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THE EARLY DEVELOPMENTAL MORPHOLOGY OF THE TRITICALE GRAIN

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SUMMARY

Grain shrivelling in triticale was investigated using a histological approach. The pericarp, integumentary, nucellar, and embryo-sac tissues were examined at daily intervals from 0 to 14 days post-anthesis. The embryo-sac tissues, the endosperm and the aleurone were found to be most pertinent, since malformed aleurone and associated faults in the endosperm are apparently responsible for shrivelled areas of the grain. Precocious release of α-amylase is also considered to be a factor in grain shrivelling.

Triticale is assuming a more important role as a feed crop, and has recently been introduced commercially as a supplement for wheat flour in human nutrition (BRIGGS, 1973). It has considerable agricultural potential and has an inherently high protein content of nutritionally balanced amino acid composition. But due to a variety of factors, the full yield potential of triticale is not yet realized. Moreover, it is characteristic of this species to exhibit some degree of kernel shrivelling which detracts considerably from both appearance and bushel weight.

Preliminary work has suggested that kernel shrivelling may be caused by the failure of certain cells in the developing endosperm to fill with starch and protein. This is particularly noticeable in the sub-aleurone layers of the endosperm, where damaged areas may be quite frequent and extensive. A comprehensive light- and electron-microscopic investigation was therefore undertaken in order to examine the developmental morphology of triticale grain from anthesis to maturity. It was hoped that this would determine the stage at which damage may first be recognized, and, if possible, its cause.

For this purpose, triticale strains 6A190, 6A250, UM6517, Rosner (accessions from the University of Manitoba) and Kangaroo x UM940 'S' and Beaver 'S' (accessions from CIMMYT, Mexico) were grown under glasshouse conditions and compared with plants of rye (*Secale cereale* L. cv. Prolific), durum wheat (*Triticum turgidum* L. var. durum cv.

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Stewart) and common wheat (*T. aestivum* L. em. Thell. cv. Manitou). Spikes were tagged at anthesis, and collected at daily intervals up to 14 days thereafter. The grains were carefully sliced approximately 1 mm thick and fixed in a glutaraldehyde-paraformaldehyde fixative (KARNOVSKY, 1965) for 24 hours, washed, then post-fixed for 15 hours in 2% osmium tetroxide. After fixation the sections were dehydrated and infiltrated with either glycol methacrylate according to the procedure of FEDER and O'BRIEN (1968) or with SPURR's (1969) low viscosity epoxy resin.

Glycol methacrylate sections were cut at 2 µm for light microscopy and stained with toluidine blue. Epoxy sections were cut in the gold range and stained with lead citrate (REYNOLDS, 1963) and uranyl acetate, for examination by electron microscopy. Grains up to 3 days post-anthesis were also cleared and examined by phase contrast microscopy as described by HERR (1971).

OBSERVATIONS

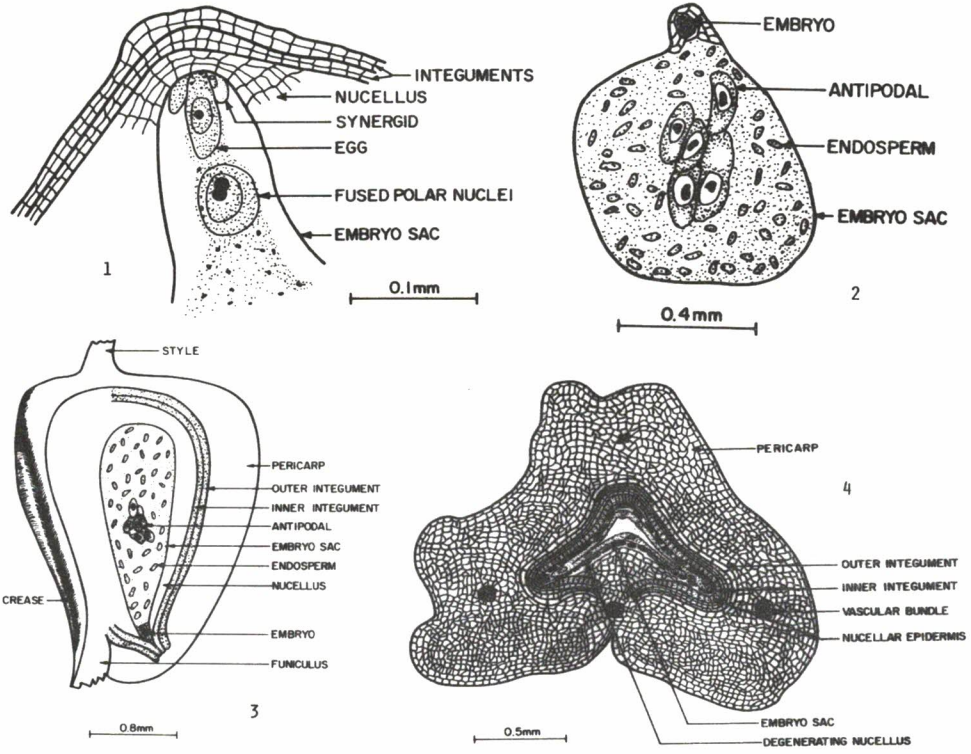
The triticale ovulary consists of a single anatropous, bitegemic, crassinucellate ovule which at anthesis contains an embryo sac consisting of an egg, two degenerating synergids, two fused polar nuclei, and several antipodals (Fig. 1). Approximately two days after anthesis, fertilization has been completed. At this stage the embryo sac contains a proembryo (usually about 4-celled), numerous free endosperm nuclei, and several large antipodals (Fig. 2, 3). The endosperm immediately surrounding the proembryo appears to be cellularized; the remainder consists of free nuclei variable in size and containing 3-7 nucleoli (Fig. 2, 3). The antipodals, which vary in number, are most commonly positioned in the center of the embryo sac surrounded by endosperm nuclei, and are usually disintegrating 2 days post-anthesis. They may be recognized by their large size and large, irregular nucleoli (Fig. 2, 3). Disintegration of the antipodals accompanies endosperm development, suggesting that antipodal lysis affords a nutritional source for this purpose.

At three days after anthesis the ovule is completely enclosed by starch-rich pericarp tissue (Fig. 4, 5), which is immediately adjacent to the outer integument. However, by the fifth day after anthesis, the pericarp has started to degenerate, possibly to provide further nutrients for the developing embryo sac.

The outer integument is composed of three cell layers, of which only the outermost persists at maturity (Fig. 4, 5). This layer develops functional chloroplasts at about three days after anthesis, and starch granules may be seen in these cells. Directly beneath the outer integument are the two cell layers of the inner integument. These layers are usually crushed by the enlarging embryo sac between 6-8 days after anthesis.

The columnar cells of the nucellar epidermis, immediately adjacent to the inner integument, persist for some time after all other nucellar tissue has disintegrated. Evidence suggests that the nucellar epidermis is unaffected by lytic enzymes which destroy other nucellar tissue. Again, this disintegration presumably provides nutrients for the developing embryo sac. Judging by electron microscopic observations (SHEALY and SIMMONDS, unpublished), the cells of the nucellar epidermis appear to be metabolically active until six or seven days after anthesis. This is similar to the situation in *Capsella* (SCHULZ and JENSEN, 1971), where the persistent synergids and chalazal proliferating cells appear to be synthetically active

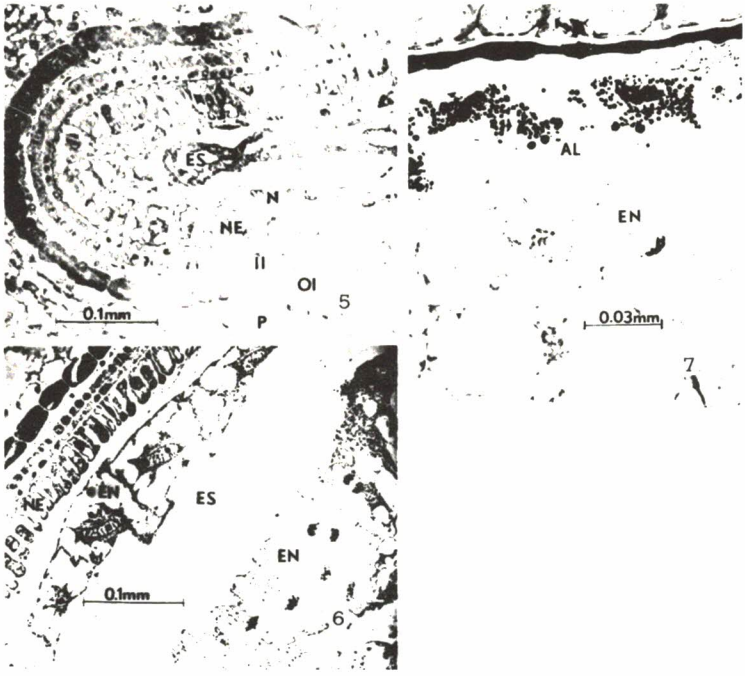
prior to their breakdown. SCHULZ and JENSEN suggest that these cells may be engaged in metabolizing and storing nutrients from surrounding ovular tissue to be utilized by the developing embryo sac.



Figures 1-4. Drawings under light microscope of portions of triticale kernels: (1) Micropylar portion, at anthesis, from cleared ovule. (2) Embryo sac dissected from ovule 48 hours post-anthesis; from cleared ovule. (3) Longitudinal section 48 hours post-anthesis. (4) Cross section at 72 hours post-anthesis; from glycol methacrylate section.

In triticale, as in most angiosperms, the endosperm results from the fusion of two polar nuclei with one male gamete. This results in a triploid primary endosperm nucleus which divides mitotically to produce numerous free nuclei (Fig. 2-5). These nuclei are positioned around the periphery of the embryo sac, which begins to cellularize by cytokinesis about 4-5 days after anthesis (Fig. 6).

The outermost layer of the endosperm becomes discernible as aleurone under the light microscope at approximately ten days after anthesis (Fig. 7). However, under the electron microscope, aleurone bodies are seen to be clearly differentiated by seven days post-anthesis. Starch grains are conspicuous and well developed by this stage, and by the tenth day they are the dominant feature of the



Figures 5-7. Electron micrographs of portions of triticale kernels: (5) Cross section through portion of ovule at 72 hours post-anthesis with pericarp (P), outer integument (OI), inner integument (II), nucellar epidermis (NE), nucellar tissue (N), and embryo sac (ES). (6) Embryo sac surrounded by nucellar epidermis 120 hours post-anthesis. Note division of endosperm cells (EN) on ventral side of embryo sac. (7) Aleurone (AL) and endosperm 14 days post-anthesis. Note that aleurone cells are incompletely filled with aleurone bodies at this stage.

endosperm. By this time tangential cell division from the cambial layer separating aleurone from endosperm cells is almost completed, and by fourteen days post-anthesis the endosperm contains its full complement of storage cells. These continue to enlarge and become increasingly filled with starch granules and protein until maturity.

Vasculature of the developing triticale grain consist of four traces which branch from a basal vascular bundle. Three traces, two lateral and one dorsal to the embryo sac, extend through the pericarp (Fig. 4). However, the dorsal trace is small and not as conspicuous as the lateral traces. These three traces degenerate with the

pericarp and leave only the fourth trace, which extends through the funiculus and supplies the developing ovule.

DISCUSSION

Two modes of endosperm development have been proposed. BRENCHLEY (1909) stated that the entire endosperm tissue arises by cellularization of a syncytial mass formed by multiple, free-nuclear divisions of the first endosperm nucleus. On the other hand GORDON (1922) supported by EVERS (1970) believed that the endosperm arises by serial tangential cell division of a cambial layer lining the embryo sac, this layer having been derived by multiple divisions of the first endosperm nucleus.

Yet other workers (SANDSTEDT, 1946; YAMPOLSKY, 1957; JENNINGS and MORTON, 1963; BUTTROSE, 1963) have suggested that the inner endosperm may arise as described by BRENCHLEY, and the outer layers as described by GORDON, and our own observations would tend to support this mechanism. Thus at 4 days after anthesis, the embryo sac appears as a closed hollow cylinder in which free nuclei are embedded in the cytoplasm forming the outer walls. Between these free nuclei are thickened zones of cytoplasm which appear under the light microscope to be cell wall initials. By five days after anthesis cellularization is well advanced in the ventral (crease) side of the embryo sac, and is commencing in the dorsal side. By six days after anthesis, cell walls have been completely laid down randomly throughout the whole of the endosperm. Indeed, on the ventral side of some embryo sacs at 5 days post-anthesis, mitosis was observed in the innermost layer of endosperm cells (Fig. 6), but not in the peripheral cells as suggested by EVERS (1970). Full clarification of the precise mechanism must await examination of sections taken at intervals more frequent than 24 hours.

The relationship between developmental morphology and grain shrivelling suggest that this quality defect arises from an interference with aleurone formation which may be first recognized at about six days and is well established by ten days after anthesis. Invagination or deletion of the aleurone and subsequent malformation of endosperm have been observed to cause shrivelled areas which range from moderate to severe. Further, the precocious release of α-amylase in these areas leads to premature digestion of starch granules and in some cases extensive loss of kernel weight (KLASSEN, 1970). Both malformed aleurone and precocious α-amylase release are severe quality defects which lead to loss in bushel weight.

In this paper we have attempted a preliminary study concerned only with the physical basis of grain shrivelling. Once the precise mechanisms responsible for this phenomenon have been identified, the way will be open for a solution to the problem by genetic means.

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