DEFECTIVE KERNEL MUTANTS OF MAIZE
II. MORPHOLOGICAL AND EMBRYO CULTURE STUDIES

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ABSTRACT

This report presents the initial results of our study of the immature kernel stage of 150 defective kernel maize mutants. They are single gene, recessive mutants that map throughout the genome, defective in both endosperm and embryo development and, for the most part, lethal (NEUFFER and SHERIDAN 1980). All can be distinguished on immature ears, and 85% of them reveal a mutant phenotype within 11 to 17 days post-pollination. Most have immature kernels that are smaller and lighter in color than their normal counterparts. Forty of the mutants suffer from their defects early in kernel development and are blocked in embryogenesis before their primordia differentiate, or, if primordia are formed, they are unable to germinate when cultured as immature embryos or tested at maturity; a few begin embryo degeneration prior to the time that mutant kernels became visually distinguishable. The others express the associated lesion later in kernel development and form at least one leaf primordium by the time kernels are distinguishable and will germinate when cultured or tested at maturity. In most cases, on a fresh weight basis, the mutants have embryos that are more severely defective than the endosperm; their embryos usually are no more than one-half to two-thirds the size, and lag behind by one or two developmental stages. in comparison with embryos in normal kernels from the same ear. One hundred and two mutants were examined by culturing embryos on basal and enriched media; 21 simply enlarged or completely failed to grow on any of the media tested; and 81 produced shoots and roots on at least one medium. Many grew equally well on basal and enriched media; 16 grew at a faster rate on basal medium and 23 displayed a superior growth on enriched medium. Among the latter group, 10 may be auxotrophs. One of these mutants and another mutant isolated by E. H. COE are proline-requiring mutants, allelic to *pro-1*. Considering their diversity of expression as evidenced by their differences in morphological appearance, degree of defectiveness and response to embryo culturing, we believe that they represent many different gene loci.

THIS report is concerned with the further characterization of 150 defective kernel mutants previously described by NEUFFER and SHERIDAN (1980). All mutants analyzed are of the type originally described by JONES (1920) and

MANGELSDORF (1923, 1926) in which both the endosperm and the embryo are severely affected. They are single gene, recessive mutants and the majority are lethal. Furthermore, the finding that the mutants occur throughout the maize genome, located on 17 chromosome arms that are subject to testing, indicates that many gene loci are represented (NEUFFER and SHERIDAN 1980).

A major goal of this project is to search for auxotrophic mutants. To do this, we have attempted to rescue lethal mutants by culturing their immature embryos. This requires that the immature mutant kernels be distinguishable and that their embryos reach a developmental stage suitable for placing into culture. This report concerns three questions relevant to this search, as well as our interest in the number of gene loci represented in the mutant collection and the nature of the defect in each mutant: (1) At what time during kernel development does the mutant condition first become apparent? (2) What are the morphological effects of the mutant condition, especially with regard to embryo development and size? (3) What are the responses of the mutant when attempts are made to rescue them from their lethal condition by placing immature embryos on nutrient media?

By answering the first question, through a search for segregating kernels on immature ears, it has been possible to determine the time of mutant expression for 150 mutants and to carry out morphological and embryo culture studies on 106 mutants.

MATERIALS AND METHODS

The induction and preliminary analysis of the mutants examined here were previously described (NEUFFER and SHERIDAN 1980).

For each mutant, a sample of normal appearing kernels was removed from mature, self-pollinated ears that contained mutant kernels and planted in the field. The resulting plants were self-pollinated, and ears were harvested beginning at 11 days post-pollination. When a segregating immature ear was identified, usually a few mutant kernels were immediately cut open and examined to see if an embryo was visible to the unaided eye. If an embryo was observed, the ear was saved for embryo culturing.

Whenever possible, data were gathered for the mutant embryos and kernels of an early segregating ear of each mutant that was used for embryo culturing. Often, this was from an ear at the earliest segregation age, but in many cases it was from an ear one to a few days older than the earliest segregation age. Usually 10 embryos were measured, weighed and examined to determine the average length, weight and range of embryo development. A developmental stage classification system based on that of ABBE and STEIN (1954) was used to record the stage of development of the mutants and normal embryos. Usually 10 mutant and 10 normal kernels were weighed to determine average fresh kernel weight.

Histological techniques: The techniques employed are essentially those of SASS (1968). Kernels were removed from an immature segregating ear, fixed for 1 week or longer in formalin:acetic acid:ethanol:water (10:5:60:25 v/v) dehydrated and embedded *via* tertiary butyl alcohol (TBA) in Paraplast Plus, taking care to extend the period in absolute TBA to 3 days or longer and using at least 3 changes of Paraplast Plus with at least 1 day in each change. The kernels were stained with eosin during the dehydration procedure to facilitate the casting of blocks and sectioning. Sections were cut longitudinally at a 15 micron thickness, mounted on gelatin subbed slides and stained in hematoxylin and safranin.

Embryo culture media and techniques: The basal medium contained the mineral salts of MURASHIGE and SKOOG (1962), 4% sucrose, 0.8% agar and was adjusted to pH 5.8 prior to

sterilization by autoclaving. The enriched medium consisted of the basal medium plus the following additives in mg/1: (amino acids) glutamine 87.6, arganine-HCl 252.8, proline 138, glutamate 88.2, asparagine 79.2, threonine 142.8, lysine-HCl 219.6, isoleucine 157.2, methionine 89.4, aspartate 79.8, phenylalanine 99.0, tyrosine 108.6, tryptophan 122.4, serine 126.0, glycine 90.0, cysteine 72.6, alanine 106.8, valine 140.4, leucine 157.2, histidine-HCl 114.6; (vitamins) biotin 1, p-amino benzoic acid 1, calcium pantothenate 1, niacin 1, pyridoxine 1, riboflavin 1, a-tocopherol acetate 1, thiamine-HCl 1.4; (nucleic acid bases) adenine 10, guanine 10, thymine 10, cytosine 10, uracil 10 and, in addition, inositol was included at 100 mg/l. In preparing the enriched medium, a solution containing the mineral salts, sucrose, tyrosine, nucleic acid bases, agar, inositol and thiamine (0.4 mg/1 final concentration) was autoclaved and mixed with a solution of the other ingredients (amino acids and vitamins) that was sterilized by filtration through a 0.2 micron Nalgene filter unit. Some modifications of the basal and enriched media were prepared by omitting various constituents. The media were dispensed at 8 ml/tube into 20 × 150 mm disposable glass culture tubes in plastic disposable racks (KIM-RAK, Kimble Products No. 73600–20150). The tubes and racks were used repeatedly.

In preparation for embryo culturing, the ears were stripped of their husks and silks and moved to a sterile transfer hood. After a 5-min submersion in 70% ethanol, the ear was placed on the work surface, and the tops of the kernels were cut off with a sterile razor blade. The embryos were dissected with a sharpened tool having a double-edged, spear-shaped blade. The embryos were temporarily placed on sterile basal medium in a Petri dish and then transferred to culture tubes with the same dissecting tool. Ten or 15 mutant embryos were placed on each kind of media to be tested. Initially, 10 normal embryos obtained from the same ear as the mutant embryos were also cultured on the same media. In more recent tests, sets of 10 or 15 normal embryos were cultured on all media being used, but normal embryos were not cultured from every ear that was used as a source of mutant embryos.

Recording of phenotypic and growth data: In general, the culture tubes were incubated at 25° with a 16-hr light, 8-hr dark cycle prior to harvesting. When placed into culture, normal embryos and many mutant embryos germinated precociously and grew into plants. After 21 days, presence of roots and shoots with unfolded leaves, the color of leaves and the relative plant height were noted. Usually the fressh weights of the roots and shoots were recorded by the use of a Sartorius electronic balance model 3705, coupled with a Hewlett Packard 97S I/O calculator.

RESULTS AND DISCUSSION

Timing of mutant expression: Most mutants became distinguishable from normal kernels at an early stage of development. The mutant endosperm generally began to lag behind its normal counterparts on a segregating ear at 11 to 17 days post-pollination, with 127 of the 150 mutants revealing a segregation pattern on immature ears during this period; 18 of the mutants became apparent as defective kernels during the period of 18 to 21 days post-pollination, while five mutants had later segregation ages (Table 1). These are conservative estimates since the mutants may express their mutant phenotype earlier than shown. The majority of the mutants are surprisingly uniform with regard to the early time and stage of kernel development when their defective condition is first manifested. A second, small group consists of those mutants revealing their mutant condition later in kernel development. For most mutants, an appearance of a visibly mutant endosperm phenotype coincides with the onset of storage product synthesis in the endosperm of normal kernels (NELSON 1978; WILSON 1978; TSAI 1979), while the time of appearance of a mutant embryo phenotype is earlier than that of the endosperm. Embryos were further behind

TABLE 1

Earliest age of ear expression of defective kernel mutants

	330D			
	931A			
	932			
	936A			
	948A			
	971			
	974A			
	1005A			
	1024A			
	1045			
	1054			
	1113A			
	1121			
	1130			
	1142A			
	1147A			
	1163			
	1167A			
	1177A			
	1208A			
	1225A			
	1239			
	1253B			
	1255B			
	1283			
	1286A			
	1287			
	1296A			
	1315A	749		
744	1316A	883A		
747B	1339A	890A		
788	1369	1007A		918A
874A	1383	1112		1060A
874B	1385	1154A		1060B
888A	1387A	1155A		1145A
912	1390A	1162		1156A
933	1392A	1202A		1157
1058	1399A	1295	740	1172B
1089	1404	1330	928A	1310B
1168A	1406	1380A	991	1313
1311C	1410	1386A	1078B	1332
1324A	1411	1391	1104B	1341

TABLE 1 (Continued)

Earliest age of ear expression of defective kernel mutants

	627D	923	1379A	1415A	1394	1175	1348	1009				
	660C	1022	1381	1418	1395A	1180A	1373A	1122A				E1119A
	792	1092A	1384A	1419	1421	1294	1388	1176A	1196	1308A	211C	E1053
	873	1092B	1417	1429A	1425A	1319A	1414	1191	1210	1333B	925A	E901A
	1076A	1331	1423	1431	1427A	1393A	1422	1309A	1299	1401	1096A	E1241
	1303	1409	1428	1435	1436A	1413	1430	1405A	1365	1426	1420	E628A
Ear Age-Days	11	12	13	14	15	16	17	18	19	20	21	22–29
Frequency	6	6	19	48	20	11	17	6	4	4	4	5

• E-number is shown for mutants observed to segregate as defective kernels on ears of indicated age. Some mutants may have an earlier age of segregation than shown.

their normal counterparts than were the endosperms; how much earlier this timing is remains to be determined.

Phenotype of immature mutant kernels: The large group of mutants showing the defective condition at an early developmental stage are uniform in expression of mutant kernel phenotype on immature ears. Almost without exception, the mutant kernels differ from the normal kernels on the same ear in being smaller, lighter colored and more translucent in appearance (Figure 1). Normal kernels are plump and light yellow at this stage of development. The endosperm of mutant kernels in this group is generally soft and of a fluid consistency. These general characteristics common to almost all of the early-expressing mutants

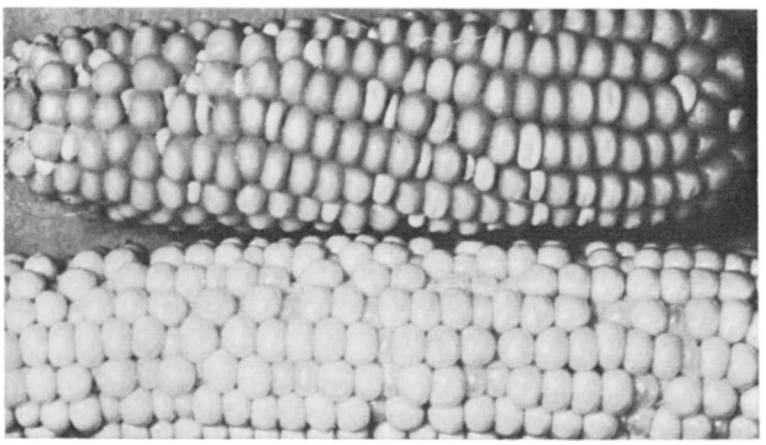


FIGURE 1.—Mature ear (upper) and immature, 16 days postpollination ear (lower) showing segregation pattern for the mutant E931A. Note that the mutant kernels on the immature ear are easily distinguished because they are smaller, lighter in color and more translucent than the normal kernels. Ears of similar age and appearance were used for culturing immature embryos in most cases.

are apparently a result of their slower development. Mutant kernels of the later-expressing type do not differ as dramatically from normal kernels in their appearance. They are closer in size and color to the normal kernels, firmer in texture, and more difficult to identify than the first group.

Some mutant kernels enlarged considerably, remained translucent and appeared swollen; when cut, these kernels show a fluid consistency and generally lack any solid endosperm material. This was termed a collapsed mature kernel phenotype. Other mutants were observed to remain smaller than the normal kernels, but they did become filled with solid endosperm material. Some of these never developed a yellow color or synthesized anthocyanin when in the appropriate genetic background and were the mutants with a colorless mature kernel phenotype. Some of these mutants developed pigmentation during kernel development and are the mutants classified as opaque, etched, or otherwise as mature kernels (NEUFFER and SHERIDAN 1980).

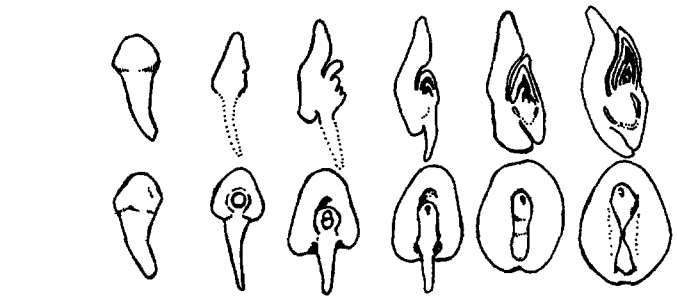
Phenotype of the immature mutant embryos: The size of the embryo was reduced by the mutant condition for most of the mutants. This is evident when the mutant and normal embryo weights from the same ear are compared (Table 2). The size reduction was more severe for the embryos than for the kernels. When the weight data for the mutant embryos (Table 2) are compared to those for the mutant kernels (data available upon request), the average embryo weight of 91 mutants was 27% that of normal, while the corresponding value for kernels of the 98 mutants that were measured was 51%. Furthermore, while over half the mutants had an embryo weight less than 20% that of normal embryos, almost half of the mutants had a kernel weight at least 50% that of normal kernels from the same ear. Since most of the kernel weight can be attributed to the endosperm, it is evident that generally the immature embryo was more severely affected than was the endosperm.

Mutant kernels could be readily located for embryo culturing, not only because the mutant kernels were generally smaller and otherwise distinctive, but also because most of the mutants had embryos about one-half to two-thirds the length of their normal counterparts (data available upon request). In the vast majority of cases, mutant embryos could be readily distinguished from normal embryos on the basis of size.

Developmental stage of immature mutant embryos: Mutant embryos were not only considerably smaller than normal embryos, but they were retarded in their rate of embryogenesis so that, generally, they lagged behind the normal embryos on the same ear by one or two developmental stages when examined at or near the time that the mutant kernels first showed the mutant phenotype. Of the mutants listed in Table 2 for which normal embryo stage is known, only two showed the same developmental stage in the mutant kernels as in the normal kernels. This result is not surprising since it was already shown that embryo development was generally more severely retarded than kernel (endosperm) development. Nevertheless, it is noteworthy that 86 of the 106 mutants listed in Table 2 had reached ABBE and STEIN's stage 1 or later and therefore possessed one or more leaf primordia by the time that the mutant kernel phenotype had become

TABLE 2

Mutant embryo developmental stage and weight expressed as a percent of normal embryo weight



Earlier or Unknown Stage	Wt	Transition Stage	Wt	Coleoptilar Stage	Wt	Stage 1	Wt	Stage 2	Wt	Stage 3	Wt	Stage 4 or later	Wt
792	14%	1309A	37%	788	4%	330D	2%	627D	26%	211C	4%	1121	75%
928A	2%	1385	1%	948A	8%	628A	--	744	6%	740	65%	1155A	43%
1092A	--	1415A	--	1162	2%	936A	22%	873	41%	874B	--	1395A	65%
1092B	18%			1399A	6%	1024A	8%	932	42%	883A	54%		
1119A	--					1045	9%	933	27%	888A	49%		
1130	--					1142A	23%	1058	2%	890A	75%		
1163	67%					1145A	108%	1060A	--	912	13%		
1167A	31%					1156A	7%	1113A	9%	923	46%		
1283	9%					1176A	6%	1239	45%	931A	32%		
1331	--					1202A	--	1255B	6%	1009	29%		
1348	--					1208A	7%	1295	12%	1054	31%		
1401	3%					1210	8%	1296A	--	1076A	61%		
1418	--					1253B	--	1299	28%	1168A	32%		
						1294	16%	1303	71%	1225A	21%		
						1311C	3%	1308A	7%	1287	8%		
						1332	8%	1313	34%	1319A	66%		
						1339A	7%	1324A	24%	1365	1%		
						1379A	3%	1333B	66%	1373A	53%		
						1380A	3%	1341	3%	1384A	26%		
						1381	1%	1369	100%	1387A	14%		
						1391	3%	1383	12%	1388	76%		
						1410	2%	1392A	21%	1405A	80%		
						1411	40%	1393A	13%	1419	12%		
						1414	3%	1394	3%	1420	55%		
						1423	4%	1404	70%	1421	--		
						1427A	5%	1406	53%	1430	90%		
						1429A	5%	1417	17%				
								1431	--				
								1435	22%				
								1436A	9%				

apparent. Clearly, then, whatever might be the nature of their defect, over 80% of these mutants underwent embryogenesis at least to the point of differentiating one or more leaf primordia.

It appears likely that most of the mutants are capable of either developing to a characteristic embryonic stage and subsequently are blocked or making only very slow progress beyond that stage during the rest of kernel maturation. Both of the above types of mutants were among the 14 defective kernel mutants studied by MANGELSDORF (1926), with some of the mutants capable of germinating and others able, at low frequency, to grow to maturity. A slowdown in embryo development was reported for two maize mutants that had a disturbed kernel development but contained viable, normal embryos—the *mn* (miniature) mutant of LOWE and NELSON (1946) and the *de-17* mutant of BRINK and COOPER (1947). With regard to mutants that result in embryo degeneration during kernel development, SASS and SPRAGUE (1950) reported two "germless" mutants that had an initial period of normal development during the first ten days post-pollination, but then the embryos began to hypertrophy and became necrotic.

The results of histological studies of immature kernels of 76 mutants (Table 2) confirmed the observations on dissected kernels of these mutants. On the basis of the combined dissection and histological observations, about 20 of the mutants are either blocked in development prior to stage 1, usually much earlier, or have begun to degenerate by the time that defective kernel segregation becomes apparent (and it is therefore possible to dissect out the mutant embryos for examination). Although segregation age is known (Table 1) for this kind of mutant, not all of them were cultured. Among this kind of mutant, a few failed to pass beyond the pro-embryo stage. A small group of mutants developed only to the transition stage, while another had only reached the coleoptilar stage at the time of collection. A few mutants produced sperhical embryos that were capable of some growth (increase in size), but lacked the capacity for differentiating either or both leaf and root primordia.

It is, nevertheless, clear that the other 130 mutants are capable of developing at least one leaf primordium (stage 1) before being developmentally blocked. Most of the mutants included in Table 2 are of this type, and 84 of them were cultured. The capacity to reach developmental stage 1 is surprising inasmuch as a majority of these mutants are not only lethal, but will not germinate even when tested as mature kernels (NEUFFER and SHERIDAN 1980).

Phenotypic responses of cultured immature mutant embryos: Mutant, immature embryos were cultured on basal and enriched media during 1977 and 1978. During 1977, embryos were also placed on *basal* medium lacking ammonium nitrate (NH_4-free basal), and during 1978 embryos were also placed on *enriched* medium lacking ammonium nitrate (NH_4-free enriched). After a culture period of about three weeks, the cultures were evaluated. Normal immature embryos under these conditions germinate precociously and grow to a height exceeding 5 cm. The cultured mutant embryos were scored as to four possible responses: no germination, 0; plumule or radicle emerged, +; small plants produced less than 5 cm high, ++; plants more than 5 cm high, +++.

A total of 102 different mutants were cultured and evaluated at least once during the two cultures seasons. Some mutants were cultured two or more times. The morphological observations of immature mutant embryos are presented in Table 2. The phenotypic growth responses of 102 mutants evaluated in 1977 and/or 1978 are shown in Table 3.

The response of the cultured embryos varied greatly among the different mutants. A consistent lack of response (0 or + score) to any medium tested was displayed by 21 of the mutants. However, many of the mutants grew well on all media.

Many mutants showed a marked growth improvement on one medium. This response included greater growth on basal medium compared with enriched medium by five mutants, as well as greater growth on enriched medium *versus* basal medium by 15 mutants. Two mutants had their greatest growth on the NH_4-free enriched medium.

A comparisons of the response scores in 1977 and 1978 to the basal and enriched media for those 56 mutants cultured both years reveals a consistent pattern (data not shown). This is true both with regard to responses to a particular medium in different years (1977 *versus* 1978) and to responses to the different media. Few mutants exhibited more than a one-step difference in scoring between years for the same medium, and almost half had identical scores on basal medium, about the same number had identical scores on enriched medium and almost one-third had identical scores for both years on both kinds of media.

The culturing of the immature mutant embryos overcame lethal blocks usually imposed during *in vivo* development and permitted germination and seedling development in many cases. Furthermore, in almost all cases, the cultured plant phenotype (Table 3) for those mutants that will germinate as mature kernels, but are lethal in the seedling stage, displayed the same seeding phenotype in culture (NEUFFER and SHERIDAN 1980).

Responses to embryo culturing—growth into plants: It is rather remarkable that about 80% of the 102 defective kernel mutants evaluated produce small or large plants with leaves and roots (a response of ++ or +++ in Table 3) upon embryo culture. Most mutant plants had green leaves; mutant embryos were therefore not only capable of growing into plants, but of growing into more-or-less normally appearing plants. Almost 60% of the 102 mutants grew into plants that reached the top of the culture tubes (a +++ response) .

A large proportion of the mutants are capable not only of forming leaf primordia, but of growing into plants, even though a large majority of the mutants are lethal under normal developmental conditions (NEUFFER and SHERIDAN

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* All data are from ears used as the source of mutant embryos for culture in either 1977 or 1978, or both years. The exceptions are five mutants, not cultured, but for which data were obtained (E1130, E1208A, E1210, E1296A and E1401).

Mutant E1330 was cultured in 1978, but is not included in this table because no data were gathered on embryos or kernels. The weight data for the embryos are mean value of five or 10 samples. The embryo stage or stages shown are for five or 10 embryos examined in each case. See ABBE and STEIN, (1954) from which the stages were redrawn, for illustration and detailed description of the embryonic stages.

TABLE 3

*Phenotypic growth response of cultured immature mutant embryos**

No growth on any medium (all lethal†)	Better growth on basal medium		Equal growth on both media (++)		Equal growth on both media (+++)			
Mutant E. No.	Mutant E. No.	Phenotype of cultured plants	Mutant E. No.	Phenotype of cultured plants	Mutant E. No.	Phenotype of cultured plants	Mutant E. No.	Phenotype of cultured plants
330D	1294	gr-sml(L)	744	pg-gs(L)	211C	w-gs(gs)	1202A	n(L)
788	1332	w-sml(L)	1045	l(l)	627D	n(L)	1225A	w(L)
792	1384A	grl-nl(V)	1092B	gr-stb(V)	740	n(L)	1239	gr-nec(L)
928A	1414	n(L)	1156A	gr-sml(?)	874B	w-gs(w)	1283	n(L)
1058	1423	n(L)	1295	v(L)	883A	n(V)	1287	pg(L)
1092A	Better growth on		1333B	gr-thd(L)	888A	gr-sml(V)	1303	gr callus(L)
1113A	enriched medium		1341	gr(V)	890A	n(V)	1313	n(L)
1119A	628A	sml-thin(L)	1380A	gr(L)	912	w-gs(gs)	1319A	n(d)
1253B	873	gr-nl-crl(L)	1381	gr-thin-crl(L)	923	str(V)	1324A	w-pg str(L)
1309A	932	gr(L)	1391	gr-nl(L)	931A	gr-nl(L)	1330	w(L)
1311C	1024A	gr-nl(L)	1394	pg-sml(L)	933	n(L)	1331	n(L)
1348	1054	gr-stb(L)	1406	sml-nl(L)	936A	n(L)	1369	n(nl)
1365	1060A	gr-nl(L)	1415A	w(L)	948A	pyg-nl(l)	1373A	n(L)
1379A	1121	gs-stb(L)	1419	gr-d(L)	1009	n(V)	1383	gr-fl base(V)

TABLE 3—Continued

No growth on any medium (all lethal†)	Better growth on basal medium		Equal growth on both media (++)		Equal growth on both media (+++)			
Mutant E. No.	Mutant E. No.	Phenotype of cultured plants	Mutant E. No.	Phenotype of cultured plants	Mutant E. No.	Phenotype of cultured plants	Mutant E. No.	Phenotype of cultured plants
1385	1145A	pg-nl(L)	1427A	gr(L)	1076A	basal ws(L)	1387A	gr-nl(V)
1393A	1162	gr-thin(L)	1429A	nec-msh(L)	1142A	n(L)	1388	n(L)
1399A	1255B	gr-nl(L)			1155A	gr(L)	1395A	n(L)
1410	1299	gr(L)			1163	n(L)	1404	n(L)
1418	1308A	gr-nl-trl(L)			1167A	n(L)	1405A	n(L)
1435	1339A	pg(w)			1168A	gr(L)	1417	gr(L)
1436A	1392A	gr(L)			1176A	gr-crl-stb(L)	1420	n(L)
	1411	w(L)					1431	n(d)
	1421	w-nl(L)						
	1430	gr-nl(L)						

* The cultured mutant embryos were scored for four possible responses: (1) embryos sometimes enlarge but no germination, o; (2) embryos enlarged and the plumule or radicle emerged, +; (3) embryos grew into small plants less than 5 cm high, ++; (4) embryos grew into plants more than 5 cm high, +++. The symbol shown for any particular treatment indicates the response of the majority of embryos. Better growth on a particular medium was shown by a growth response score one step (or more) higher than on the other media, *i.e.*, +++ on enriched *versus* ++ on basal indicated better growth on enriched medium. The meanings of the symbols used to describe the phenotype of the plants produced by cultured embryos are: crl—curled leaf; d—dwarf, short because of shortened internodes; fl-base—fleshy base of leaves; gr—green; gs—green striped; l—luteus yellow seedling; msh—multiple shoots; n—normal; nec—necrotic, affected tissue dies; nl—narrow leaf; pg—pale green; pyg—pale yellow green; sml—small plant; stb—stubby shoot with reduced leaves; str—striped; thin—thin leaf; thd—thread-like leaf; trl—translucent; w—white; ws—white stripe, leaf blade is white except along the vascular bundles; v—virescent seedling. The symbol in parentheses, *i.e.*, (L), following the cultured plant phenotype symbols indicates the mature kernel viability or, if the mature kernel germinates, the seedling phenotype. Their meanings are: L—inviable; V—growth into normal green seedling; see above for other symbols.

† All of these mutants are lethal because of inviability except for E1119A, which dies in the early seedling stage. All mutants scored an o or + response in culture.

1980). A number of causes may underlie these results, but the loss of viability in many instances must occur during the later stages of kernel maturation.

Shoot growth responses of cultured immature mutant embryos: Fresh shoot weight of immature mutant embryos when cultured on the 1977 and 1978 media was measured. The mutants varied considerably in their growth response. However, 80 of the 102 mutants cultured produced shoots that were harvested and weighed during one or both of these years. Since a comparison of growth response on the different media, especially basal *versus* enriched, was of particular interest, the results of these culture studies are expressed as a percentage. This was calculated by dividing the weight of fresh shoots obtained on basal medium into the weight of fresh shoots obtained on enriched medium, or the other media tested. The results of these tests are shown in Table 4.

In considering the culturing of any particular mutant, the response of the immature mutant embryos to culturing varied among the 10 or 15 embryos placed on each culture medium. Often, one or more embryos would fail to germinate, while the remainder would growth into plants. Also, in some cases, one or more of the embryos would produce only roots, while the other embryos of the set would produce the entire plants. For some cultures, contamination by fungi or bacteria reduced the number of cultures that could be evaluated.

A major purpose of the culture experiments was to screen for nutritionally deficient mutants. In comparing the results obtained with enriched medium with those obtained with basal medium, we have arbitrarily assumed that growth on enriched must exceed 150% of that on basal to be significantly greater. On this basis, 19 mutants grew to a greater extent on enriched medium than on basal medium in 1977 and/or 1978; three mutants had their greatest growth on NH_4-free enriched medium, 16 had greater growth on basal medium than on enriched medium (a growth response on enriched of less than 67% that on basal medium), and five mutants grew to the greatest extent on NH_4-free basal medium (Table 4).

The mutants that grow on basal medium, but not on enriched medium, are presumably inhibited by one of the organic media additives. Since normal embryos grow satisfactorily on this enriched medium, the levels of organic additives present should not be toxic. The mutant embryos are apparently abnormally sensitive to growth inhibition by them. Because of the changed sensitivity to one or more of these substances in the mutants, their presence in the mutant kernels may inhibit and ultimately result in the death of the embryonic tissues.

The mutants that preferred NH_4-free media may be sensitive to and inhibited by the presence of ammonium ion, not only in culture medium, but also *in vivo*, and the normal ammonium ion concentration in the developing kernel may be excessively high for the development, growth and survival of such mutants. The positive response of such mutants to NH_4-free basal medium is somewhat puzzling since the only nitrogen source in this medium is nitrate ion, which must be converted to ammonium ion before it can be utilized by the cultured embryo. Of course, the rate of conversion might be such as to maintain a sufficiently low level of ammonium ion in the embryonic cells that a toxic level would not be reached.

TABLE 4

*Shoot growth of cultured immature mutant embryos expressed as percent of growth on basal medium**

NH_4-free basal medium	Enriched medium, 1977	Enriched medium, 1978	NH_4-free enriched medium
0–33%	0–33%	0–33%	0–33%
932(04/05), 1176A(06/05), 1239(08/08), 1339A(09/02), 1381(08/06), 1430(06/06),	936A(06/09), 1045(07/09), 1167A(08/05), 1332(03/02), 1387A(09/07), 1427A(01/01), 1431(07/09)	888A(14/13), 1225A(05/07), 1253B(01/02), 1423(07/10),	740(07/08), 1045(04/03), 1145A(05/06), 1168A(06/05), 1176A(05/04), 1287(07/05), 1380A(14/09), 1384A(05/07), 1391(10/15), 1423(04/10), 1436A(10/10)
34–67%	34–67%	34–67%	34–67%
740(07/09), 744(09/05), 874A(03/08), 912(07/09), 933(08/08), 936A(08/09), 1045(06/09), 1121(10/10), 1162(06/02), 1168A(04/09), 1294(05/03), 1313(07/08), 1324A(06/07), 1387A(06/07), 1391(06/06), 1420(04/04), 1423(05/09), 1427A(02/01), 1429A(05/06), 1431(07/09), 1435(09/04), 1436A(04/04)	627D(07/03), 890A(10/07), 923(06/06), 931A(09/10), 933(06/08), 1156A(06/09), 1176A(09/06), 1239(08/08), 1294(04/03), 1313(06/08), 1324A(07/07), 1369(09/07), 1383(05/08), 1395A(04/07), 1420(03/04), 1423(05/10), 1429A(04/09)	211C(10/09), 740(08/08), 936A(09/08), 1176A(10/04), 1287(08/05), 1299(14/03), 1303(05/03), 1319A(07/09), 1333B(15/14), 1384A(08/07), 1395A(06/10), 1417(07/06)	211C(10/09), 744(12/15), 888A(03/13), 912(13/14), 936A(09/08), 948A(10/11), 1054(13/15), 1225A(05/07), 1253B(02/02), 1295(12/17), 1299(08/03), 1303(08/03), 1369(15/09), 1387A(15/15), 1395A(08/10), 1406(07/02), 1410(05/03), 1411(13/14), 1414(13/14), 1417(06/06), 1431(15/15)
68–99%	68–99%	68–99%	68–99%
931A(10/10), 1142A(08/07), 1163(10/08), 1167A(01/05), 1283(07/08), 1303(09/10), 1331(06/07), 1395A(06/07), 1411(09/08), 1417(07/07),	740(09/09), 1009(03/05), 1142A(06/07), 1162(04/02), 1163(08/08), 1168A(07/09), 1283(07/08), 1391(06/06), 1406(07/07), 1415A(06/07), 1436A(04/04)	627D(14/12), 744(13/15), 874B(14/11), 923(06/06), 1145A(08/06), 1239(10/06), 1283(02/06), 1295(11/17), 1330(10/08), 1381(06/09), 1410(05/03), 1411(14/14), 1414(11/14), 1415A(04/02), 1419(04/11), 1429A(03/08),	874B(15/11), 890A(15/14), 923(06/06), 1239(10/06), 1324A(09/09), 1330(06/08), 1373A(15/15), 1381(08/09), 1405A(10/10), 1419(03/11)
		100–149%	
		890A(14/14), 912(14/14), 931A(07/09), 1168A(06/05), 1294(14/12), 1324A(10/09), 1331(12/11), 1339A(13/15), 1369(15/09), 1380A(12/09),	

TABLE 4—Continued

NH_4-free basal medium	Enriched medium, 1977	Enriched medium, 1978	NH_4-free enriched medium
100–149%	100–149%	100–149%	100–149%
883A(09/09), 890A(09/07), 923(05/06), 1009(04/05). 1054(04/08), 1332(01/02), 1373A(09/10), 1383(02/08), 1392A(03/01)	883A(08/09), 888A(05/08), 912(04/05), 932(08/05). 1303(09/10), 1373A(08/10), 1381(07/06), 1392A(02/01), 1394(01/04), 1421(04/06), 1435(06/04)	1383(14/14), 1387A(14/15), 1388(10/10), 1391(07/15), 1404(07/08)	627D(13/12), 873(10/10), 1294(10/12), 1331(12/11), 1333B(15/14), 1383(15/14), 1415A(03/02), 1429A(06/08), 1430(05/07)
150% and up	150% and up	150% and up	150% and up
627D(07/03), 873(05/06), 888A(07/08), 1156A(06/09), 1202A(03/07), 1255B(07/06), 1287(06/04), 1369(07/07), 1394(03/04), 1406(03/06), 1415A(07/07), 1421(04/06)	744(05/05), 873(08/06), 1054(02/08), 1121(05/10), 1202A(07/07), 1255B(08/06), 1331(04/07), 1411(07/08), 1417(07/07), 1430(04/06)	873(10/10), 948A(08/11), 1024A(10/12), 1045(05/03), 1054(14/15), 1121(09/10), 1162(10/06), 1255B(06/06), 1308A(15/14), 1373A(15/15), 1392A(10/10), 1405A(10/10), 1406(07/02), 1430(14/07), 1431(15/15)	931A(05/09), 1024A(14/12), 1121(09/10), 1162(06/06), 1255B(08/06), 1308A(14/14), 1319A(08/09), 1392A(12/10), 1404(07/08)

* The number of plants harvested and used to determine the fresh weight of shoots is shown in parentheses next to the E number of each mutant, with the number to the right of the diagonal line indicating the number for basal medium and the number to the left of the slash indicating the number from the other medium tested (enriched, NH_4-free enriched, or NH_4-free basal).

These cultures were the same as those used to evaluate the phenotypic responses presented in Table 3. Generally, the length of culture period in 1977 was about three weeks, but in cases of contamination of the cultures the culture period was sometimes curtained; in some cases of slow growing mutants, the culture period was lengthened. All of the cultures in 1978 were harvested after 21 or 22 days. Some of the mutants that were cultured and are listed in Table 3 are not included in this table because they failed to produce shoots on both basal and one other kind of medium.

The search for auxotrophs: A more detailed analysis of their shoot growth response indicates that 10 mutants are especially promising in our search for nutritionally deficient or auxotrophic mutants. They are those lethal mutants with superior growth on enriched media (Table 4) that also had a mean shoot growth value in excess of 100 mg on this medium (data not shown). Growth of this extent is comparable to that of normal immature embryos on this medium. All of these mutants produced green shoots, and at least seven embryos were evaluated on each kind of medium for each mutant. Nine of these mutants (E873, E1054, E1121, E1202A, E1373A, E1392A, E1405A, E1417 and E1430) had superior growth on the complete enriched medium and the 10th (E1404) grew best on the NH_4-free enriched medium. The interest in this type of conditional mutant in flowering plants had received attention and discussion (NELSON and BURR 1973; REDEI 1975; NELSON 1978; NEUFFER 1978), but, with one exception, the only nonleaky and well-characterized auxotrophic mutants in flowering plants are thiamine-requiring mutants.

The exception to the above statement is the proline-requiring mutant of maize (*pro-1*) reported by GAVAZZI, NAVA-RACHI and TONELLI (1975). This single gene, recessive mutant has an absolute requirement for proline when rescued by embryo culturing of mature embryos. Mutant kernels when planted produce a seedling that always dies in the one- or two-leaf stage (GAVAZZI, NAVA-RACHI and TONELLI 1975; RACCHI *et al.* 1978).

One of the EMS-induced mutants included in this study and an additional spontaneously occurring mutant have been tested for allelism. The results of these tests demonstrate that both of the mutants are proline-requiring mutants allelic to the *pro-1* mutant. These two mutants are E1121 and *pro-341*, isolated by E. H. COE. When heterozygous plants of these two mutants were crossed with pollen from heterozygous *pro-1* plants, ears were obtained bearing a 3:1 ratio of normal to mutant kernels. The same results were obtained when heterozygous plants of the two new mutants were crossed.

Immature mutant embryos of *pro-1*, E1121 and *pro-342* that were 27-, 22- and 25-days-old, respectively, were cultured for 21 days. The mean fresh shoot weight on basal medium was 265, 265 and 298 mg, respectively; on basal medium supplemented with a two-fold concentration of the amino acid supplement, their weights were 467, 412 and (no data) mg, respectively; and on basal medium supplemented with 3 millimoles of proline, their weights were 594, 419 and 811 mg, respectively. Furthermore, the supplementing of basal medium with 3 millimoles of ornithine, a proline precursor (MESTICHELLI, GUPTA and SPENSER 1979), had no beneficial growth effect. These results show that the screening techniques employed will detect auxotrophic mutants.

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LITERATURE CITED

ABBE, E. E. and O. L. STEIN, 1954 The growth of the shoot apex in maize: embryogeny. Am. J. Bot. **41**: 285–293.

BRINK, R. A. and D. C. COOPER, 1947 Effect of the de17 allele on development of the maize caryopsis. Genetics **32**: 350–368.

GAVAZZI, G., M. NAVA-RACHI and C. TONELLI, 1975 A mutation causing proline requirement in maize. J. Theor. Appl. Genet. **46**: 339–346.

JONES, D. F., 1920 Heritable characters of maize. IV. A lethal factor—defective seed. J. Heredity **11**: 161–167.

LOWE, J. and O. E. NELSON, JR., 1946 Miniature seed—a study in the development of a defective caryopsis in maize. Genetics **31**: 525–533.

MANGELSDORF, P. C., 1923 The inheritance of defective seeds in maize. J. Heredity **14**: 119–125.

——, 1926 The genetics and morphology of some endosperm characters in maize. Conn. Agr. Exp. Sta. Bull. **279**: 509–614.

MESTICHELLI, L. J. J., R. N. GUPTA and I. D. SPENSER, 1979 The biosynthetic route from ornithine to proline. J. Biol. Chem. **254**: 640–647.

MURASHIGE, T. and F. SKOOG, 1962 A revised medium for rapid growth and biosassays with tobacco tissue cultures. Physiol. Plant. **15**: 473–497.

NELSON, O. E., 1978 Gene action and endosperm development in maize, pp. 389–403. In: *Genetics and Breeding of Maize*. Edited by D. B. WALDEN. Wiley-Interscience, New York.

NELSON, O. E. and B. BURR, 1973 Biochemical genetics of higher plants. Ann. Rev. Plant Physiol. **24**: 493–518.

NEUFFER, M. G., 1978 Induction of genetic variability. pp. 579–600. In: *Genetics and Breeding of Maize*. Edited by D. B. WALDEN. Wiley-Interscience, New York.

NEUFFER, M. G. and W. F. SHERIDAN, 1980 Defective kernel mutants of maize. I. Genetic and lethality studies. Genetics **95**: 929–944.

RACCHI, J. L., G. GAVAZZI, D. MONTI and P. MANNITO, 1978 An analysis of the nutritional requirements of the *pro* mutant in *Zea mays*. Plant Science Letters, **13**: 357–364.

REDEI, G. P., 1975 Induction of auxotrophic mutations in plants. pp. 325–350. In: *Genetic Manipulations with Plant Material*. Edited by L. LEDOUX. Plenum Press, New York.

SASS, J. E., 1968 *Botanical Microtechnique*. Iowa State College Press. Ames.

SASS, J. E. and G. F. SPRAGUE, 1950 The embryology of "germless" maize. Iowa State College. J. Sci. **24**: 209–218.

TSAI, C. Y., 1979 Early termination of zein accumulation in *opaque-2* maize mutants. Maydica **27**: 129–140.

WILSON, C. M., 1978 Some biochemical indicators of genetic and developmental controls in endosperm. pp. 405–419. In: *Genetics and Breeding of Maize*. Edited by D. B. WALDEN. Wiley-Interscience, New York.

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