Grain Shrivelling in Secondary Hexaploid Triticale. II. Morphology of Mature and Developing Grains Related to Grain Shrivelling[1]

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ABSTRACT

Morphological characteristics of plump, medium shrivelled, and highly shrivelled grains of eight secondary hexaploid triticales were examined by scanning electron microscopy. Although enzymatically damaged starch grains were observed in three of the eight triticales, the extent of damage was not considered large enough to cause grain shrivelling. Furthermore, visible-light microscopic examination of developing grains from plump, medium shrivelled, and highly shrivelled seeds of the triticale cultivar Rahum showed that grain shrivelling is more likely to result from failure of endosperm cells to completely fill the endosperm cavity. No evident relationship between grain shrivelling and the number and identity of rye chromosomes was observed.

Morphological characteristics associated as a cause or manifestation of grain shrivelling in triticale were studied by several investigators. Dronzek et al (1974) studied grain morphology of a shrivelled triticale cultivar by scanning electron microscopy (SEM) and observed some empty regions where no starch or endosperm formation occurred. Some starch damage near the aleurone and crease of the grain was also observed. Shealy and Simmonds (1973) observed aleurone malformation to be first recognized at six days postanthesis (p.a.) and well established by 10 days p.a. Simmonds (1974) suggested that distorted aleurone cells released enzymes that degraded the meristematic layer, producing empty areas beneath the nucellar epidermis that finally resulted in malformation and shrivelling in the grain. All these authors suggested that premature release of α-amylase and subsequent digestion of starch granules is a phenomenon that contributes to grain shrivelling in triticale. In contrast, Lorenz et al (1978) observed that the only abnormality that possibly produces a shrivelled appearance in triticale is partial separation of the pericarp from the endosperm. We previously examined α-amylase activity and carbohydrate content of mature and developing grain of plump, medium shrivelled, and highly shrivelled kernels of secondary hexaploid triticales (Peña and Bates 1982). Our results indicated that α-amylase activity was not a major factor affecting grain shrivelling in triticale, so we explored the grain morphology of the same materials used in the biochemical study mentioned above in an effort to determine what factors affect grain shrivelling in triticale. Results of our morphological observations by SEM of plump, medium shrivelled, and highly shrivelled mature grains of eight secondary hexaploid triticales, as well as light microscope observations of developing grains originated from plump, medium shrivelled, and highly shrivelled seeds of the hexaploid triticale cultivar Rahum are presented.

MATERIALS AND METHODS

All grain samples used were grown in Mexico during the winter of 1977–1978 as part of the International Maize and Wheat Improvement Center's (CIMMYT) triticale yield trials. All triticales studied were advanced secondary hexaploid triticales containing $2n = 6\times = 42$ chromosomes.[2] Variety, or cross name, and identification number of the materials are listed in Table I. Number and identity of rye chromosomes in the triticale cv. Rahum are presented in Table II; the same characteristics of the remaining seven triticale cultivars are presented in Table III.

SEM

All triticale materials were categorized into three classes: plump (a), medium shrivelled (b), and highly shrivelled (c). Wheat, rye, and categorized triticale grains were fractured with a razor blade to obtain median transverse cross-sections. Four grain sections of each cultivar were mounted on specimen stubs with silver paste, coated with carbon and gold-palladium to a thickness of approximately 200 Å, and viewed and photographed in an ETEC autoscan electron microscope.

Light Microscopy

To study morphological changes during grain development, we grew seeds of the three classes (a, b, and c) within the secondary hexaploid triticale cv. Rahum separately under controlled environmental conditions. As the heads emerged, each was bagged, and the anthesis date recorded. At six different stages of maturity (5, 10, 15, 20, 26, and 35 days p.a.), two spikes were harvested and fixed in glacial acetic acid-formaldehyde-picric acid (Bouins fixative) or formaldehyde-propionic acid-70% ethanol (FPA fixative). Dehydration, infiltration, and embedding were performed according to the ethanol-tertiary butanol-paraffin schedule of Sass (1958). Transverse sections (four from each grain class) 10 μm thick were prepared on a Spencer 815 rotary microtome (American Optical Co., Buffalo, NY), stained with Safranin and Fast Green FCF, and permanent slides prepared in Preservaslide (Curtin Matheson Scientific). Cross sections were viewed and photographed with a Reichert Zetopan microscope equipped with an automatic camera.

TABLE I
Triticale, Wheat, and Rye Samples

Variety	Variety or Cross Name	Identification No.
Triticale		
424	Rahum	P-1
430	Mapache	P-2
823	PM 28 Bulk-Cml "s"	
	X-21349-2N-OY	P-3
1628	Drira-Arm "s"	
	X-21367-4N-OY	P-4
2427	IRA[2] × M_2A	
	X-11308-B-2M-3Y-2Y-4M-OY	P-6
1303	1A-Kla × Cal	
	X-14920-2Y-OM	P-7
1401	Bgc-Bulk e2	
	X-11066-A-6M-100Y-101B-10YY-OY	P-10
Wheat		
	Calidad	P-8
	Hermosillo	P-9
Rye		
	Snoopy	P-11

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[2]K. Mujeeb. 1978. Personal communication.

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RESULTS AND DISCUSSION

SEM-Morphological Relationship to Grain Shrivelling

Morphological observations of cross-section, aleurone, pericarp and starchy endosperm of all cultivars studied, are represented by Figs. 1 through 5.

Plump Grains (Class A)

The pericarp is attached tightly to the grain; however, the pericarp separates from the seed coat in some points to produce a slightly shrivelled appearance (Figs. 1a and 3a), which agrees with observations reported by Dronzek et al (1974) and Lorenz et al (1978). Separation of the pericarp from most of the seed coat surface is also a common characteristic of rye (Fig. 5d). In most of the triticale lines, where the seed coat and the pigment strand join, an empty space varying from small to large can be observed between the pericarp and either the seed coat or the nucellar epidermis (Figs. 2a, 3a, and 4a). This factor should be considered when grain plumpness is used as a criterion for triticale seed improvement because the volume of the grain is not completely filled with starchy endosperm and the selection would be erroneous. This suggestion agrees with observations of Salminen and Hill (1978) that masked differences between shrivelled and plump-grain triticales may result from variability in sink size and total dry matter production among the lines.

TABLE II
Number and Identity of Rye Chromosomes Present in the Secondary Hexaploid Triticale Cultivar Rahum[a]

Sample	Frequency of Plants	Rye Chromosomes 1R	2R	3R	4R	5R	6R	7R	No. of Rye Chromosome Pairs
P-1a		—L[b,c]				—			
	15	+		+		+	+	+	5
P-1b						—			
	10	+		+		+	+	+	5
		—L		**S[d,e]	*—[f,g]				
	5	+		+	+	+*	+	+	6
					**S	—			
	8	+		+	+	+	+	+	6
					**S				
	5	+		+	+	+	+	+	6
P-1c					**S				
	3	+		+	+	+	+	+	6
					**S	*—			
	2	+		+	+	+	+	+	6

[a] Data provided by Jozef Pilch (1979, unpublished).
[b] — = reduced terminal heterochromatin on both chromosomes.
[c] L = chromosome, long arm.
[d] ** = no terminal heterochromatin on both chromosomes.
[e] S = chromosome, short arm.
[f] * = no terminal heterochromatin on one chromosome.
[g] — = reduced terminal heterochromatin on one chromosome.

TABLE III
Number and Identity of Rye Chromosomes Present in Seven Secondary Hexaploid Triticale[a]

Sample	No. of Plants Analyzed	Rye Chromosomes 1R	2R	3R	4R	5R	6R	7R	No. of Rye Chromosome Pairs
P-2	10	+	+			+	+	+	5
		—L[b,c]				—L			
P-3	10	+	+	+	+	+	+	+	7
					**S[d,e]				
P-4	10	+	+	+	+	+	+	+	7
								—L	
P-5	10	+	+	+	+	+	+	+	7
P-6	10	+	+			+	+	+	5
P-7	10	+	+			+	+	+	5
P-10	10	+	+			+	+	+	5

[a] Data provided by Jozef Pilch (1979, unpublished).
[b] — = reduced terminal heterochromatin on both chromosomes.
[c] L = chromosome, long arm.
[d] ** = no terminal heterochromatin on both chromosomes.
[e] S = chromosome, short arm.

Medium Shrivelled Grains (Class B)

Class b grains of all triticale lines (except P-5b) showed aleurone cells and starchy endosperm similar to the normal, plump grains (Figs. 1–4, e and f, respectively). The pericarp separation from the seed coat was more pronounced and at more points than in the plump class, which enhanced the shrivelling appearance (Figs. 1d–4d). For this particular characteristic, the medium shrivelled triticale grain class resembled its rye parent more than its wheat parent (Fig. 5a and 5d). The shrunken aspect of the medium shrivelled grains was even more intensified by depressions at some points of the endosperm surface (Figs. 1d through 4d, respectively). No depressions were observed in the rye or wheat endosperms (Fig. 5a and d), indicating that endosperm depressions in triticale grain are morphological abnormalities rather than parentally inherited characteristics. The voids between the pericarp and seed coat at the pigment strand junction observed previously in the plump grain class occurred similarly in the medium shrivelled grain classes (Figs. 2d and 4d). In two of the samples, the cheeks of the grain were abnormally separated from each other (Fig. 2d), a characteristic that could result from early developmental problems (Bennett 1977, Kaltsikes and Roupakias 1975, Kaltsikes et al 1975). Grains of the triticale P-5b exhibited characteristics that differed from those in all others of the same grain class. Cells of the aleurone layer were normal except for the dorsal side of the grain, where they were disrupted at depressions in the starchy endosperm (Fig. 3d). Perhaps the grain had a weak structure produced by a void that extended from the head of the crease to the depression points, or perhaps the grain lost moisture during maturation, so that an invagination of nucellar epidermis and seed coat crushed and disrupted the aleurone cells. This could have initiated some catabolic hydrolysis of proteinaceous membranes and starch granules of the endosperm (Fig. 3f).

Highly Shrivelled Grains (Class C)

Five of the triticale grains of the class c (P-2c, P-3c, P-6c, P-7c, and P-10c) had aleurone cells apparently intact. Compression of aleurone cells at various points along the endosperm surface was the only abnormality observed (Fig. 4g and 4h). In two other lines (P-1c and P-4c), the aleurone was crushed and disrupted at specific points of the endosperm, resulting either from a cavity in the endosperm extending from the crease to a large portion of the central endosperm (Fig. 1g) or from a localized collapse of a large area of the endosperm (Fig. 2g). In the remaining triticale (P-5c), aleurone cells were crushed and disrupted at several points along the endosperm surface. Only about half of the grain sink was filled (Fig. 3g).

The highly shrivelled appearance of the grains of this class developed primarily from pericarp shrivelling and separating from the seed coat because depressions were greater in number and in depth than those in grains of classes a and b. The endosperm depressions may result from a collapse of nucellar epidermis and seed coat into an insufficiently filled portion of the starchy endosperm, perhaps dependent on how the starchy endosperm was packed (Figs. 1i, 3i, and 4i). This suggestion is based on morphological characteristics observed in the samples P-1c, P-3c, P-5c, and P-10c. The sample P-1c, for example, showed a cavity about one-third the size of the total cross section with the walls

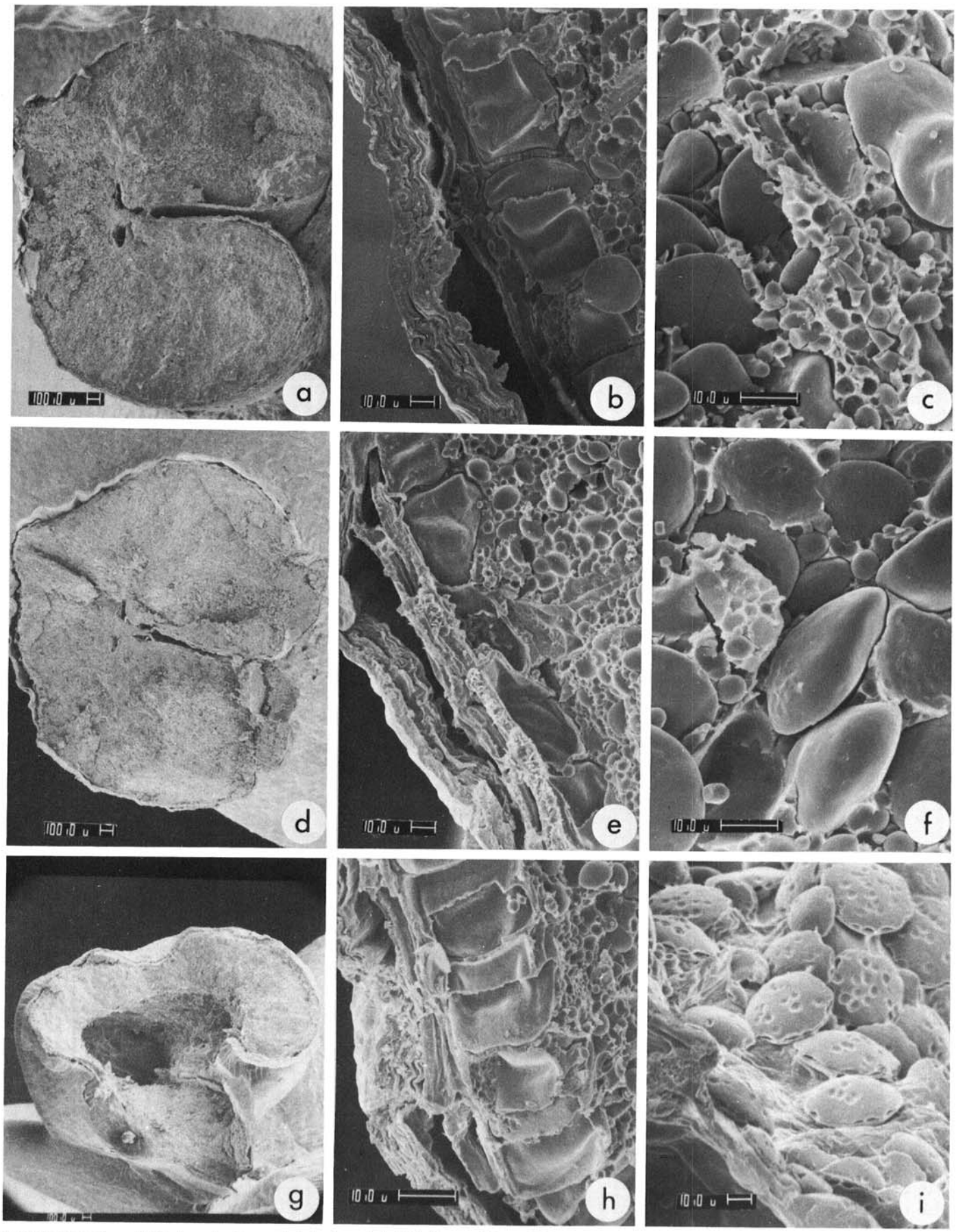

Fig. 1. Cross section of triticale grain classes: P-1a (**a, b,** and **c**), P-1b (**d, e,** and **f**), and P-1c (**g, h,** and **i**).

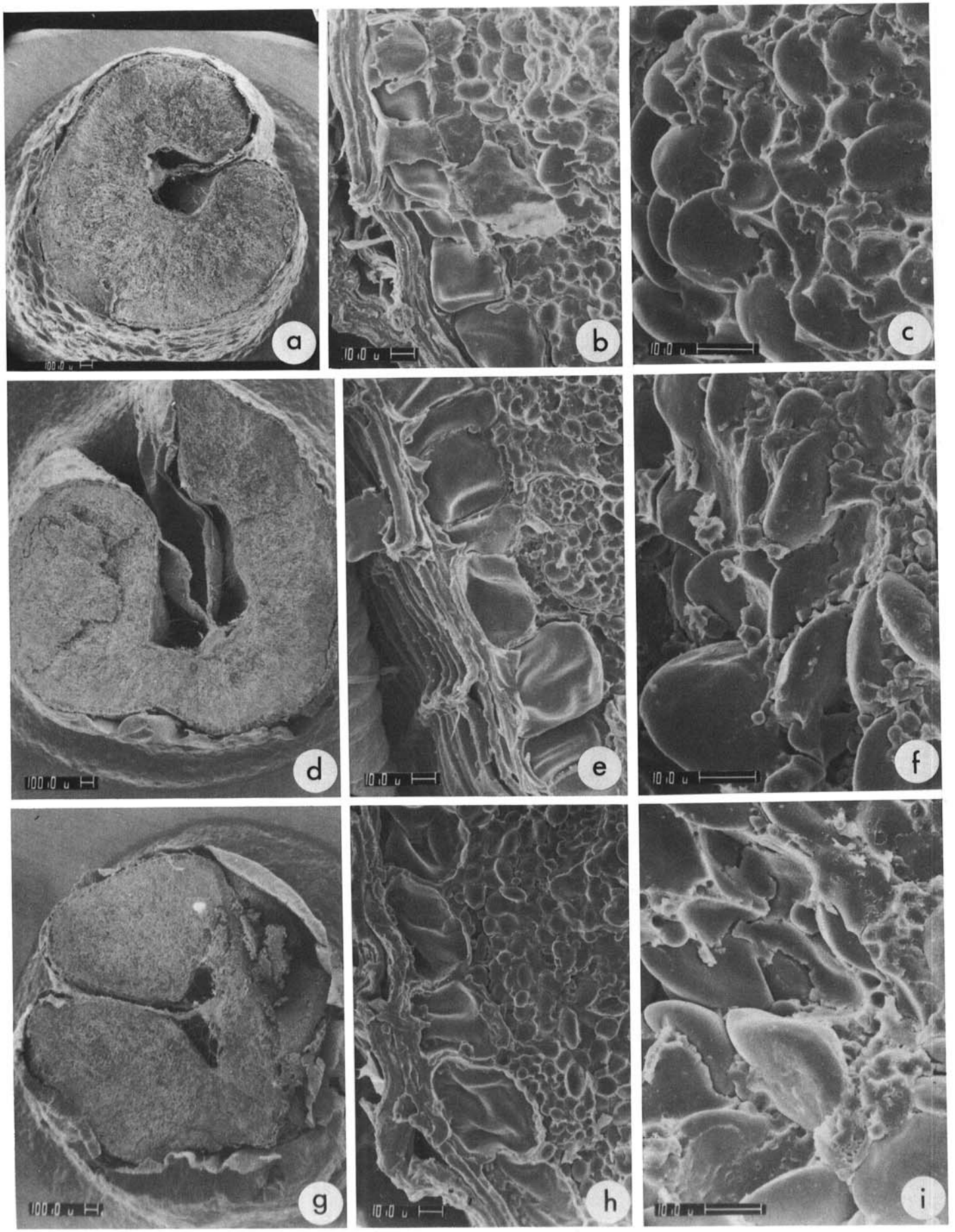

Fig. 2. Cross section of triticale grain classes: P-4a (**a, b,** and **c**), P-4b (**d, e,** and **f**), and P-4c (**g, h,** and **i**).

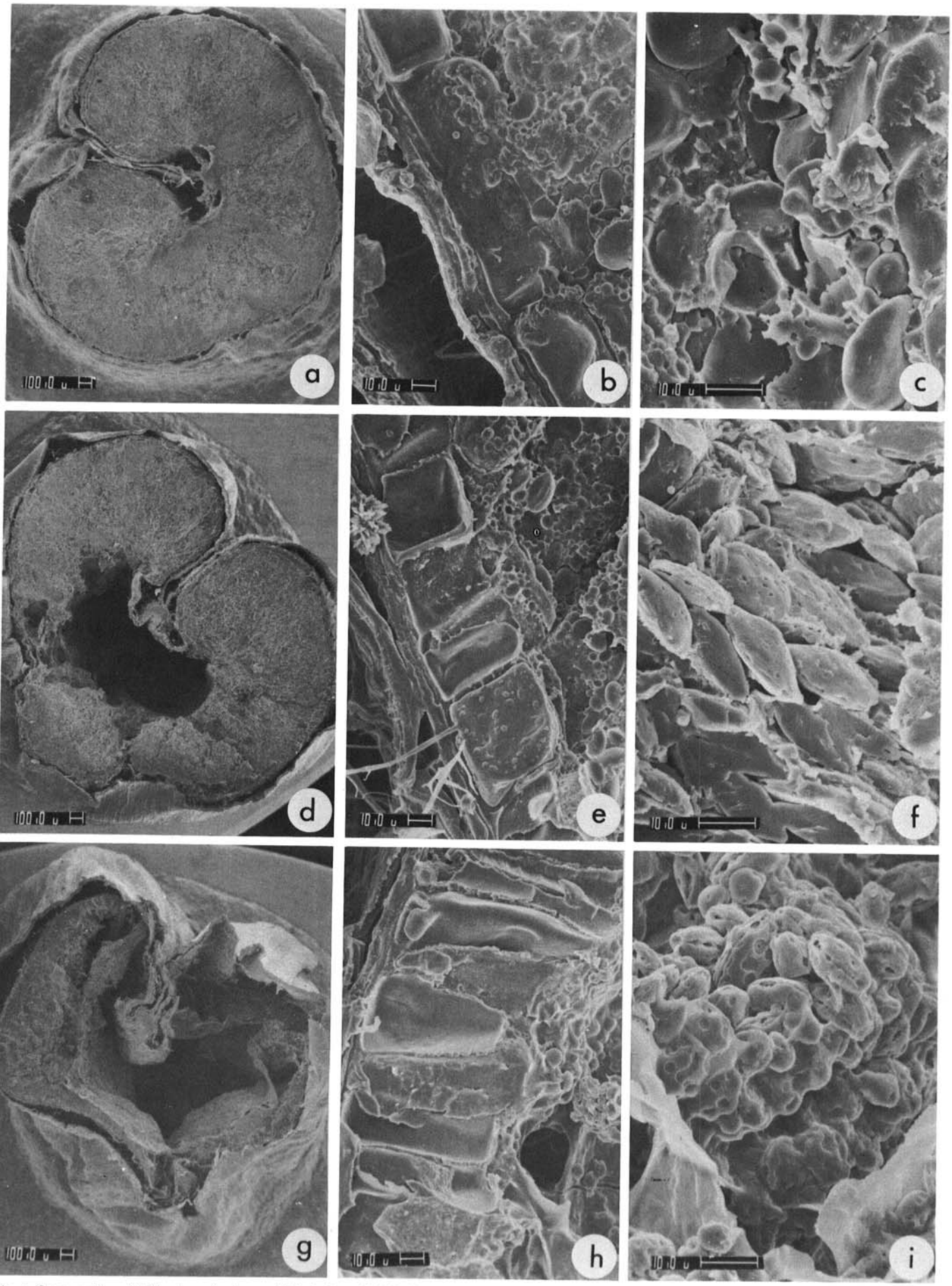

Fig. 3. Cross section of triticale grain classes: P-5a (**a, b,** and **c**), P-5b (**d, e,** and **f**), and P-5c (**g, h,** and **i**).

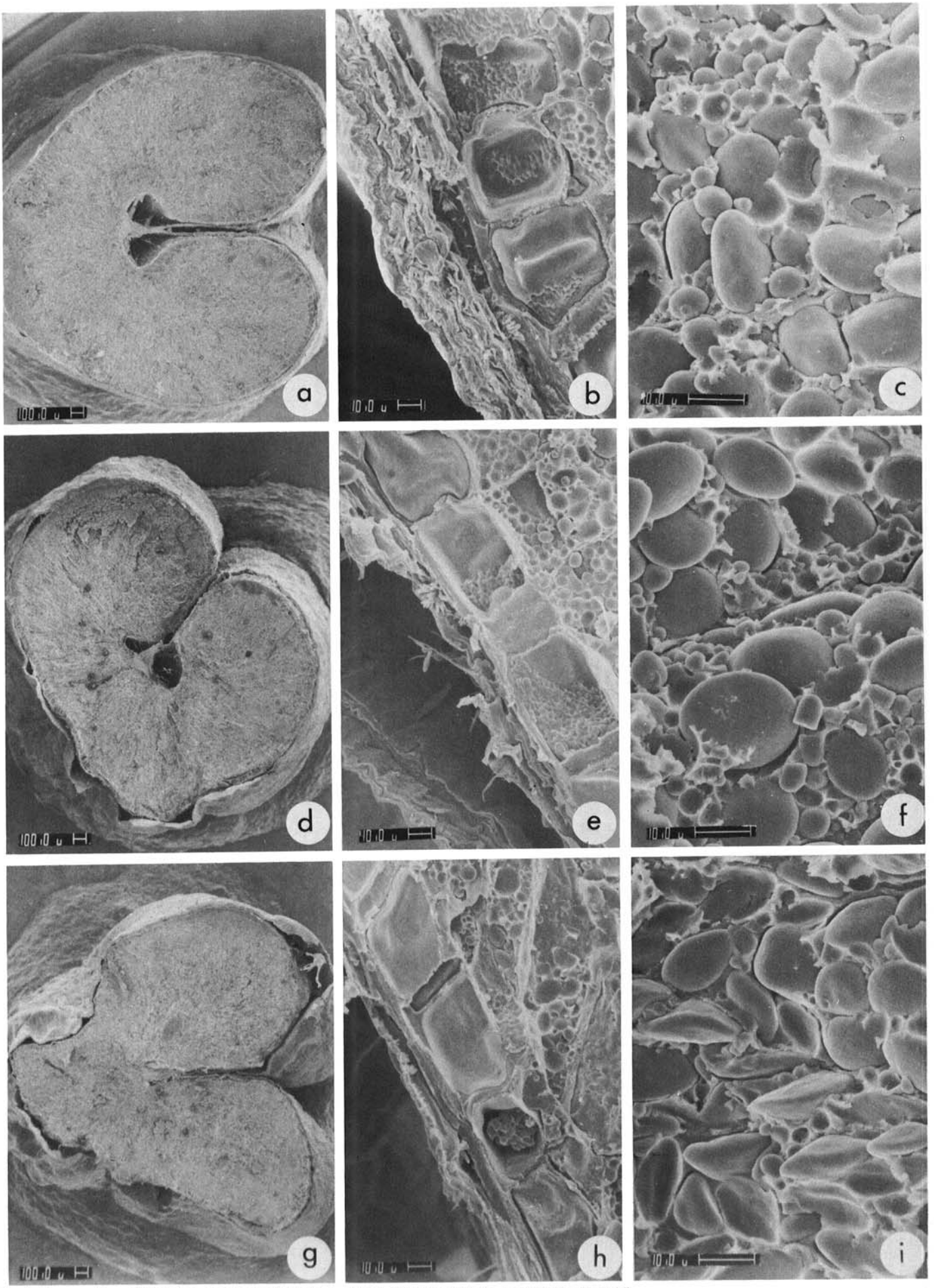

Fig. 4. Cross section of triticale grain classes: P-7a (**a, b,** and **c**), P-7b (**d, e,** and **f**), and P-7c (**g, h,** and **i**).

composed of endosperm (Fig. 1g and 1i); and sample P-5c showed a compact endosperm that formed a rim around only about two-thirds of the grain periphery, and more than half of the grain was composed of a cavity subdivided by the seed coat (Fig. 3g).

Enzymatically damaged starch granules were observed in only three of the eight triticale lines. Furthermore, only the starch damage in sample P-5c could be considered extensive enough to affect grain shrivelling (Fig. 3i). In the other two samples, starch damage was insufficient to cause a collapse of endosperm cells (Fig. 1i). Therefore, failure of endosperm cells to completely fill their grain sinks is the phenomenon most likely responsible for the grain shrivelling in triticale.

Developmental Morphology and Its Relationship to Grain Shrivelling

Light microscopic observations of general morphological characteristics of developing grains produced by plants from P-1a, P-1b, and P-1c seeds are presented in Figs. 6 and 7. At five days p.a., morphological characteristics of all three samples were similar. At this stage, the innermost pericarp cells were degenerated at the ventral port of the grain (Fig. 6a). Several layers of cells, outer and inner integuments, and nucellar tissue surrounded the enlarging embryo sac in which the antipodal cells were centrally located. Endosperm cellularization had begun and was observed from the periphery inward (Fig. 6a). Similar developmental morphology of triticale grains was reported earlier (Dedio et al 1975, Shealy and Simmonds 1973, Simmonds 1974).

In the next developmental stages, progressively increased lysis of pericarp cells occurred, so that after 25 days p.a., only two or three pericarp cell layers were observed in all samples (Fig. 7c). Outer and inner integuments developed similarly in all samples. At 20 days p.a., the outer integument was no longer observed, whereas the inner one remained as a thin layer of crushed cells (Fig. 7a), which at maturity is recognized as the seed coat of the grain (Simmonds 1974). The nucellar tissue, composed initially of several cell layers, remained as a single-celled layer (nucellar epidermis) until 15 days p.a. After that time, crushed cells of the nucellar epidermis were observed only in areas where they had intimate contact with the endosperm cells (Fig. 6e). The same phenomenon was observed by Simmonds (1974) soon after seven days p.a. From 20 days p.a. onward, the triticale nucellar epidermis was a thin layer surrounding the endosperm. We found no significant differences in

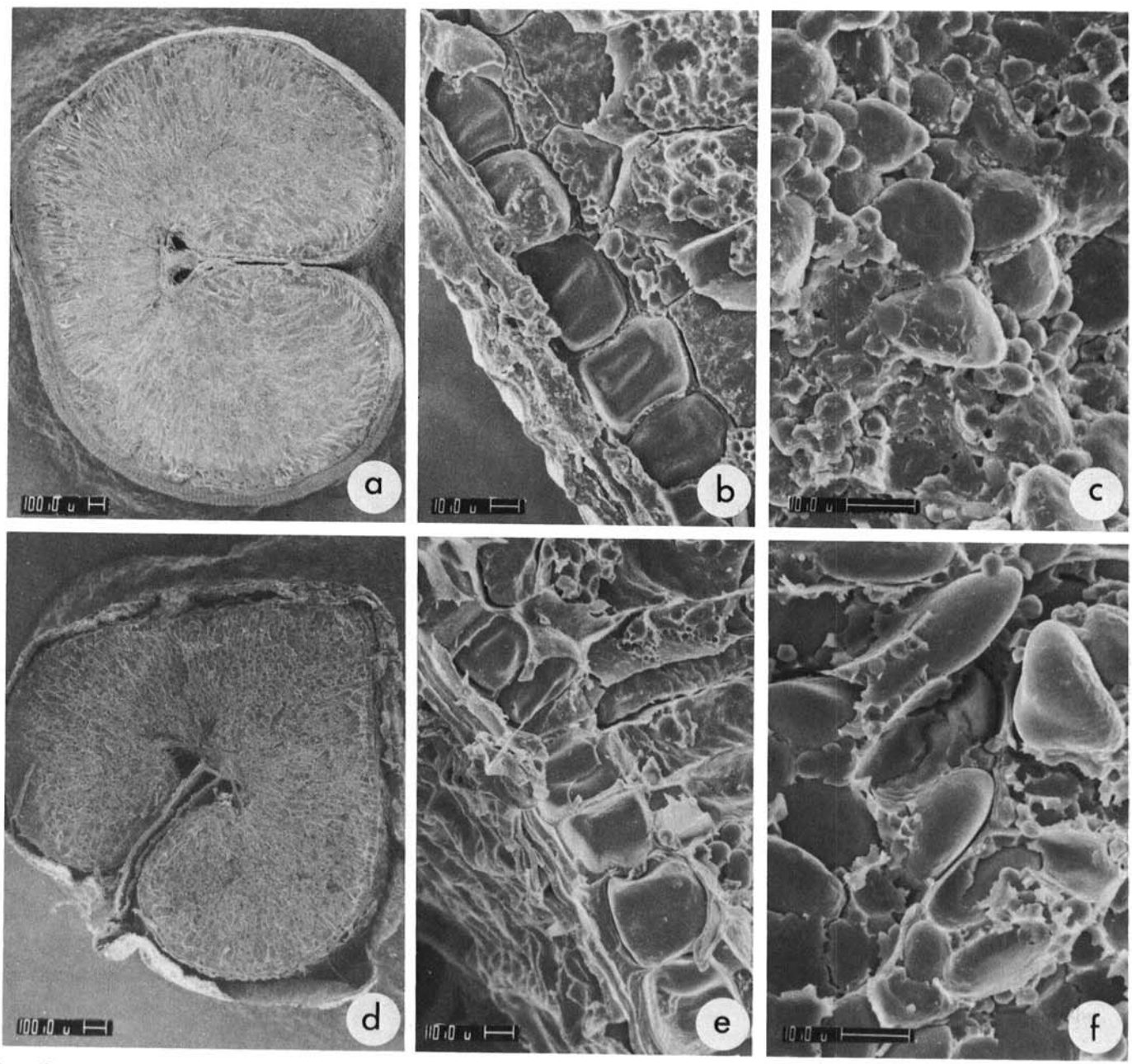

Fig. 5. Cross section of wheat and rye grains: wheat P-8 (**a, b,** and **c**) and rye P-11 (**d, e,** and **f**).

this structural component among grains from all samples. Meristematic characteristics of the outermost endosperm cells continued in all samples until at least 15 days p.a. (Fig. 6d), as Simmonds (1974) observed. Subsequent endosperm development resulted from cell enlargement and deposition of starch and protein, as suggested by the clear aleurone cell surrounding the endosperm. In some areas, a pronounced empty space occurred between the endosperm and the rest of the seed coat (Fig. 7a and c).

Development of the starchy endosperm from five days p.a. until 35 days p.a. differed among grains produced by P-1a, P-1b, and P-1c. At 10 days p.a., the mass of cellularized endosperm did not fill the endosperm cavity; this characteristic was much more pronounced in grains from P-1c (Fig. 6b and 6c). Thereafter, failure of the endosperm cavity to completely fill resulted in endosperm cells lining only a certain extent of the total volume, which left an empty space between the endosperm and the crease area of the grain. This empty space was always less in grains from P-1a and P-1b (Fig. 7d) than in those from P-1c (Fig. 7e). Aleurone cells were disrupted in most of the area surrounding the cavity (Fig. 7a). When the mass of endosperm did not fill the grain sink, the seed coat did not enlarge or expand as it does under normal conditions. Thus, at 26 days p.a., the pericarp cells remained separated from the rest of the grain (except at the crease head) and separated to a greater extent in grains from P-1c (Fig. 7c) than in those from P-1a and P-1b (Fig. 7b). At 35 days p.a., grains from P-1c showed surface depressions, and the pericarp and seed coat had collapsed to produce a wrinkled appearance (Fig. 7d). Grains from P-1a and P-1b (Fig. 7d) showed the same abnormal characteristics but much less than those from P-1c. Based on SEM observations at maturity and on sections at 35 days p.a., endosperm depressions, pericarp shrivelling, and invagination of the seed coat in the crease area would increase because of loss of water.

When the number and identity of rye chromosomes in each of the triticale genotypes (Table II) were compared to extent of grain shrivelling, no evident relationship was found. Grain classes of the triticale P-1 showed differences in rye chromosome composition (Table III). The plump grain class had five rye chromosome pairs; 2R and 4R were substituted by chromosomes of wheat (perhaps by D-genome chromosomes). The medium shrivelled class had two groups of genotypes, one similar to P-1a grains and the other to that of P-1c grains that had one additional rye chromosome (4R). Some morphological differences in chromosome 5R separated the P-1c class into four subclasses. The absence of the 4R chromosome in plump grains may be a significant influence in grain shrivelling, as Darvey (1973) and Kaltsikes and Roupakias (1975) suggested. Different triticale genotypes originating from a single cultivar may be explained only as partial sterility and consequent outcrossing in the experimental fields where several different cultivars are grown in the same area. This also could explain the observations that plump and shrivelled grains are randomly distributed in a single spike and that different plants from the same cultivars can produce different seed types.

CONCLUSION

The biochemical results of our previous study (Peña and Bates 1982) and the SEM-morphological observations show definitely that α-amylase activity does not affect grain shrivelling. Although α-amylase activity increases within a triticale cultivar as shrivelling increases, the activity can be explained by two reasons. First, the quantity of starchy endosperm in the highly shrivelled grains is less than in plump grains, as observed by SEM and light microscopy, which results in a smaller ratio of starchy endosperm to aleurone. Thus, α-amylase concentration increases as does α-amylase activity

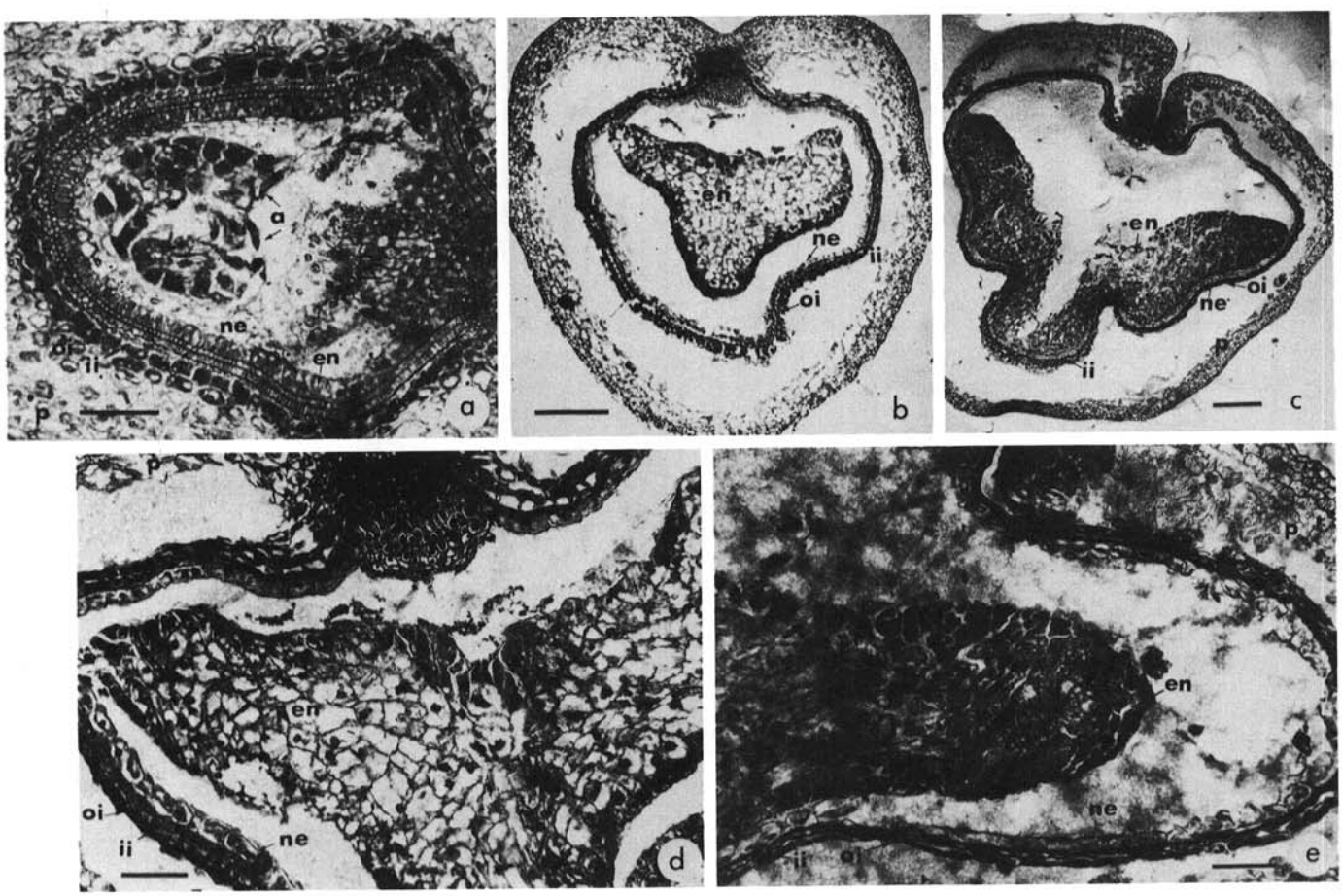

Fig. 6. Cross section of triticale grain classes at several developmental stages. a = antipodals, oi = outer integument, ii = inner integument, p = pericarp, ne = nucellar epidermis, al = aleurone, en = endosperm, **a,** Grains from medium shrivelled seeds at five days post-anthesis; **b,** grains from medium shrivelled seeds at 10 days post-anthesis. **c:** grains from highly shrivelled seeds at 10 days post-anthesis. **d:** grains from medium shrivelled seeds at 15 days post-anthesis. **e:** grains from highly shrivelled seeds at 15 days post-anthesis.

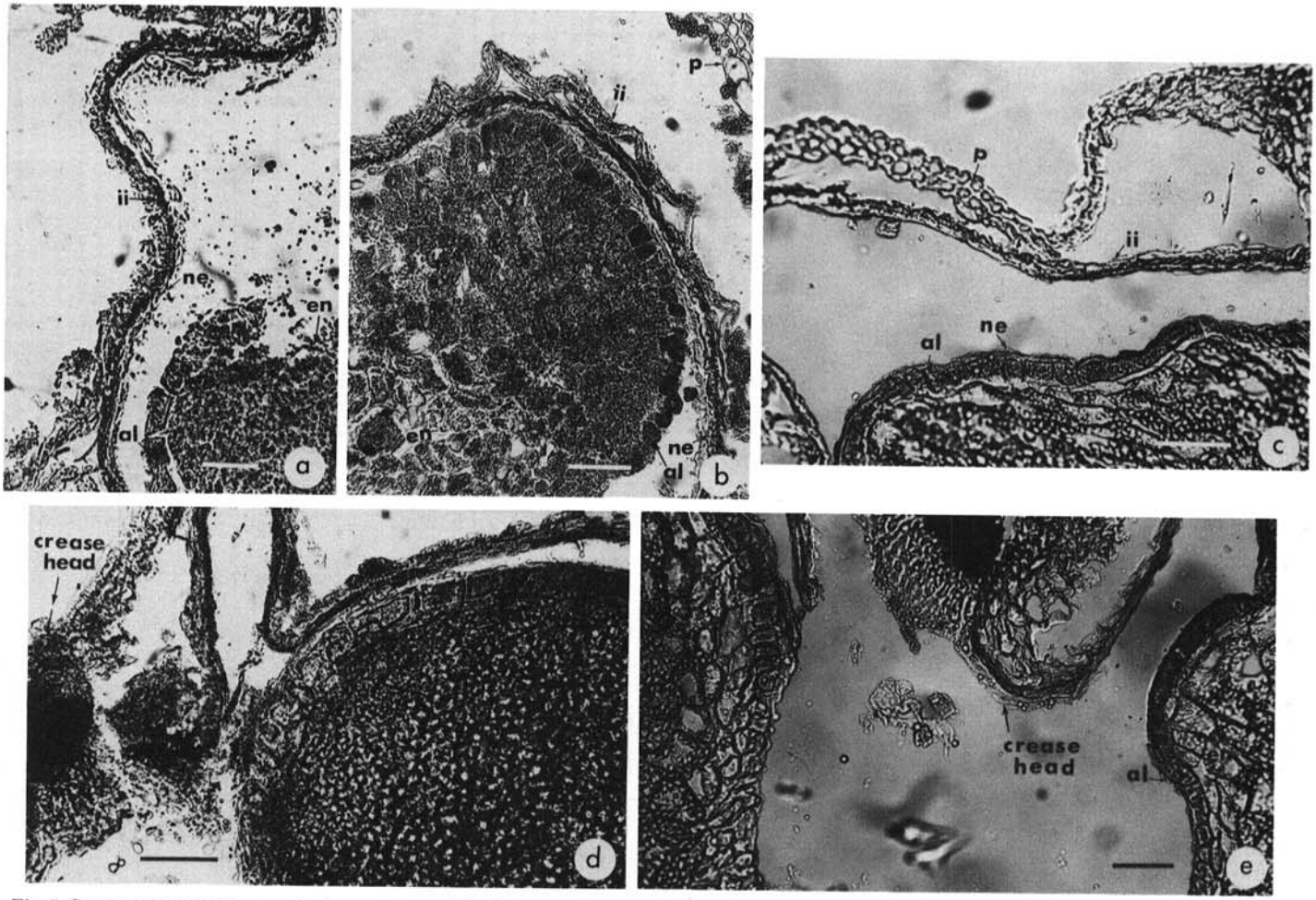

Fig. 7. Cross section of triticale grain classes at several developmental stages. a = antipodals, oi = outer integument, ii = inner integument, p = pericarp, ne = nucellar epidermis, al = aleurone, en = endosperm. **a,** Grains from highly shrivelled seeds at 20 days post-anthesis; **b,** grains from medium shrivelled seeds at 26 days post-anthesis; **c,** grains from highly shrivelled seeds at 26 post-anthesis; **d,** grains from medium shrivelled seeds at 35 days post-anthesis; **e,** grains from highly shrivelled seeds at 35 days post-anthesis.

on a per kernel basis. Second, the same mechanisms that affect grain shrivelling may also increase α-amylase activity in some triticale genotypes, but these are of a secondary nature because the attacked starch granules maintain their shape and do not collapse. Our observations and supplementary rye chromosome data, along with early findings of Kaltsikes and Roupakias (1975), Bennett (1977), and a recent review of the developmental aspects of grain shrivelling in triticale (Thomas et al 1980), led us to conclude that grain shrivelling in secondary hexaploid triticale has its origin in incompatibility problems between wheat and rye chromosomes in the triticale genotype. The extent of grain shrivelling is established at the earliest stages of endosperm development when aberrant nuclei are formed. As a consequence, the number of cells that remain at maturity is reduced. The reduced number of endosperm cells leads to an early termination of dry matter accumulation because no metabolic sink area for storage exists. Consequently, physiological maturity occurs early. Because the embryo sac was prepared to be completely filled by the expanding endosperm cells and cannot be when the number of endosperm cells is reduced, the pericarp, seed coat, and aleurone layers collapse into empty spaces during the later stages of grain maturation. The final result is endosperm voids and depressions that can disrupt aleurone cells facilitating enzymatic attack (primarily α-amylase) on starch granules in adjacent cells, and pericarp shrivelling that characterizes most mature triticale grains.

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