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Morphology and ultrastructure of 11 barley shrunken endosperm mutants

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Summary. Eleven Na-azide induced barley shrunken endosperm mutants expressing xenia (*sex*) were characterized genetically and histologically. All mutants have reduced kernel size with kernel weights ranging from 11 to 57% of the wild type. With one exception, the mutant phenotypes are ascribable to single recessive mutant alleles, giving rise to a ratio of 3:1 of normal and shrunken kernels on heterozygous plants. One mutant (B10), also monofactorially inherited, shows a gene dosage dependent pattern of expression in the endosperm. Among the 8 mutants tested for allelism, no allelic mutant genes were discovered. By means of translocation mapping, the mutant gene of B10 was localized to the short arm of chromosome 7, and that of B9 to the short arm of chromosome 1. Based on microscopy studies, the mutant kernel phenotypes fall into three classes, viz. mutants with both endosperm and embryo affected and with a non-viable embryo, mutants with both endosperm and embryo affected and with a viable embryo giving rise to plants with a clearly mutant phenotype, and finally mutants with only the endosperm affected and with a normal embryo giving rise to plants with normal phenotype. The mutant collection covers mutations in genes participating in all of the developmental phases of the endosperm, i.e. the passage from syncytial to the cellular endosperm, total lack of aleurone cell formation and disturbance in the pattern of aleurone cell formation. In the starchy endosperm, varying degrees of cell differentiation occur, ranging from slight deviations from wild type to complete loss of starchy endosperm traits. In the embryo, blocks in the major developmental phases are represented in the mutant collection, including arrest at the proembryo stage, continued cell divisions but no differentiation, and embryos deviating only slightly from the wild type.

Key words: Barley – Grain development – Mutants – Ultrastructure – Genetics

Introduction

In plants, many of the mutations reported to alter differentiation patterns also affect the developing seed, for example, in maize (Mangelsdorf 1923, 1926; Brink and Cooper 1947; Lowe and Nelson 1947; Sass and Sprague 1950; Manzocchi et al. 1980a, b; Neuffer and Sheridan 1980; Sheridan and Neuffer 1980, 1982), *Arabidopsis* (Meinke and Sussex 1979a, b) and barley (Jarvi and Eslick 1975; Ramage and Crandall 1981a, b, c; Felker et al. 1985). In cereal grains, two organs provide excellent possibilities for studying the genetic control of development, namely the endosperm, consisting of aleurone and starchy endosperm cells, and the embryo. Both organs pass through morphologically distinct stages. The control of kernel formation is expected to involve a high number of gene loci, housekeeping as well as tissue specific. Recently, Neuffer and Sheridan (1980) induced defective maize kernel mutants in an estimated 285 gene loci distributed on 17 of the 18 chromosome arms tested. Among the mutants that were affected in both grain tissues, some were lethal (Type 1) and some were viable, but showed a mutant plant phenotype (Type 2). In addition, some mutants were affected only in the endosperm (Type 3) or in the embryo (Type 4).

In barley, shrunken kernels may be caused by mutations in single genes expressed either maternally, i.e. in the vegetative plant tissues (*seg*: shrunken endosperm caused by the maternal genotype, Jarvi and Eslick 1975; Ramage and Scheuring 1976; Ramage and

Crandall 1981c), or in the kernel itself (*dex*: defective endosperm expressing xenia, Ramage and Crandall 1981a, b; *sex*: shrunken endosperm expressing xenia, Eslick and Hockett 1976). In crosses, the two groups can be distinguished by the occurrence on heterozygous *seg*-plants of exclusively normal kernels, whereas on heterozygous *dex*- or *sex*-plants 25% of the kernels are shrunken. In a histological study of kernels from *seg*-mutants, Felker and co-workers (1985) revealed two types of mutants, those exhibiting a premature termination of grain filling caused by the death of tissue of maternal origin, and those exhibiting characteristic abnormalities in the endosperm growth pattern but with a normal development of maternal-origin tissues. To our knowledge, similar studies on the histology of *dex*- or *sex*-mutants has not been published.

The aim of the present communication is to describe histological and ultrastructural variation in the endosperm and embryo of 11 Na-azide induced *sex*-mutants of barley. In addition, a histological comparison is made between our mutants and 12 of the *dex*-mutants of Ramage and Crandall (1981b). Our motivation for isolating a new set of barley shrunken kernel mutants is to limit background variation by mutant induction in a well characterized barley cultivar. Hopefully, in combination with the molecular techniques currently in use, such mutants may become useful tools in the identification and characterization of genes determining plant development.

Materials and methods

Mutant induction and isolation

After soaking at 4 °C for 17 h, kernels of *Hordeum vulgare* L. var. 'distichum', cv. 'Bomi', were treated for 2 h in solutions containing 1.0, 0.5, 0.25 or 0.1×10^{-3} M Na-azide, respectively, mainly following the procedure of Nilan et al. (1975). At the highest concentration, the distilled water as well as the Na-azide solution was saturated with oxygen. In the treatments with 0.5 and 0.25×10^{-3} M Na-azide, air was used instead of oxygen. Potential kernel developmental mutants were selected as M_2-spikes containing 25% or more shrunken kernels. Mutant B (Blindern) 1, B2 and B3 were isolated from the treatment with 1.0×10^{-3} M, B7–B16 from the 0.5×10^{-3} M treatment and B17 in the experiment with 0.25×10^{-3} M Na-azide. No mutants were found in the control series.

To assess the mutagenic effect of the Na-azide treatments, 100–300 M_2-families not segregating for shrunken kernels were randomly selected and checked for chlorophyll mutants. The percentages of M_2-families segregating for chlorophyll defects were 20.1, 9.7 and 3.9 at 1×10^{-3}, 0.5×10^{-3} and 0.1×10^{-3} M Na-azide, respectively. The corresponding germination percentages of the same treatments were 19, 26 and 41, respectively. Grains from heterozygous *dex*-plants were received from Dr. Ramage.

Germination tests

Germination tests were carried out at room temperature on moist filter paper after surface sterilization of the kernels in 70% ethanol for 5 min.

Segregation analysis, allelism testing and gene mapping

The weights of normal and shrunken M_3-grains from heterozygous plants were determined after air drying at room temperature for 2 months. Segregation ratios between normal and shrunken M_3-grains were registered on spikes from 5–8 heterozygous M_2-plants. Allelism testing of 8 mutants was performed by grouping the mutants into three classes based on the degree of grain filling viz. B2, B10 and B17; B7, B9 and B13; and B15, B16. Within these groups, crosses were carried out between heterozygous plants since most of the mutants are lethal (Table 1). In addition, for the viable mutants B1, B2 and B11, homozygous recessive plants were crossed in all three possible combinations. Mutant genes were considered to be non-allelic if only normal kernels were present in the F_1-grain generation. All crosses were progeny tested in the F_2-grain generation to confirm the results from the F_1-generation.

To locate the mutant genes of B9, B10 and B13 for their chromosomal position, mono-heterozygous plants for each mutant gene were crossed to a set of 13 homozygous reciprocal translocation lines (Ramage 1971; Linde-Laursen 1984). Normal F_2-grains from selfed semisterile F_1-plants were then sown in numbers ranging from 40 to 230. After selfing, the F_2-plants were classified into one of the following classes: fully fertile and normal grains (FAA), fully fertile with shrunken and normal grains (FAa), semisterile and normal grains (SAA) and semisterile with normal and shrunken grains (SAa). In the case of free recombinations between the translocation breakpoints (FS) and the segregating mutant gene (Aa), the theoretical distribution between the four classes is 1:2:1:2, respectively. If complete linkage exists, the corresponding distribution is 1:0:0:2.

Light and electron microscopy

Fixation, embedding, sectioning and staining were performed as described by Olsen and Krekling (1980).

Scanning electron microscopy

Kernels were prepared for scanning electron microscopy as described in Klemsdal et al. (1986).

Results

Genetic analysis

Based on the system for phenotypic classification of maize defective kernel mutants (Neuffer and Sheridan 1980), our barley mutants B7, B9, B10, B15, B16 and B17 are of Type 1 (Table 1). In these, the phenotype of both the endosperm and the embryo deviates from wild type 'Bomi' and the embryo is non-viable. Mutant kernel dry weight varies from 11 to 44% of the wild type (Table 1). Type 2 mutants include B8 and B13, in which both endosperm and embryo are affected and the viable embryo gives rise to a mutant plant phenotype. In B8, all mutant embryos that germinated were albino, dying at an early stage. B13 mutant plants

Table 1. Classification of mutants, dry weight of homozygous recessive mutant grains (% of normal) and M_3-segregation ratios for grain filling (normal: shrunken). Type 1 – endosperm and embryo affected, embryo non-viable; Type 2 – endosperm and embryo affected, embryo viable, plant phenotype clearly mutant; Type 3 – endosperm affected, mutant plant phenotype normal

Type	Mutant	Grain wt	Segregation ratio
1	B7	11 ± 2	3:1
	B15	12 ± 1	3:1
	B16	16 ± 1	2.2:1[a]
	B9	21 ± 2	3:1
	B10	38 ± 4 22 ± 2	3.2:1[b]
	B17	44 ± 2	3:1
2	B8	21 ± 2	3:1
	B13	54 ± 3	3:1
3	B1	36 ± 2	3:1
	B2	40 ± 4	3:1
	B11	57 ± 4	3:1

[a] Significant deviation from 3:1 and 9:7
[b] Not a simple recessive mendelian inheritance. Explanation in the text

Table 2. Allelic relationship between mutant loci

Mutant	Non-allelic to
B1	B11, B2
B2	B10, B11, B17
B7	B9, B13
B9	B13
B15	B16

are fertile dwarfs. Grain dry weight in B8 and B13 is 21 and 54% of the wild type, respectively (Table 1). In the Type 3 mutants B1, B2 and B11, the homozygous recessive endosperm is shrunken, and plants from the corresponding grains normal. Grain dry weight is 36, 40, and 57% of normal, respectively, for B1, B2 and B11. In all mutants except B16, normal and shrunken M_3-grains segregate into a ratio of 3:1, indicating the involvement of a single recessive gene mutation (Table 1).

For B10, although segregating 3:1, heterozygous M_3-plants were obtained from one-half of the normal M_3-grains ($P>20\%$), indicating deviation from a simple recessive mendelian pattern of inheritance. Among the shrunken M_3-grains, two size classes, weighing 38 and 22% of normal kernels (Table 1), were present in the ratio of 1.4:1. Most of the heaviest shrunken kernels germinated, all giving rise to plants segregating for grain filling. None of the lightest shrunken grains germinated. For mutant B16, the observed 2.2:1 ratio between normal and shrunken grains deviates significantly both from a 3:1 ($P<1\%$) and a 9:7 ($P \ll 0.1\%$) ratio. Heterozygous spikes of this mutant contain a high frequency of sterile flowers.

In the allelism tests, including 10 of the 45 possible crosses between the 8 mutants involved, no allelic mutant genes were discovered (Table 2).

In the crosses aimed at mapping the mutant genes of B9, B10 and B13, the locus of B9 was found to be tightly linked to one of the translocation breakpoints of S33 (T2–7b) (FAA:FAa:SAA:SAa = 31:0:0:52), placing the B9-locus either on the distal part of the short arm of chromosome 1 or on the distal part of the long arm of chromosome 6. In the only other successful cross involving these two chromosome arms, S28 × B9 (S28; short arm of chromosome 4, centromere region of chromosome 6), no linkage is indicated. Thus, unless the two translocation breakpoints on the short arm of chromosome 6 are unlinked, the mutant gene of B9 is on chromosome 1. In the crosses involving B10, tight linkage was confirmed with all stocks involving the short arm of chromosome 7, i.e. S24, S26, S29 and S32. In the cross B10 × S32, the distribution between the four F_2-classes FAA:FAa:SAA:SAa was 107:0:3:242, respectively. In the cross involving S29, which has one of its translocation breakpoints in the satellite region of chromosome 7, the distribution between FAA:FAa:SAA:SAa were 76:29:10:23, respectively.

In the crosses with B13, no conclusive evidence for linkage was obtained.

Microscopy

Wild type 'Bomi'.

As a basis for the description of the mutants, sections of endosperm and embryo from wild type 'Bomi' grains at different stages of development are shown in Fig. 1. The endosperm develops from the triploid primary endosperm cell of the embryo sac by several nuclear divisions, giving rise to a syncytium lining the embryo sac 2–3 days after anthesis (Fig. 1 A, B, C). By day 9 endosperm cells fill up the lumen of the embryo sac (Fig. 1 E, F). At this stage aleurone cells can be distinguished from the starchy endosperm cells. Starch granules are observable in the starchy endosperm cells. In the starchy endosperm, cell division ceases around day 14 and in the aleurone at day 21 (Kvaale and Olsen 1986). Cell filling continues until maturity (Fig. 1 G).

The barley embryo develops from the diploid zygote by several cell divisions, first giving rise to an undifferentiated proembryo (Merry 1941) (Fig. 1 B, D). At day 9 after anthesis, differentiation has begun, and the parts giving rise to the scutellum, coleoptile and stem meristem can be distinguished (Fig. 1 F). In the

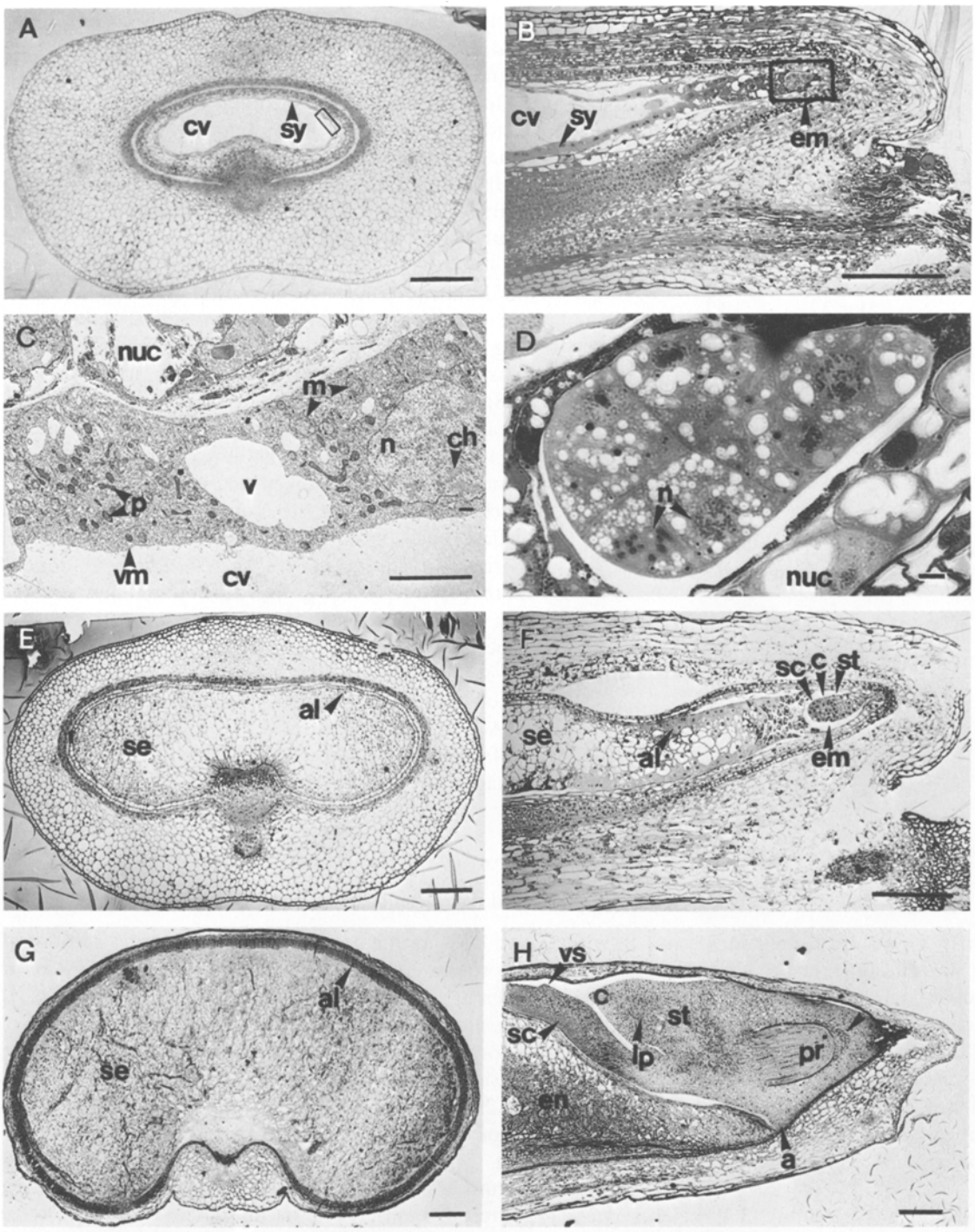

Fig. 1. Light micrographs (except **C** = transmission electron micrograph) of transverse sections of endosperm (**A, C, E, G**) and longitudinal sections of embryo (**B, D, F, H**) from wild type 'Bomi' grains, showing 3-day-old endosperm (**A**) and embryo (**B**) (**C** and **D** are details of frames in **A** and **B**, respectively), 9-day-old endosperm (**E**) and embryo (**F**) and 25-day-old endosperm (**G**) and embryo (**H**). Bar in **A, B, E, F,** and **H** represents 0.25 mm and in **C** and **D** 5 μm. a = angle, al = aleurone cells, c = coleoptile, ch = chromatin, cr = coleorhiza, cv = central vacuole, en = endosperm, em = embryo, lp = leaf primordia, m = mitochondrion, n = nucleus, nuc = nucellus, p = plastid, pr = primary root, sc = scutellum, se = starchy endosperm cells, st = stem meristem, sy = syncytium, v = vacuole, vm = vacuole membrane, vs = ventral shield

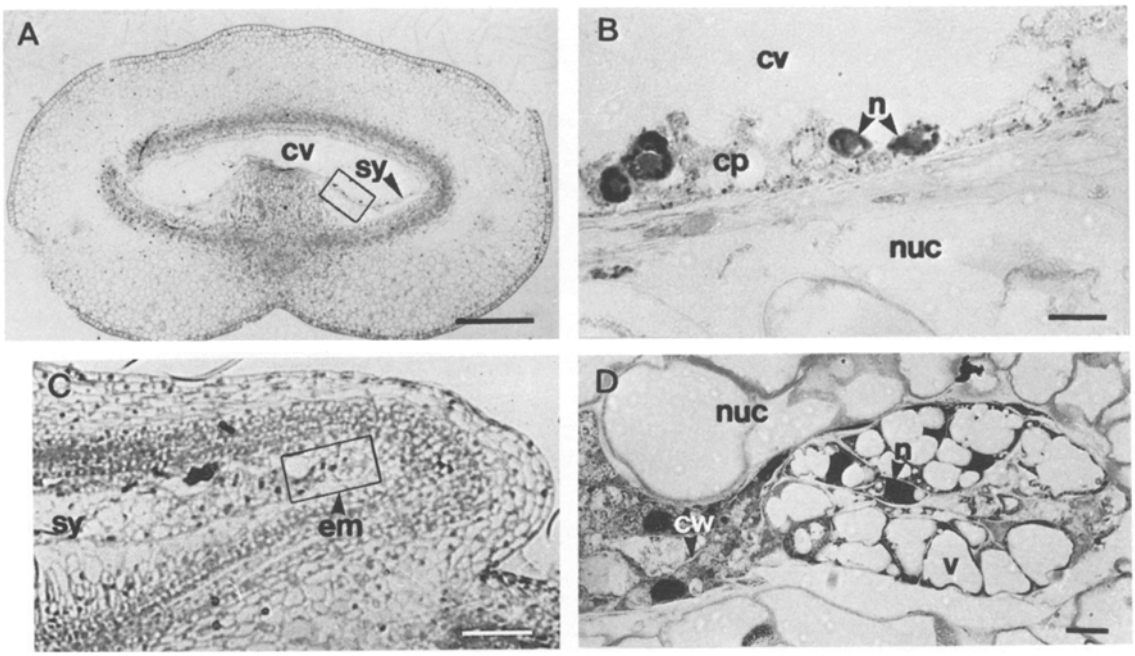

Fig. 2. Light micrographs of sections from 15-day-old homozygous mutant B15 (Type 1) endosperm (**A** and **B**) and embryo (**C** and **D**) showing endosperm and embryo arrested at an early developmental stage. **A** Transverse section of ovule. **B** Frame of **A** enlarged showing the nuclei of the endosperm syncytium. **C** Longitudinal section of the proximal part of the grain containing the embryo (frame). **D** Frame of **C** enlarged; embryonic cells. Bar in **A** represents 0.25 mm, in **B** and **D** 10 μm and in **C** 0.1 mm. cp = cytoplasm, cw = cell wall, otherwise as in Fig. 1

period between 9 and 25 days, the coleoptile covers the stem meristem and leaf primordia, and root primordia develop. The ventral shield on the scutellum and the characteristic angle between the scutellum and the coleoptile become apparent (Fig. 1H). Growth and development of more leaf and root primordia continues until maturity.

Type 1 mutants

B7, B15 and B16. Of the Type 1 mutants, B7, B15 and B16 are all arrested at the syncytial stage of endosperm development (Fig. 2A, B), leading to low grain weights (Table 1). Due to the similar phenotypes of these three mutants, only sections from 15-day-old B15 grains are shown. No cell walls are observable in the central part of the embryo sac, but some undifferentiated cells can occasionally be seen in the proximal part, close to the embryo (cw in Fig. 2D). As in the endosperm, embryo development stops prematurely, at a stage corresponding approximately to the proembryo of wild type embryos (Fig. 2C, D). For comparison to the wild type, see Fig. 1A–D. The cells of the mutant embryos are large, vacuolized and contain large nuclei with dense chromatin (Fig. 2D). Homozygous mutant grains are recognizable in heterozygous spikes 5 days after anthesis.

B9. In the endosperm of 25-day-old mutant grains, the cell content varies considerably in the different parts, ranging from only a few cell layers of undifferentiated cells (Fig. 3A,B) to a large number of starch containing cells (Fig. 3C). Since aleurone cells have not been detected in homozygous B9 endosperm at any developmental stage, development seems to be arrested before 9 days after anthesis (compare Fig. 1E, F).

The embryo of the 25-day-old homozygous B9 grain (Fig. 3D, E) resembles a wild type proembryo, consisting of a few undifferentiated, vacuolized cells (Fig. 1D).

In spikes from plants that are heterozygous for the B9 allele, homozygous B9 kernels can be detected 12 days after anthesis. To investigate the effects of this mutant locus on the earliest stages of endosperm development, 41 randomly chosen ovules from heterozygous plants were harvested 3 days after anthesis. Studies of transverse sections of the ovules under the light microscope revealed two phenotypes, one with a syncytium similar to that of the wild type grains (Fig. 1A), the other with a more condensed cytoplasm, particularly on the border facing the central cavity (Fig. 4A). The two types occurred in a ratio of 31 to 10, strongly indicating that the condensed type represents homozygous B9 grains. The impression of a more condensed cytoplasm in B9 given by the light micro-

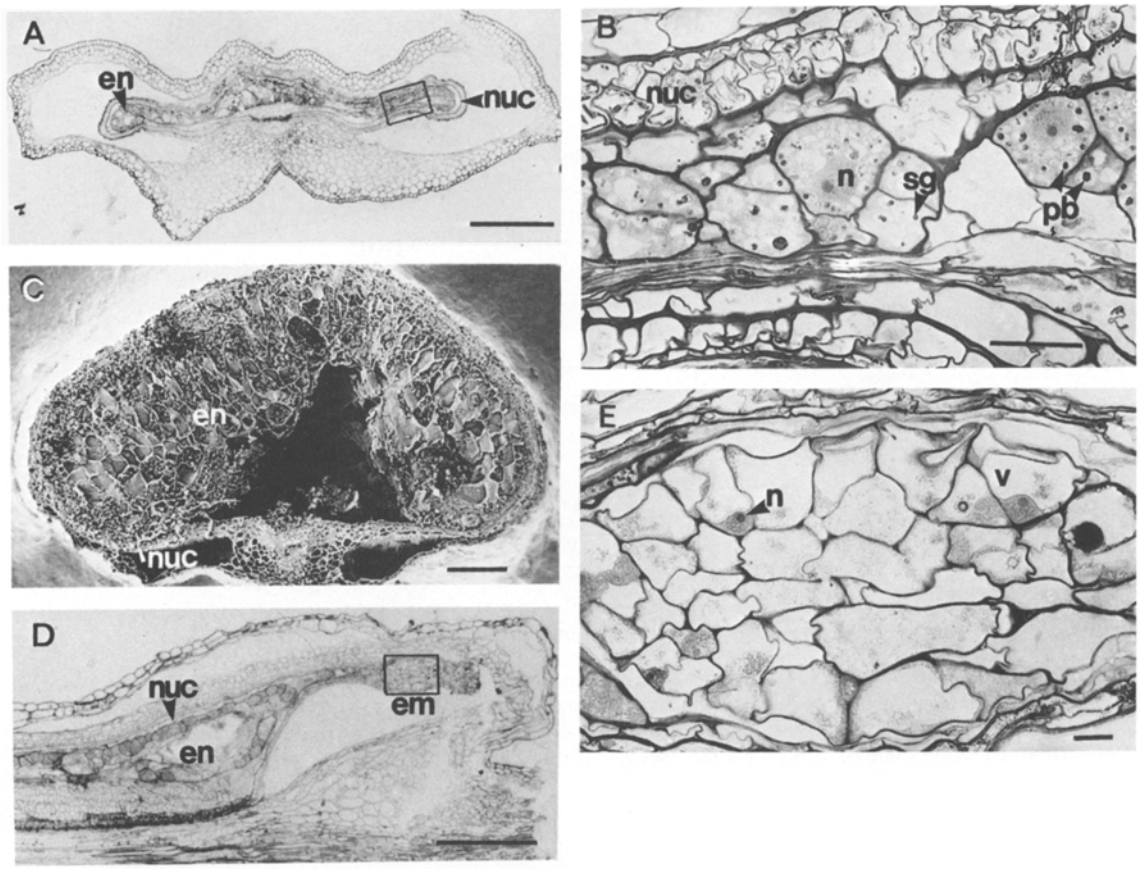

Fig. 3. Light micrographs (**C** = scanning electron micrograph) of sections of 25-day-old homozygous mutant B9 (Type 1) grains. **A** Transverse section of endosperm showing the collapsed embryo sac. **B** Details of frame in **A** showing the undifferentiated nature of the endosperm cells. **C** Transverse section of a more well developed mutant grain than in **A**. **D** Longitudinal section of the proximal part of the grain including the embryo (frame). **E** Frame in **D** enlarged, showing details of the embryo. Bar in **A, C,** and **D** represents 0.25 mm, in **B** and **E** 10 μm. pb = protein body, sg = starch granule, otherwise as in Fig. 1

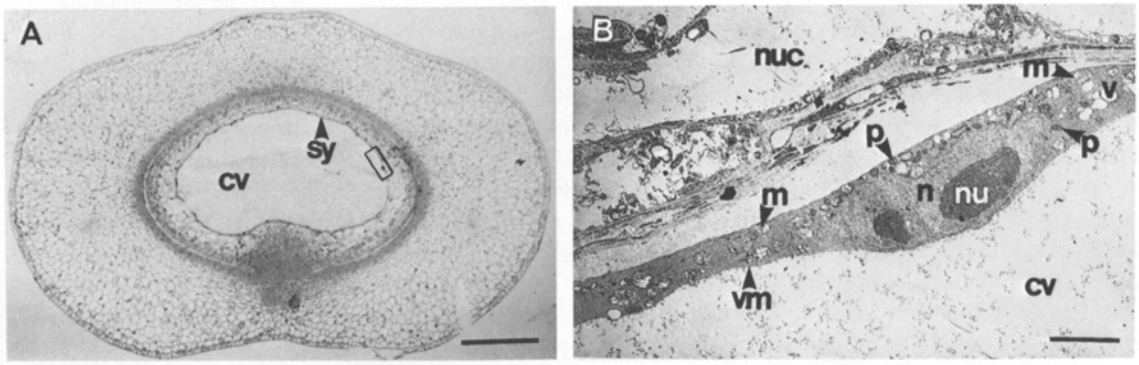

Fig. 4. Light micrograph of a transverse section of a 3-day-old homozygous mutant B9 (Type 1) ovule (**A**). Transmission electron micrograph of frame in **A** shows details of the syncytium (**B**). For comparison to wild type, see Fig. 1 A and C. Bar in **A** represents 0.25 mm, and in **B** 5 μm. nu = nucleolus, otherwise as in Fig. 1

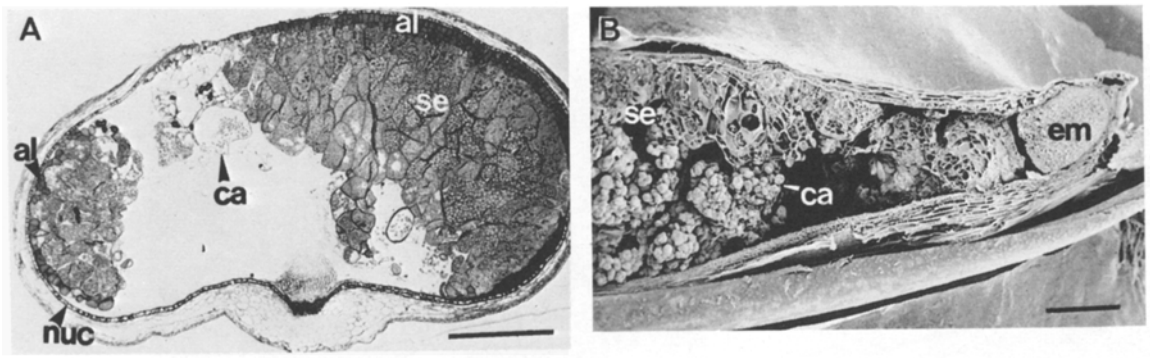

Fig. 5. Light micrograph of transverse section (**A**) and scanning electron micrograph of longitudinal section (**B**) of 28-day-old mutant B10 (Type 1) grain. During the preparation of tissue for microscopy, callus cells are washed out, resulting in the lower number of such cells in **A** than in **B**. Bars represent 0.5 mm. ca = callus-like cells, otherwise as in Fig. 1

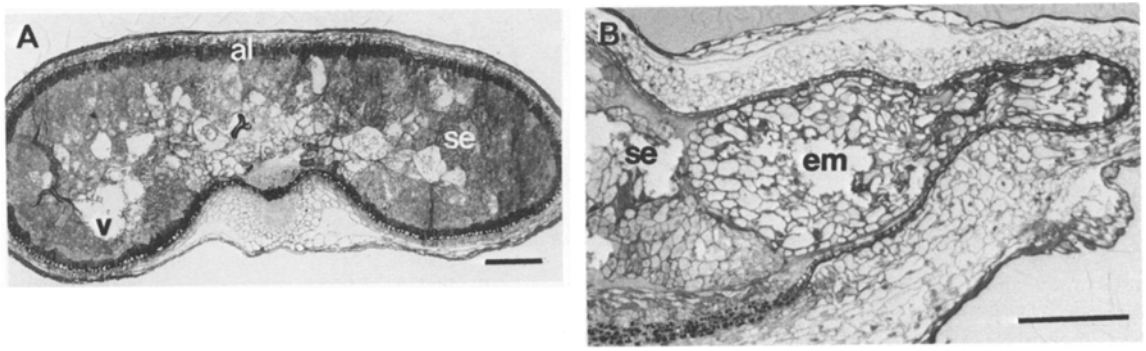

Fig. 6. Light micrographs of a transverse section of the endosperm (**A**) and longitudinal section of the embryo (**B**) of a 27-day-old homozygous mutant B17 (Type 1) grain. Bars represent 0.25 mm. Legend as in Fig. 1

scope is confirmed by the transmission electron microscope (Figs. 1C and 4B). In all the mutant nuclei observed, the nucleolus is highly condensed, as shown in Fig. 4B.

B10. In spikes from heterozygous B10 plants, mutant grains are discernible 10 days after anthesis. At this stage, the developing grains collapse when punctured. The reason for this clearly appears in cross sections showing that the endosperm cavity is only partly filled with cells (Fig. 5A, B). In addition to aleurone and starchy endosperm cells, a third cell type, not present in wild type endosperm, is present (Fig. 5A, B). These cells closely resemble undifferentiated callus cells usually seen in plant tissue cultures.

B17. In homozygous mutant B17, which has the heaviest Type 1 mutant grains, embryo differentiation is more severely affected by the mutant gene than is differentiation of the endosperm (Fig. 6). The aleurone layer appears normal and is two cell layers thick (Fig. 6A). The starchy endosperm cells vary greatly in size and content; some contain large vacuoles, others are densely packed with starch and protein. The embryo of mutant B17 seeds compares well with a 9-day-old wild type embryo in shape and degree of differentiation (Fig. 1F) but is larger and consists of more cells (Fig. 6B).

Type 2 mutants

B8. Mutant B8 grains contain an almost normal endosperm and embryo (Fig. 7A, D). In the starchy endosperm, small starch granules are missing and fewer protein bodies are observable than in wild type grains (Fig. 7C). The aleurone layer is irregular in shape and has thinner cell walls than wild type aleurone (Fig. 7B). At later stages, the dorsal aleurone cells of B8 grow inward and divide the starchy endosperm into two halves.

B13. In homozygous B13 grains the aleurone layer is one to two cell layers thick and the starchy endosperm cells are of varying shape and size (Fig. 8A). Twenty-five days after anthesis, the starchy endosperm cells contain large vacuoles, and the nuclei are still visible

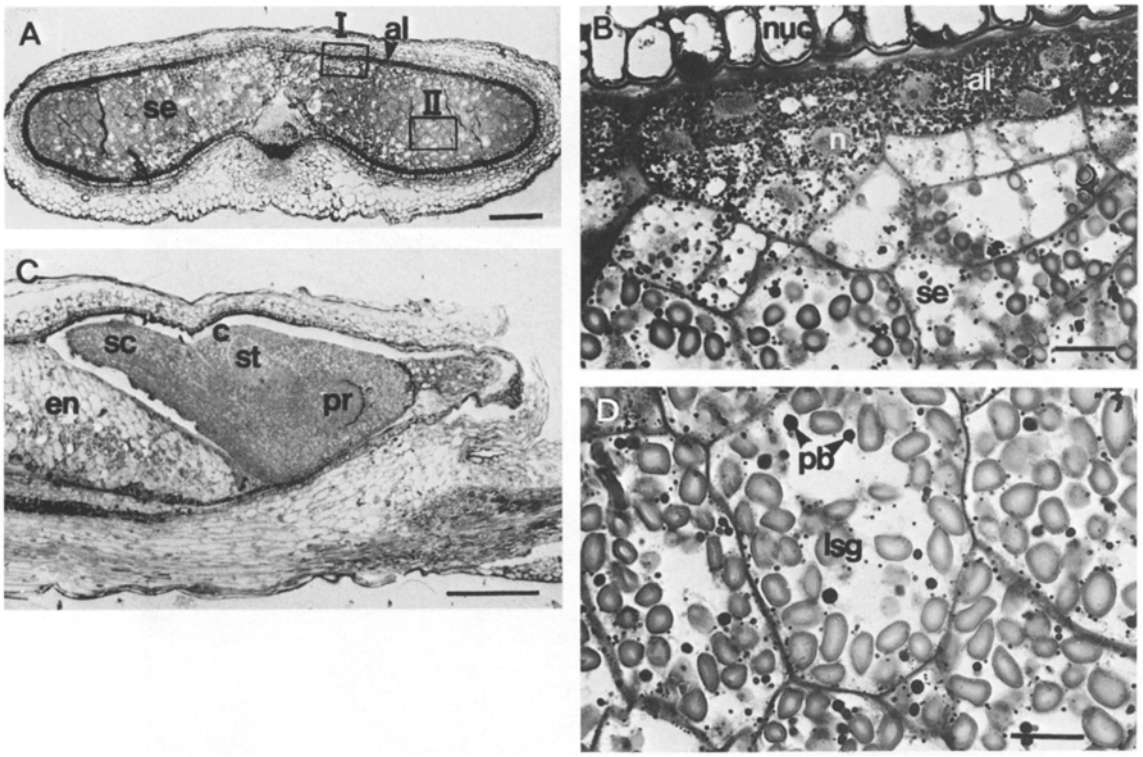

Fig. 7. Light micrographs of a 22-day-old homozygous mutant B8 (Type 2) grain showing a transverse section of the endosperm (**A**), aleurone and starchy endosperm cells of frame I in **A** (**B**), starchy endosperm cells of frame II in **A** (**C**) and longitudinal section of the proximal part of the endosperm including the embryo (**D**). Bar in **A** and **B** represents 0.25 mm, and in **B** and **C** 20 μm. lsg = large starch granule, pb = protein body, otherwise as in Figs. 1 and 4

(Fig. 8 B). At this stage, the cells contain large starch granules and some protein bodies. In older grains, 35 days after anthesis, the starchy endosperm cells are tightly packed with large starch granules and a continous protein matrix (Fig. 8 C).

At day 25 after anthesis, the embryo is highly immature (Fig. 8 D), resembling the 9-day-old wild type embryo (Fig. 1 F). Ten days later, however, transverse sections of the embryo reveal the formation of a shoot apical meristem but no root meristem (Fig. 8 E). Homozygous B13 plants are dwarfs measuring, on the average, only half the height of wild type plants at maturity.

Type 3 mutants

All Type 3 mutants (B1, B2 and B11) develop a differentiated endosperm and embryo. In the starchy endosperm, large starch granules can be seen but small granules are absent (data not shown). In these mutants, the most irregular grain tissue is the aleurone (Fig. 9). In B1, the orientation of the aleurone mitotic spindle seems affected (Fig. 9 A) whereas in B2, the cell walls are thinner than in the wild type aleurone (Fig. 9 B). In B11, the aleurone consists of enlarged, densely packed cells (Fig. 9 C).

In longitudinal sections of 22-day-old embryos of mutant B1, B2 and B11, few deviations from wild type embryos were discovered (see Fig. 1 H). In general, it seems that development is delayed in these mutants by approximately 10 days in mid-phase between anthesis and maturity. In spite of these differences, homozygous mutant embryos give rise to plants with normal phenotypes.

dex-mutants

In order to compare the grain phenotype of the *dex*- and the B-mutants, cross sections from 12 *dex*-mutants were examined under the light microscope (data not presented). The grains were harvested at the earliest stage at which they could be identified in the spikes of heterozygous plants. From this study, it appears that *dex* 4d, *dex* j, *dex* k, *dex* m, *dex* n, and *dex* o are arrested at the free nuclear stage of development, resembling B7, B15 and B16 (Type 1). In *dex* g, *dex* l, *dex* i, *dex* 3e and *dex* 1a a cellular endosperm lacking an aleurone layer develops, as in B9 (Type 1), whereas

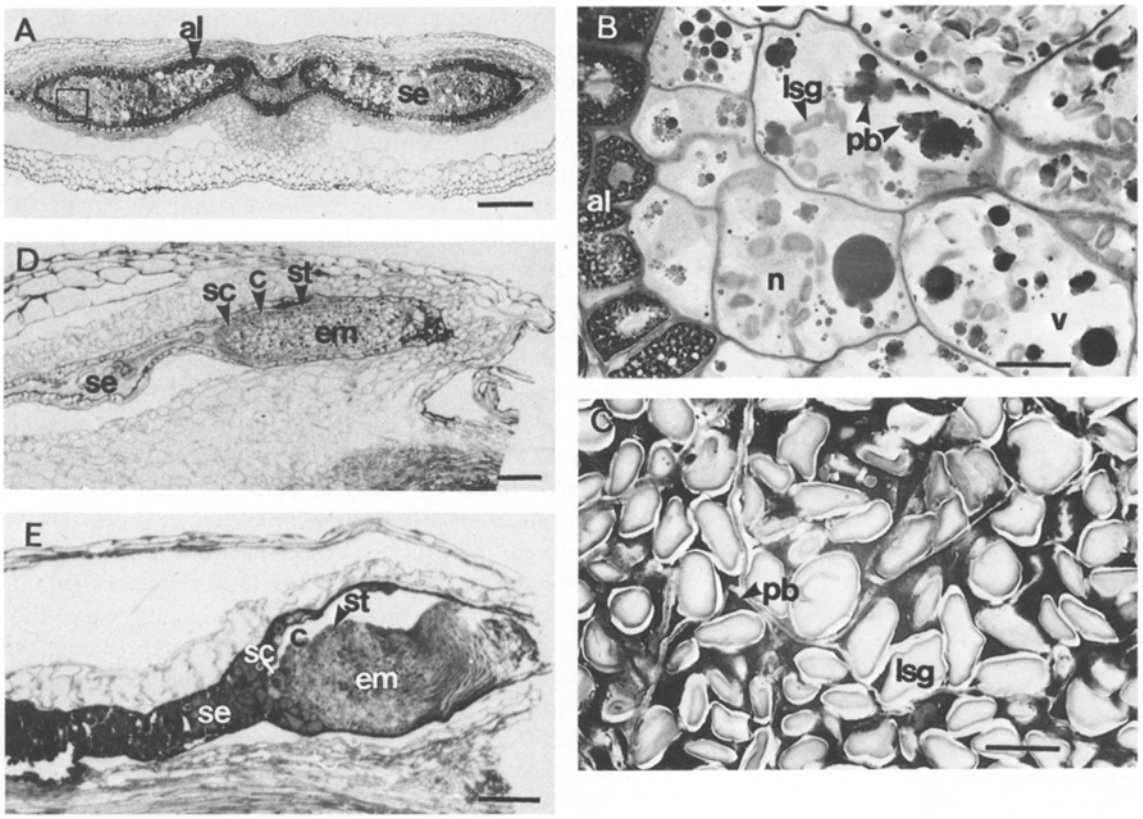

Fig. 8. Light micrographs of a transverse section of a 25-day-old mutant B13 (Type 2) grain (**A**) and details of the aleurone and starchy endosperm of frame in **A** (**B**). **C** shows details of starchy endosperm cells from a 35-day-old grain. At this stage, the starchy endosperm cells are heavily packed with protein bodies. Longitudinal sections of the proximal part of B13 grains harvested at day 25 and 35, respectively, are shown in **D** and **E**. Bar in **A** represents 0.25 mm, in **B** and **C** 20 μm and in **D** and **E** 0.1 mm. Legend as in Figs. 1 and 7

dex 2b develops a fairly normal endosperm. Neither the morphology of the *dex* embryos, nor the allelic relationship to our mutants have been examined.

Discussion

In our view, the barley endosperm is an attractive model system for studies in developmental biology due to its simple organization. The mutants presented above, representing the first to be isolated in a collection of 70, include mutations in genes (lethal as well as viable) affecting all of the major developmental phases of the endosperm as well as the embryo.

As in maize (Neuffer and Sheridan 1980), barley grain formation is likely to involve many genes. The 12 mutants described here, and the *dex*-mutants of Ramage and Crandall, are therefore likely to represent only a fraction of the total number of genes necessary for the formation of a wild type barley grain. Further gene mapping studies are under way to assign more of the B-mutant loci to their respective chromosome loci.

The term *dex*, standing for defective endosperm mutants expressing xenia, was coined by Ramage and Crandall (1981 a). According to their definition, defective endosperm mutants have endosperm so defective as to prevent stand establishment under normal field conditions. This definition is somewhat imprecise, particularly when in vitro embryo rescue techniques are applied. Until more precise terms can be found, therefore, we prefer the symbol *sex*, standing for shrunken endosperm mutants expressing xenia (Eslick and Hockett 1976), for our mutants.

For most of the mutants, a simple mendelian pattern of inheritance is present. One mutant, B10, however, forms an exception to this rule, and deserves special mentioning. The observation that shrunken, as well as smooth grains, give rise to heterozygous plants indicates the requirement of 2 wild type alleles to attain smooth endosperm. Thus, assuming equal viability of all gametes, a segregation ratio of 1:1 of smooth and shrunken grains in heterozygous spikes is expected. One explanation of the observed 3.2:1 segregation ratio (Table 1) may be a severe deficit of female recessive

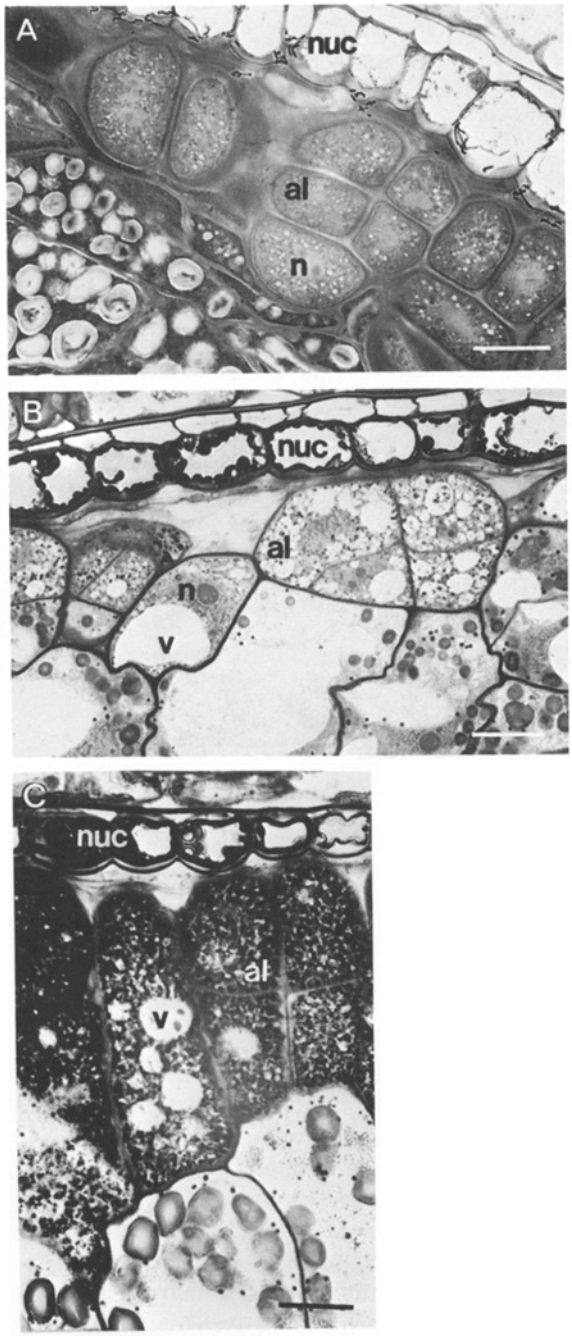

Fig. 9. Light micrographs of transverse sections of 24-day-old grains showing the irregular forms of the aleurone layers of homozygous mutant B1 (Type 3) (**A**), B2 (Type 3) (**B**) and B11 (Type 3) (**C**). Bars represent 20 μm. Legend as in Fig. 1

gametes. Such a deficit may result from preferential degeneration of recessive tetrad cells during female gametogenesis. A similar effect of an embryo lethal mutant gene on pollen viability is reported in *Arabidopsis thaliana* (Meinke 1982). In the developing silique, this leads to a deficit in the number of homozygous mutant embryos.

At present, in mutant B16, no explanation can be offered for the 2.2:1 segregation ratio, deviating significantly from 3:1, and showing a relative excess of homozygote recessive genotypes.

A further classification in maize of the Type 1 shrunken endosperm mutants according to their growth response in tissue culture showed that 81 out of 102 were nutritional (Sheridan and Neuffer 1980), i.e. the developmental defect could be overcome by cultivation of a medium supplying the missing nutrient. Among the 21 mutant embryos that were unable to grow on any of the media tried, 15 were arrested at an early developmental stage and thus potentially true developmental mutants. However, since Type 1 maize mutants also have defective endosperm, the majority of the latter mutants are likely to be of the cellular type, i.e. with mutations affecting essential cellular processes like membrane biogenesis or protein synthesis, as defined by Meinke and Sussex (1979a). Due to the lack (so far) of data from in vitro culture experiments, we cannot classify any of our mutants as nutritional. Among the Type 1 maize mutants that were not nutritional, most, if not all, were cellular, being affected in both grain tissues. Although not positively identified, cellular mutants are therefore also likely to be present among our Type 1 and 2 mutants. One possible candidate for such a mutant is B9, in which the endosperm cells are condensed and seem to grow more slowly than wild type cells. Also, the Type 3 barley mutants B1, B2 and B11, although not affecting the plant phenotype in an obvious way, are likely to be either nutritional or cellular, since development is not arrested at a particular stage.

It is not known at which stage of grain development the mutant genes are first expressed. Our results from microscopy at early stages of endosperm development in mutant B9, however, show that mutant grains can be distinguished from wild type grains as early as 3 days after anthesis, i.e. at the free nucleate stage of endosperm development. In addition, for B7, B15 and B16, which are all arrested at the syncytial stage, an early effect of the mutant genes can also be inferred.

Several of the maternally inherited *seg*-mutants described by Felker and his co-workers (1985) resemble our mutants histologically. Among the *seg*-mutants, two groups exist, i.e. those with premature termination of grain filling due to necrosis of maternal tissue (*seg*-1, 3, 6, 7), and those with characteristic abnormalities in the endosperm, but normal development of maternal tissue (*seg*-2, 4, 5, 8). One of these mutants (*seg*-4) have a completely unorganized aleurone layer and callus-like cell formations in the endosperm, resembling our B10 mutant. While impaired transport of certain assimilates from the maternal tissue to the endosperm cells, or lack of some maternal origin factor required for normal development, is suggested as possible explanations of the abnormal development in the *seg*-mutants (Felker et al. 1985), utilization of the same assimilates or response to the same maternal

factors might be affected in the *sex*- and *dex*-mutants. The nature of such maternal factors is open to speculation, although plant hormones are obvious candidates. Changes in plant hormone levels in a maize defective endosperm mutant is reported by Torti et al. (1986). In their study, the shrunken grained recessive mutant de-B18 has at least a 15 times lower level of IAA than wild type grains.

In the future, several ways of utilizing the barley *sex*-and *dex*-mutants can be envisaged. First, by selection and mapping of many non-allelic mutants, the number, chromosomal location and phenotypic effect of the major genes contributing to grain formation can be assessed. Secondly, the mutants may be suitable objects for ultrastructural studies, particularly concerning the early stages of grain formation. In addition to in vitro culture experiments to identify nutritional *sex*-mutants, in vitro techniques may be applied to select mutants with improved capacity to regenerate plants from immature embryos.

In barley, over the last years, a number of grain proteins have been isolated and characterized. For some of these, the corresponding genes have been cloned. In the *sex*-mutants, by the use of the proper antibodies and probes, the regulation of gene transcription and mRNA processing may be studied. The identification of the primary genetic lesion in such mutants may, however, have to await further refinements in molecular biology techniques, such as transposon "tagging" of mutant genes (see, for example, Fedoroff et al. 1984). Only then may it be possible to identify the genes responsible for the barley *sex*-phenotypes, some of which may be expressed at very low levels.

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