on later growth.

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