SCANNING ELECTRON MICROSCOPY OF MATURING TRITICALE, WHEAT, AND RYE

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ABSTRACT

Kernels of wheat, rye, and triticale that were harvested at different stages of maturity were viewed with a scanning electron microscope. The changes in the pericarp, aleurone, and endosperm regions of the kernels were observed for the last four weeks of kernel development. Complete collapse of all pericarp tissues was the only indication of abnormal development in the maturation of triticale. The observed changes in the endosperm and the aleurone layers of triticale proceeded as in maturing wheat and rye.

The scanning electron microscope (SEM) has been used increasingly in cereal research. The instrument is capable of producing three-dimensional images and combines the advantages of the light microscope with the better resolving power and higher magnification range of the transmission electron microscope.

SEM photographs of the structures of barley (1), oats (2), buckwheat (3), pearl millet (4), sorghum (5), and milled rice (6), as well as wheat, flour, and dough (7,8), have been published. In this study, kernels of wheat, rye, and triticale that were harvested at different stages of maturity were studied with the SEM for a comparison of kernel structures at different developmental stages and to investigate morphological changes during shriveling of the triticale kernel at maturation

MATERIALS AND METHODS

A tall spring triticale (6-TA-204), a hard red spring wheat (Colano), and a rye (Prolific) were seeded at the Colorado State University Agronomy Research Center in Fort Collins on April 10, 1976. Several rows were harvested on July 15, 22, and 29 and August 6 and 12. The kernels were kept frozen until needed. They were then defrosted and prepared for SEM without further treatment.

Representative kernels from each developmental stage were carefully cross-sectioned with a razor blade, placed on an aluminum disk specimen stage, and gold coated (500 Å). They were viewed with a Super I International Scientific Instrument Model SMS-2-1 scanning electron microscope. Pictures were taken using Polaroid Type 52 film.

RESULTS AND DISCUSSION

Each stage of development of the wheat and rye varieties was normal in its gross morphology and in its microscopic structure as observed with SEM during

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the last four weeks of kernel development. The wheat and rye varieties, which were grown for this study under agronomic and climatic conditions of the high altitude region of the United States, are generally ready for harvest approximately 40 days after anthesis. The rye variety requires a slightly longer time than does the wheat. Moisture content of the grains at the time of harvest is given in Table I. Four weeks before full maturity, triticale kernels were plump and relatively large compared with kernels of the parental species—wheat and rye (9). As kernel moisture decreased during maturation, the kernels decreased in size and became shriveled.

Changes in the Pericarp During Kernel Development

The pericarp, which surrounds the entire grain, is several layers thick at anthesis. An epidermis bounds it both externally and internally. Degeneration of the pericarp begins about three to four days after anthesis (10) and is seen as a breakdown in the regular array of cells. By seven days after anthesis, the cell walls are extensively degraded. Approximately 14 days after anthesis, only two or three layers of parenchyma tissue remain undistrubed (10). The development of rye grain is similar to that of wheat (11).

The two innermost layers of the pericarp do not degenerate with other cells of the parenchyma, although they change markedly in appearance as the grain matures. Changes in the pericarp of wheat and rye are illustrated in Fig. 1 and those of triticale in Fig. 2. The sequence of events in the destruction of the pericarp has been described by Percival (12) and Peterson (13) for wheat and by Hector (14) for rye. The mechanism responsible for the destruction of the pericarp tissue is not known, but is presumably enzymatic (10). Dedio *et al.* (15), for instance, have shown that dissolution of the starch granules in the pericarp of triticale is affected by an α-amylase that is specifically released in this tissue between 5 and 22 days after anthesis.

At anthesis, the inner epidermis of the pericarp consists of a layer of somewhat elongated cells. About eight days after anthesis, their cell walls have thickened slightly and individual cells have become separated from one another. As seen in Fig. 1, these cells lie with their long axis parallel to the long axis of the grain. In the mature grain they are referred to as tube cells. As the kernels of wheat and rye

TABLE I
Moisture Content (%) of Grains at Harvest[a]

Harvest Date	Appromimate Days After Anthesis	Triticale (6-TA-204)	Wheat (Colano)	Rye (Prolific)
July 8	11	60.0	47.8	67.0
July 15	18	55.1	44.2	56.7
July 22	25	49.6	27.2	45.7
July 29	32	34.2	13.1	35.9
Aug. 6	40	15.1	12.9	27.0
Aug. 12	46	12.0	...	20.8

[a] Grains were seeded April 10, 1976.

dry out, the tube cells are pressed into close contact with the testa, which is the crushed and desiccated remains of the integumen.

The pericarp of triticale kernels (Fig. 2) shows rather large, distinct tube cells four weeks before maturity. At maturity, these tube cells have collapsed

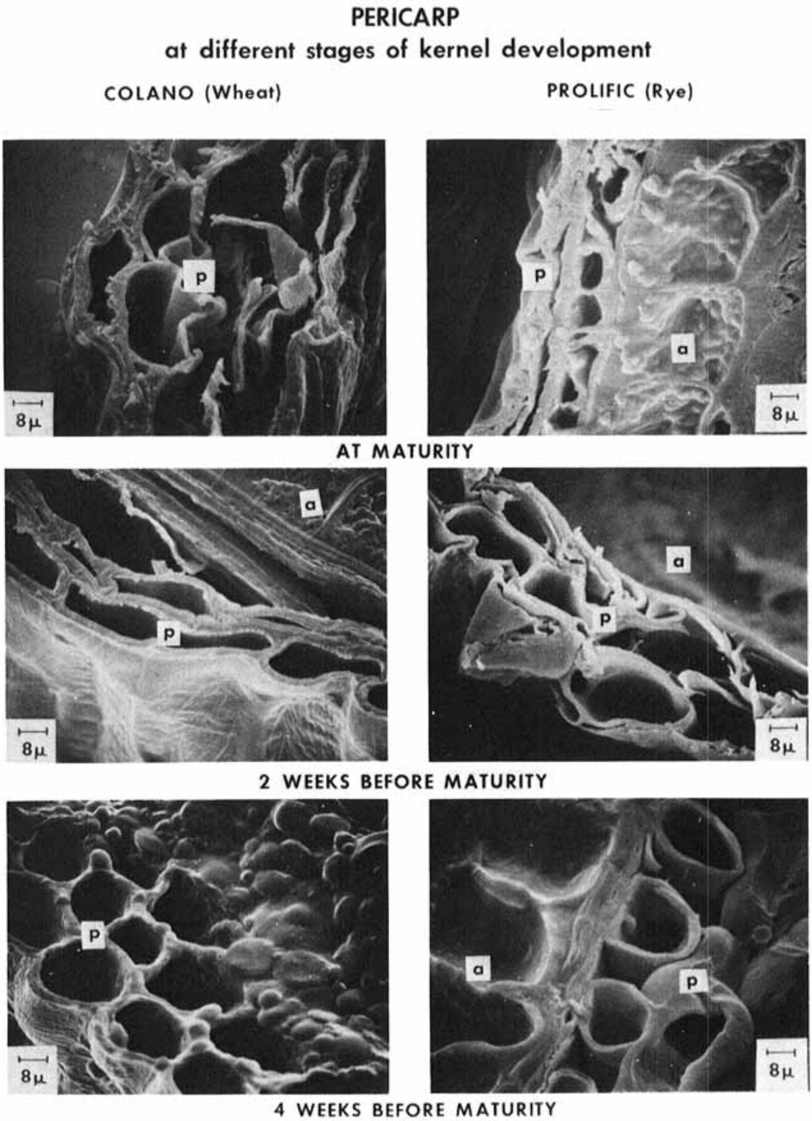

Fig. 1. Pericarp of wheat (Colano) and rye (Prolific) at different stages of kernel development. P, pericarp; a, aleurone; e, endosperm.

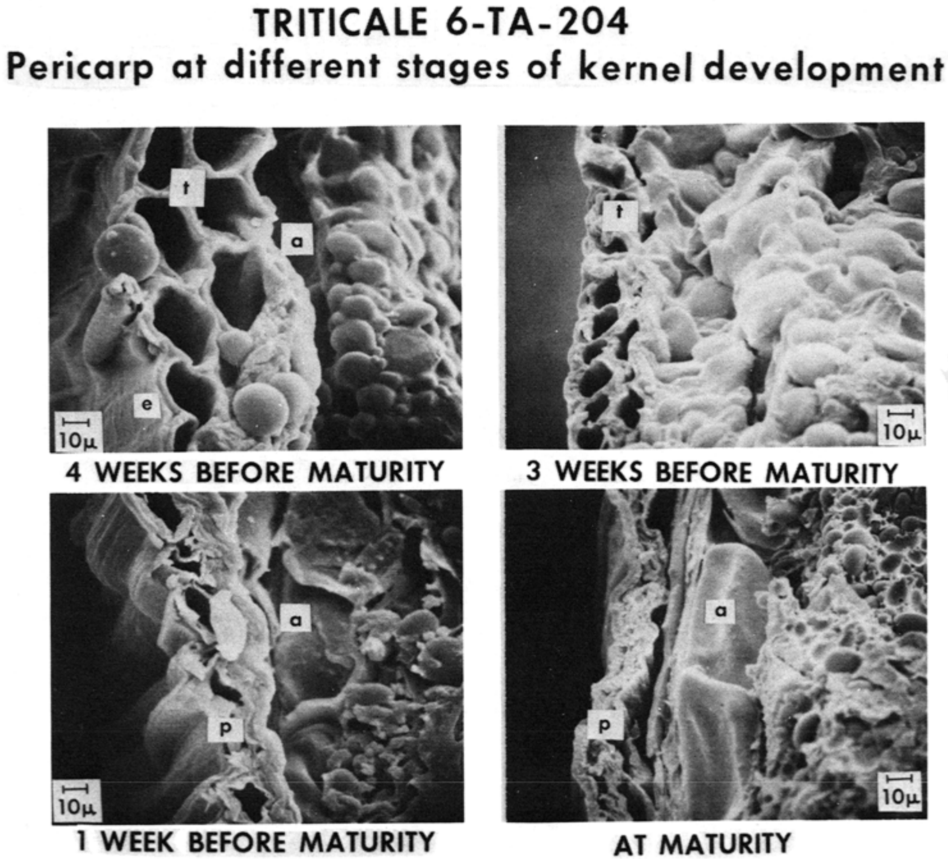

Fig. 2. Pericarp of triticale (6-TA-204) at different stages of kernel development. T, tube cells; e, epidermis; p, pericarp; a, aleurone.

completely and the enlarging endosperm has compressed the contents. The pericarp has shrunk and shriveled and is separated from the aleurone layer at certain points. The uneven outer surface becomes apparent, giving these kernels a shriveled appearance. The pericarp layer is rather dense and compact at that stage. The large cells on the inside of the pericarp, as seen in kernels one week

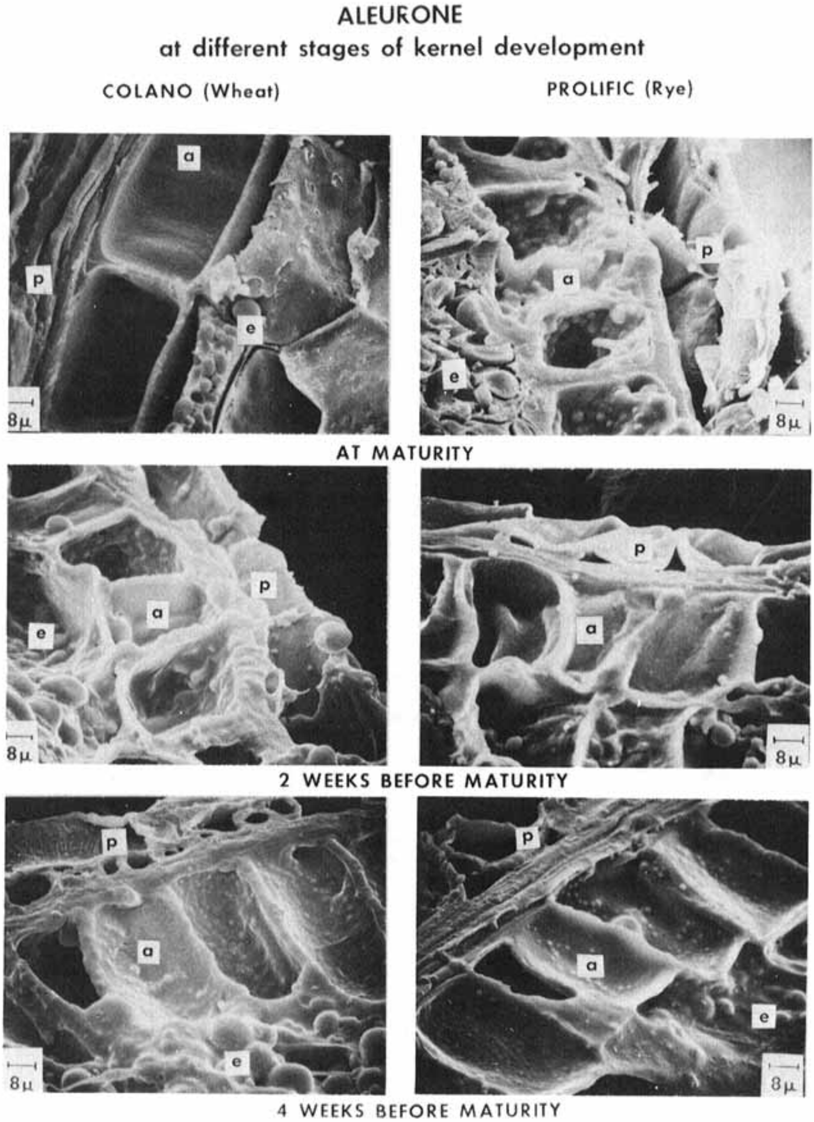

Fig. 3. Aleurone layer of wheat (Colano) and rye (Prolific) at different stages of kernel development. A, aleurone; p, pericarp; e, endosperm.

before maturity and at maturity, are cells of the aleurone layer. The whole of the pericarp is thin and papery in the ripe grain. The crushed and desiccated remains of the integumen constitute the testa or seed coat.

Changes in the Aleurone Layer During Kernel Development

The aleurone is botanically the outer layer of the endosperm and is one cell layer thick in wheat, rye, and triticale. It completely surrounds the starchy endosperm. Changes in the aleurone layer of wheat and rye are shown in Fig. 3 and of triticale in Fig. 4. The cells are square to slightly rectangular in each of the grains. The cell walls appear fragile four weeks before maturity but seem to increase in rigidity as the grains mature.

In mature rye, numerous aleurone granules characterize the aleurone layer. Lipid droplets, or spherosomes, surround these granules and comprise the lipid storage reserve of the grain (10). These aleurone granules are clearly visible in the mature rye sample (Fig. 3). Aleurone granules have also been found in mature wheat (16) and are present in the aleurone cells of mature kernels of triticale (17). These granules probably were dislodged when mature kernels of wheat and triticale were prepared for SEM. The separation of the pericarp from the aleurone layer of mature triticale is apparent (Fig. 4). This is probably partly responsible for the shriveled appearance of these kernels.

Simmonds and Campbell (10) have reported that an invagination into the underlying starchy endosperm tissue may disturb the normally regular arrangements of the cells in the peripheral layer of rye. Similar observations have been made in triticale (17). The phenomenon has been considered to be responsible for the shriveling of the triticale kernels. In triticale, a thickening and intrusion of the nucellar epidermis between adjacent dividing cells and degradation of the meristematic layer seem to cause the invaginations (10). Neither of these phenomena was observed in this study.

Changes in the Starchy Endosperm During Kernel Development

The starchy endosperm represents the bulk of the kernel. Starch deposition in the developing endosperm is first noticeable about ten days after anthesis. As the grain develops, existing granules enlarge and new granules are initiated (10). Deposition does not appear to start in any particular region. In the mature grain, however, the distribution of starch granules is considerably greater in the central endosperm than in the subaleurone layer (10).

Starch granules in kernels of wheat and rye that were harvested at different stages of kernel development are shown in Fig. 5. Starch granules of wheat and rye are generally of two principal size distributions—large lenticular granules and smaller spherical granules. This is shown in Fig. 5. Dronzek *et al.* (18) suggested that the smaller spherical-type granules of starch serve as nuclei on which more starch is deposited, preferentially in the equatorial plane, and that the ring structures that are formed grow toward each other in the equatorial plane and finally fuse. The center granule of the rye starch at full maturity (Fig. 5) might be an illustration of this phenomenon. The SEM work reported here did not show any evidence in granules of wheat or triticale that might correspond to this mode of granule development.

Starch granules in kernels of triticale at different developmental stages are shown in Fig. 6. Tightly packed spherical granules of different sizes are seen four

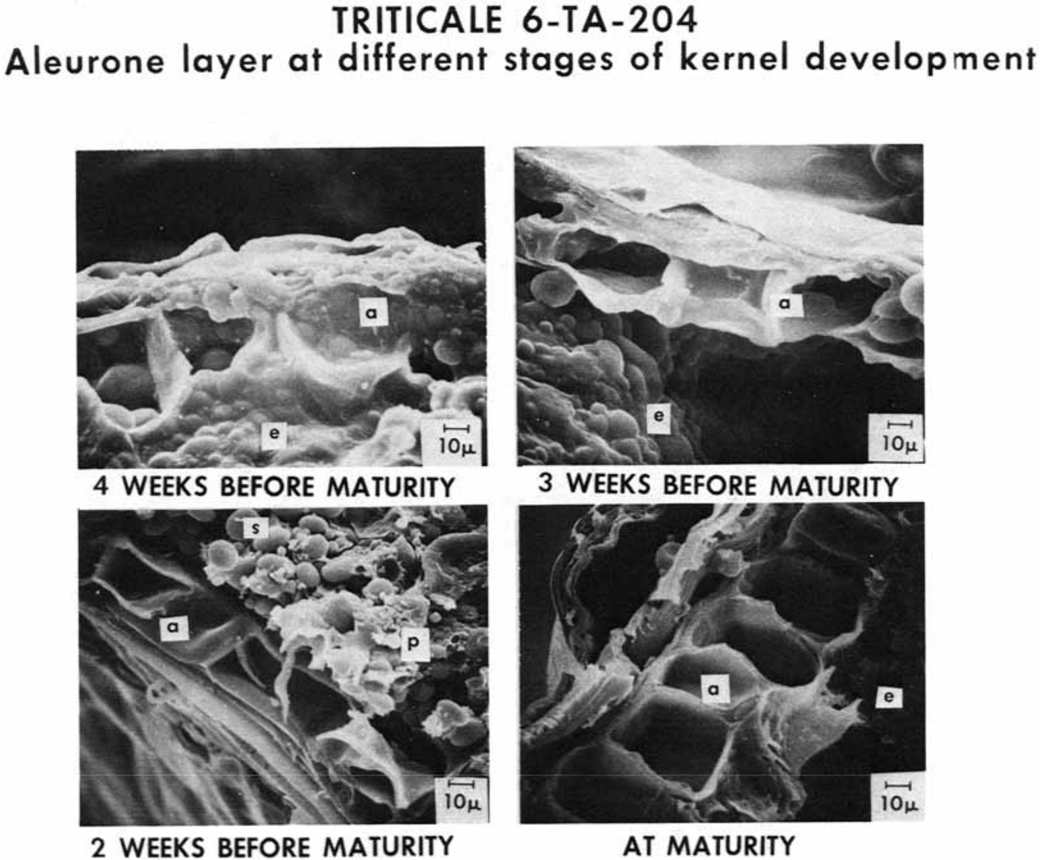

Fig. 4. Aleurone layer of triticale (6-TA-204) at different stages of kernel development. A, aleurone; e, endosperm; p, protein bodies; s, starch granules.

weeks before maturity. Presumably proteinaceous material fills the intergranular spaces. The granules are embedded in or covered by a viscous layer that likely is mostly water, since the moisture content, which is determined on the entire kernel, is approximately 50% at this stage of maturity. As the kernels mature and moisture content decreases, this viscous layer disappears and a somewhat more

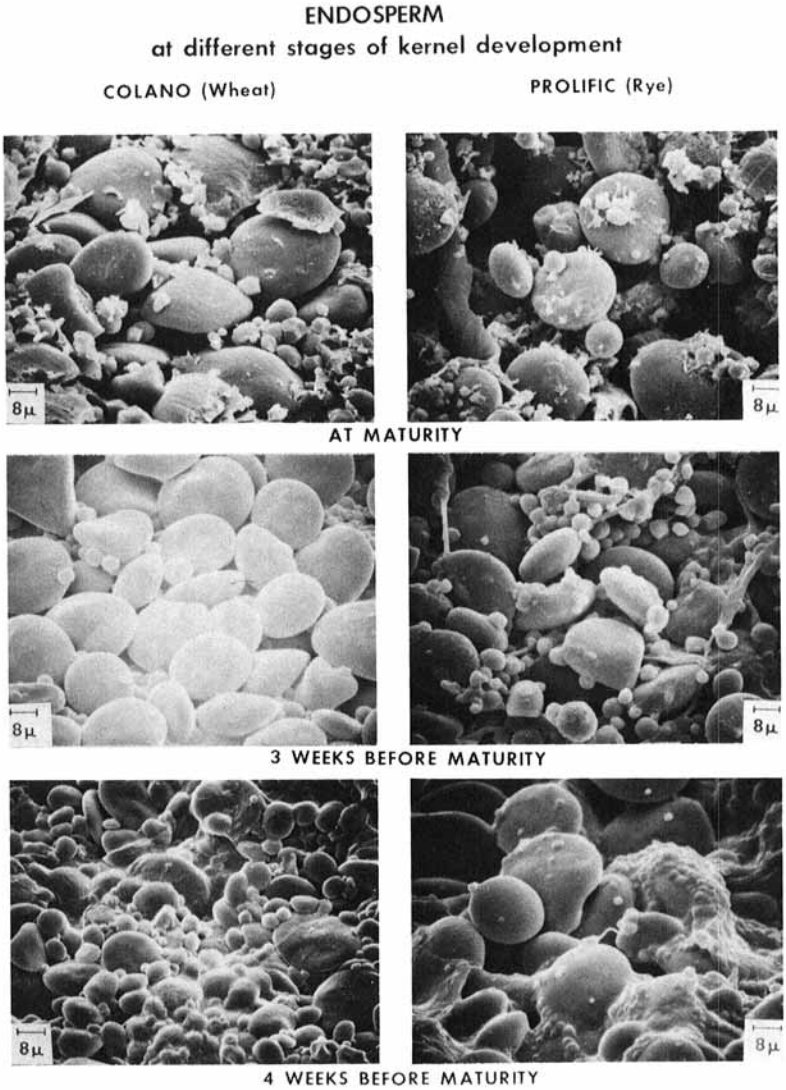

Fig. 5. Endosperm of wheat (Colano) and rye (Prolific) at different stages of kernel development.

TRITICALE 6-TA-204
Endosperm at different stages of kernel development

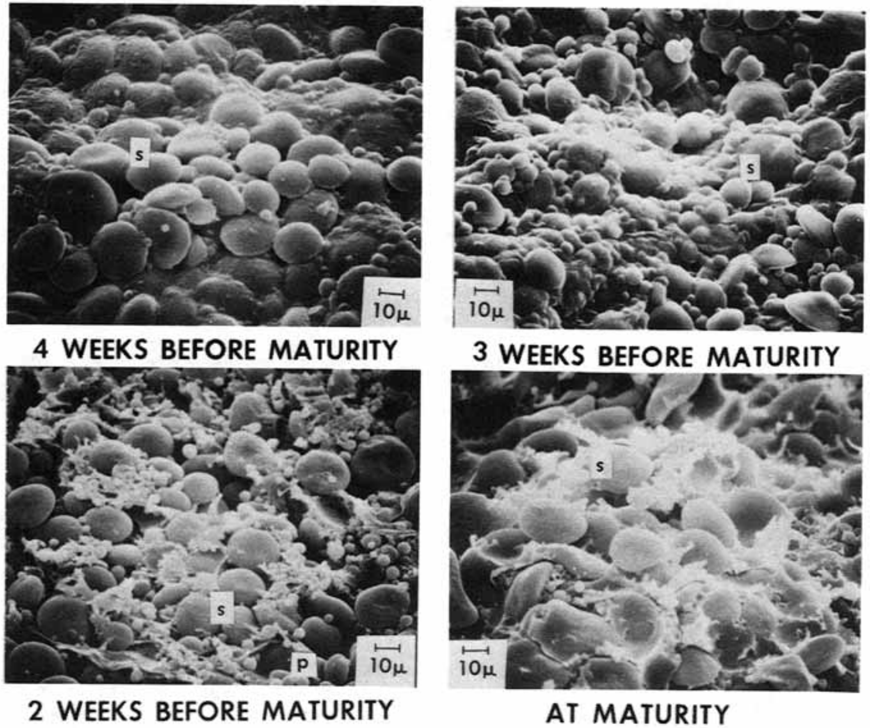

Fig. 6. Endosperm of triticale (6-TA-204) at different stages of kernel development. S, starch granules; p, protein bodies.

distinct protein matrix, in which granules of starch are randomly scattered, becomes apparent.

Several of the starch granules show indentations two weeks before maturity. Such indentations on starch granules have been explained as being caused by protein bodies on the flexible starch granules (19). Figure 6 shows small protein bodies next to the starch granules.

In this study, granules of starch were observed to dislodge easily when mature kernels of triticale, which had a moisture content of 12%, were cross-sectioned for SEM. This could account for the relatively small number of starch granules embedded in the matrix (Fig. 6).

Overall, the developmental sequence of starch granules in the endosperm of triticale during the last five weeks before maturity was similar to that observed for starch granules of wheat and rye.

CONCLUSION

Collapse of cellular layers within the pericarp was the only indication of abnormal development in the maturation of triticale that could possibly contribute to the shriveled appearance of mature triticale. The changes in the endosperm and the aleurone layer proceeded as observed for kernels of wheat and rye during maturation.

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