Anatomy and Histochemistry of *Echinochloa turnerana* (Channel Millet) Spikelet

D. W. IRVING, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, CA 94710

Cereal Chem. 60(2):155–160

ABSTRACT

Mature spikelets of *Echinochloa turnerana* were studied by means of scanning electron, fluorescence, and brightfield microscopy to delineate anatomical features and to localize storage components in the seed. The pericarp was a thin, two-cell layered structure and contained anisotrophic substances in cell walls. The cuticle was localized beneath the pericarp, and in the mature caryopsis the seed coat had been completely obliterated, as is common in the gramineae. The aleurone was a layer of thick-walled cells. Beneath the embryo, at the base of the fruit, were large aleurone transfer cells and a pigment strand. Embryo morphology was typical of cereal grains. The scutellum, coleorhiza, and coleoptile contained protein bodies, carbohydrate, and small vacuoles. The starchy endosperm contained nearly spherical starch granules, lipid, and protein.

Numerous studies on the structure of grass caryopses have included observations on the aleurone layer (Fulcher et al 1972, 1977), embryo (Bechtel and Pomeranz 1978a, Rost 1972), coleoptile (O'Brien 1967; O'Brien and Thimann 1967a, 1967b), scutellum (Smart and O'Brien 1979a, 1979b; Swift and O'Brien 1972a, 1972b), and endosperm (Bechtel and Pomeranz 1978b, 1979, 1981; Wood and Fulcher 1978). However, these studies were predominantly concerned with the commercial grains barley, oats, wheat, and rice.

Echinochloa turnerana (channel millet), a native to the arid channel region of inland Australia, has been recognized as a promising grain and forage crop for arid regions (NAS 1975). In its native habitat, it was recognized as one of the most productive, palatable, and nutritious fodder grasses. *E. turnerana* was very drought tolerant; deep flooding was a requirement for germination and allowed the plant to develop completely without additional watering (NAS 1975); ie, the soil retained enough moisture to sustain the developing plant. The plant is as salt tolerant as sorghum, barley, and bermuda grass (Shannon et al 1981).

Information on the anatomical structure of *E. turnerana* is crucial if the plant is to be used commercially. This study deals with spikelet anatomy of *E. turnerana* and localization of important storage components in the caryopsis, namely protein, carbohydrate, and lipid.

MATERIALS AND METHODS

Seed Samples

Mature panicles of *E. turnerana* were received from J. Ison, Department of Primary Industries, Brisbane, Queensland, Australia, in December 1976.

Scanning Electron Microscopy

Spikelets were left intact and sequentially dissected by removing successive floral parts. Samples were then attached to aluminum specimen stubs and sputter-coated with gold in a PS-2 coating unit (International Scientific Instruments, Inc.). Specimens were viewed and photographed in an International Scientific Instruments, Inc. Super I scanning electron microscope (Irving et al 1981). Caryopses were placed in a fixative containing 6% glutaraldehyde and 3% acrolein in 0.025M Na_2HPO_4-NaH_2PO_4, pH 6.8, evacuated approximately 3 hr, and fixed for 48 hr (0–4° C). Following dehydration, tissue was ethanol-cryofractured in liquid nitrogen (Humphreys et al 1974) and critical-point dried in a Polaron E-3000 critical-point dryer using CO_2 as the transition fluid. Specimens were then mounted, gold-coated, and viewed as above.

Light Microscopy

Brightfield Microscopy. Caryopses were halved (some longitudinally and others transversely) and fixed in formalin-acetic acid-alcohol (Jensen 1962), dehydrated, and embedded in paraffin. Sections 10 μm thick were cut on a rotary microtome and stained with Safranin O and Fast Green FCF (Berlyn and Miksche 1976). Sections were photographed in a Nikon SMZ-10 stereo zoom microscope using Kodak 4 × 5 in. film.

Glycol methacrylate sections were prepared as for fluorescence microscopy and stained with acid fuchsin followed by toluidine blue O, as described by Feder and O'Brien (1968).

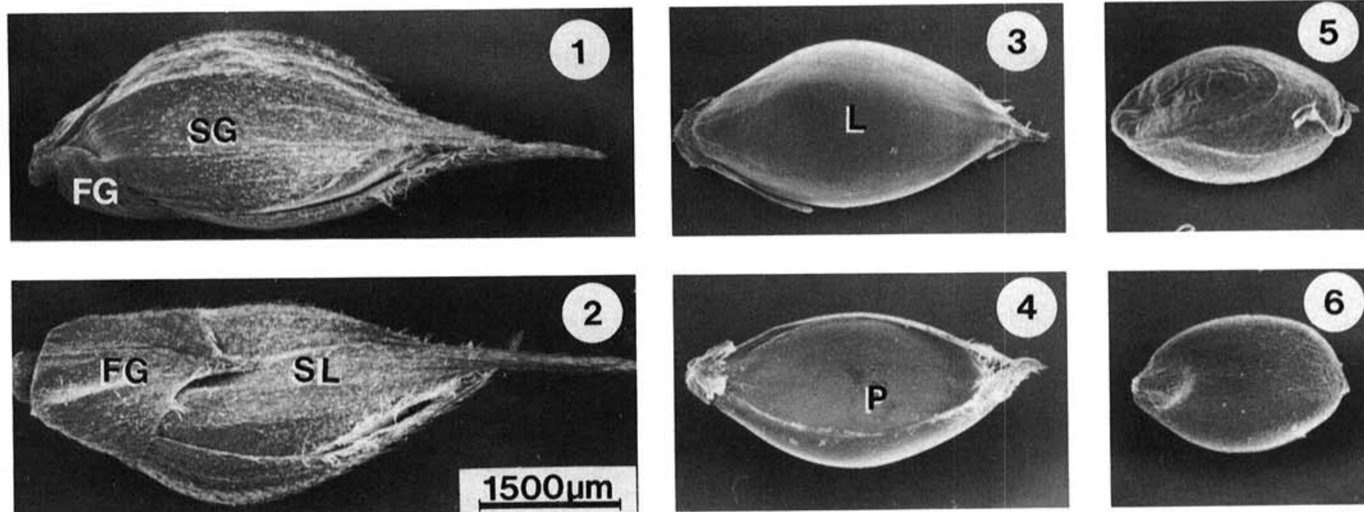
Fluorescence Microscopy. Caryopses of *E. turnerana* were cut in half, fixed in 6% glutaraldehyde in 0.025M Na_2HPO_4-NaH_2PO_4 buffer for 18 hr, pH 6.8 (0–4° C), and dehydrated with two changes each of 2-methoxyethanol, ethanol, *n*-propanol, and *n*-butanol (21° C) (Feder and O'Brien 1968). Tissues were infiltrated with glycol methacrylate (monomer, 0.9% benzoyl peroxide, and 5.0% polyethylene glycol) at 0–4° C for two to three days, and embedded in glycol methacrylate (JB-4 medium, Polysciences, Warrington, PA) (Lulham 1979), using only the above mixture. Polymerization was performed at 40° C. Material was sectioned 2–5 μm thick, using a Sorvall Porter-Blum MT-2 ultramicrotome equipped with glass knives.

Two staining methods were employed to determine the location of protein in tissues. A modification of the ninhydrin-Schiff's reaction for total protein as described by Jensen (1962) and Pearse (1960) was employed in the first method. The ninhydrin reaction involves production of a stable, nondiffusible aldehyde from protein groups that then react with Schiff's reagent, yielding a fluorescent product. Glycol methacrylate sections were first treated with dinitrophenylhydrazine (saturated solution in 15% acetic acid) to block free aldehydes (Feder and O'Brien 1968), and then placed in 0.5% ninhydrin in absolute ethanol at 37° C for 24 hr. Sections were washed in ethanol and water and treated with Schiff's reagent for 1–2 min. Sections were washed briefly in running water, air-dried, and mounted. The second method for protein localization involved staining for 1–2 min in aqueous acid fuchsin (Fulcher and Wong 1980). As a control measure in protein localization, sections were incubated in either 1% trypsin (lyophilized, Worthington Biochemical Corp., Freehold, NJ) in 0.05M tris-HCl (pH 8.0) or in 0.4% pronase (B grade, Calbiochem, San Diego, CA) in water (18 hr at 40° C). Additionally, sections were treated in tris-HCl buffer or in water without enzyme. Treatment of slides with enzyme or buffer was followed by acid fuchsin staining as described previously.

Carbohydrate was localized by treatment with dinitrophenylhydrazine, then with periodate-Schiff's, according to methods of Fulcher and Wong (1980).

A technique developed by Pease (1973) involving the polymerization of glutaraldehyde, urea, and glycol methacrylate was used to preserve lipids for localization. Sections were stained with 0.001% aqueous Nile blue A (Fulcher and Wong 1980), dried, and mounted in immersion oil. Whereas Fulcher and Wong suggested observation of wet-mounted sections, superior resolution was obtained using sections mounted dry with nonfluorescing immersion oil.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Association of Cereal Chemists, Inc., 1983.



Figs. 1–6. Scanning electron micrographs of a mature spikelet of *Echinochloa turnerana* showing first and second glumes, embryo side (**Fig. 1**), and other side showing first glume and sterile lemma (**Fig. 2**). Spikelet with outer bracts removed and showing the hardened lemma (**Fig. 3**) and palea (**Fig. 4**). Caryopsis, embryo side (**Fig. 5**), and opposite side (**Fig. 6**). Magnification is the same in all micrographs. FG = First glume, L = lemma, P = palea, SG = second glume, SL = sterile lemma.

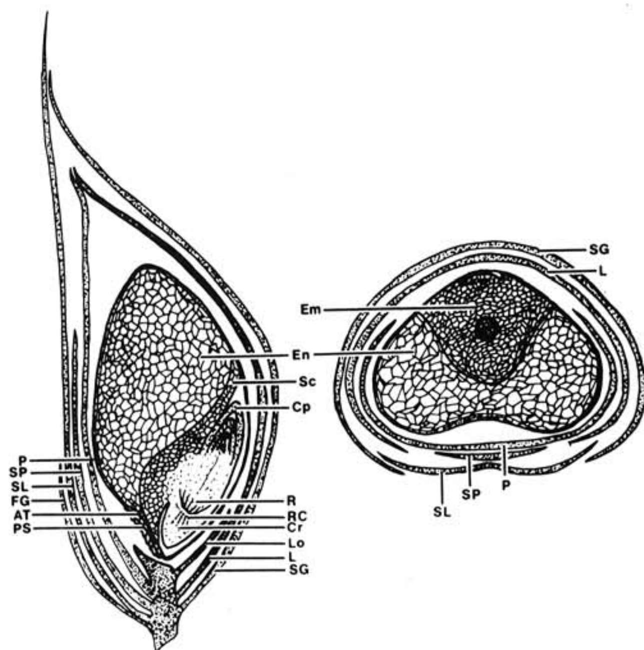
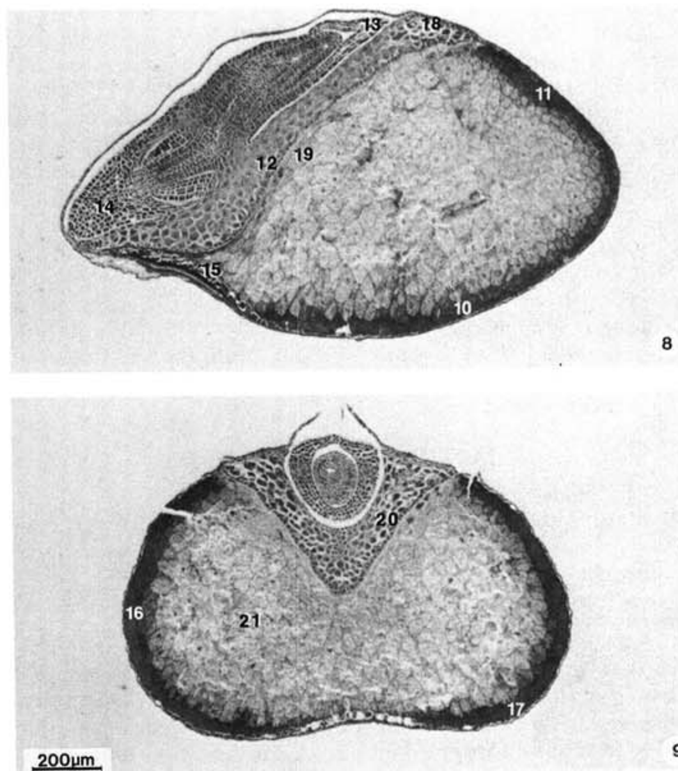


Fig. 7. A diagrammatic representation of longitudinal and cross sections of *Echinochloa turnerana* spikelet. AT = Aleurone transfer cells, Cp = coleoptile, Cr = coleorhiza, Em = embryo, En = endosperm, FG = first glume, L = lemma, Lo = lodicule, P = palea, PS = pigment strand, R = radicle, RC = root cap, Sc = scutellum, SL = sterile lemma, SG = second glume, SP = sterile palea.

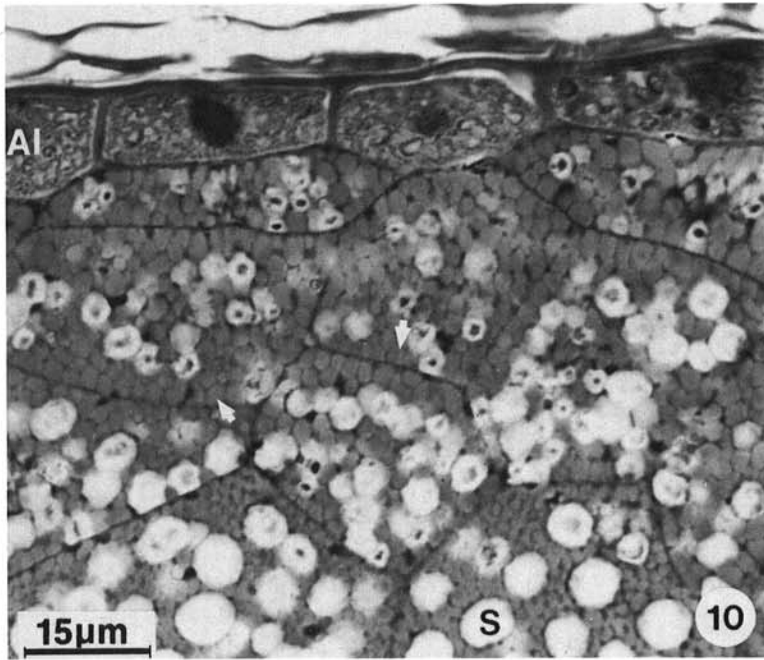


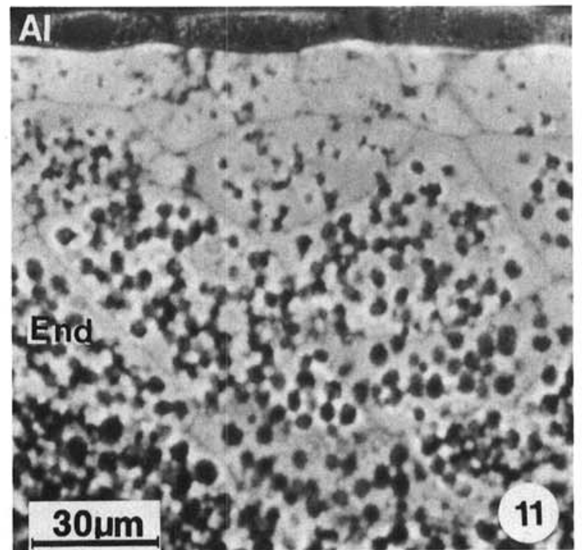
Figs. 8–9. Safranin O and Fast Green FCF-stained longitudinal (**Fig. 8**) and cross (**Fig. 9**) sections of mature *Echinochloa turnerana* caryopsis. Magnification is the same in both micrographs. Numbers indicate approximate tissue location of Figs. 10–21.

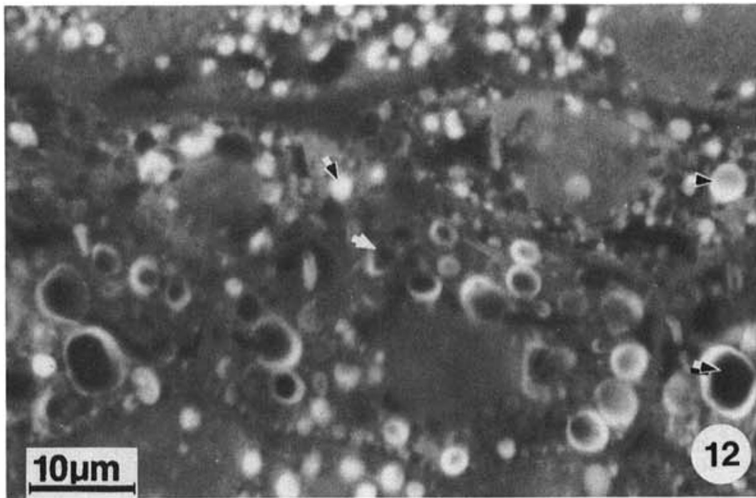
All preparations were viewed and photographed using a Nikon Fluophot epifluorescent microscope and a G-excitation (500–550 nm) filter combination. Photomicrographs were made with Kodak 35 mm Plus X Pan (ASA 125) or Tri-X Pan (ASA 400) film.

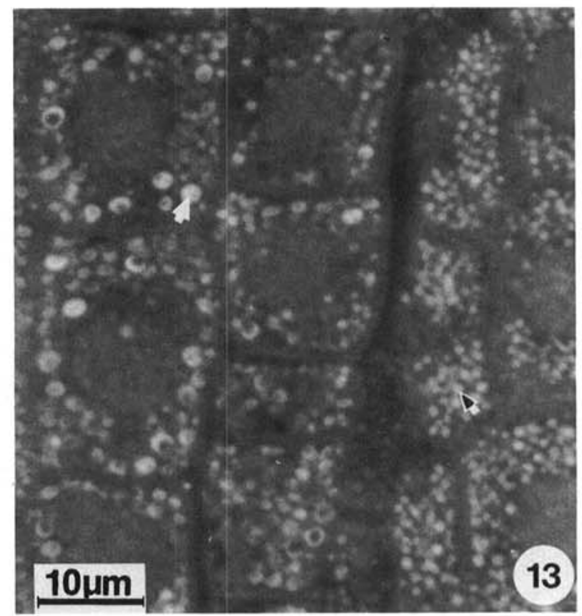
RESULTS AND DISCUSSION

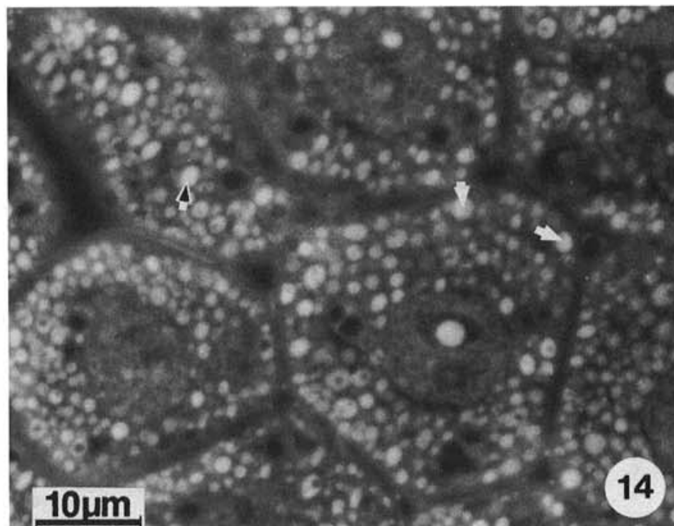
A typical mature spikelet of *E. turnerana* measured 4–5 mm in length by 1.5 mm wide, and had two glumes. The first glume was reduced and could be seen at the base of the spikelet (Fig. 1) on the embryo side (Figs. 1, 3, 5) of the spikelet, and extended its apex less than halfway on the other side of the spikelet (Fig. 2). The second glume (Fig. 1) was large and covered the lemma (Fig. 3). The sterile lemma (Fig. 2) had its base beneath the first glume and its apex beyond that of the second glume. The sterile palea (not shown) occurred beneath the sterile lemma. The lemma (Fig. 3) and palea (Fig. 4) were hardened, and completely enclosed the caryopsis (Figs. 5 and 6), which was approximately 1.5 mm in length and 1.0 mm in width. *E. turnerana* spikelet anatomy, as depicted in Fig. 7, was similar to that of Japanese millet *E. utilis* as described by Zee and O'Brien (1971). Longitudinal (Fig. 8) and cross (Fig. 9) sections











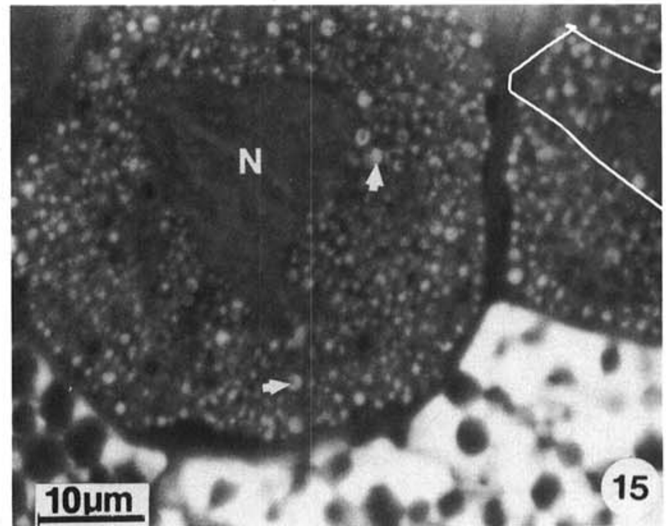


Fig. 10. Brightfield micrograph of the starchy endosperm and aleurone of *E. turnerana* following staining with acid fuchsin and toluidine blue. Protein bodies appear dark in brightfield; starch is unstained or white. **Figs. 11–15.** Protein bodies appear bright when stained and viewed with fluorescence microscopy. Fluorescence view of protein bodies after acid fuchsin staining showing starchy endosperm and aleurone (**Fig. 11**), the dark areas interspersed in the protein are starch granules. Longitudinal sections of the scutellum (**Fig. 12**), coleoptile (**Fig. 13**), coleorhiza (**Fig. 14**), and aleurone transfer cells (**Fig. 15**). Al = Aleurone, End = endosperm, N = nucleus, S = starch. Arrows indicate protein bodies.

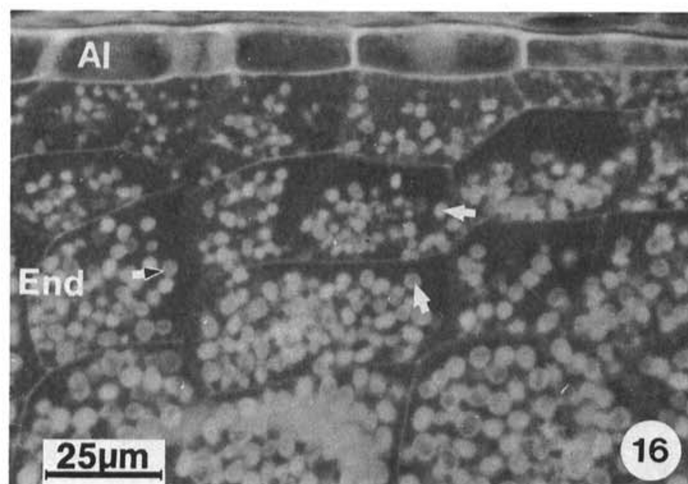
revealed the general structure of the caryopsis. As is typical of the gramineae, the proportionately large endosperm was filled with starch granules and had an outer layer higher in protein plus an aleurone layer. Embryonic structure showed all the features of grasses including a scutellum, coleoptile, and coleorhiza.

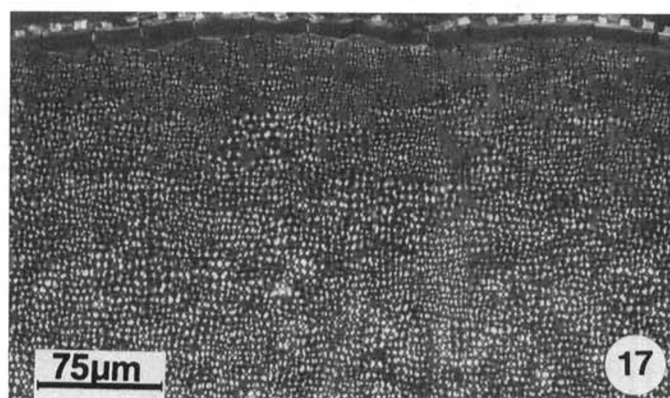
Localization of protein with fluorescent microscopy using either ninhydrin-Schiff's treatment or acid fuchsin staining indicated that protein-staining affinity and intensity varied throughout the caryopsis tissues. The first few cell layers beneath the aleurone layer were found to have the greatest affinity for protein stains (Figs. 10 and 11), where tightly packed protein bodies (Fig. 10) were visible; affinity for protein stains diminished toward the center of the caryopsis. This protein distribution pattern is typical of other cereal grains. Cytoplasmic components (protein bodies) of cells in the aleurone layer, a single cell layer thick, also had affinity for protein dyes (Figs. 10 and 11).

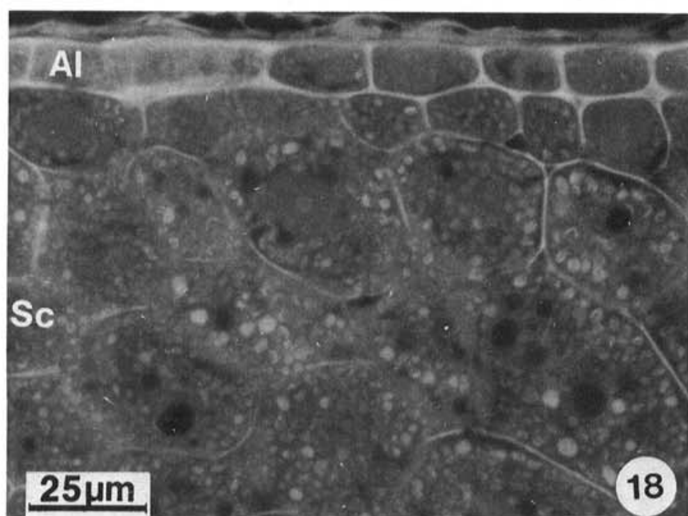
Sections were treated with proteolytic enzymes (not shown) for further characterization of acid fuchsin-positive structures as a definitive localization of protein bodies. Trypsin-treated sections showed complete digestion of protein in embryonic tissues; thus, no reaction was observed upon staining with acid fuchsin. Endosperm protein bodies were not digested by trypsin, but were digested by pronase. To determine whether autolysis occurred during proteolytic enzyme treatment and perhaps interfered with the reaction, control sections were incubated under the same conditions as for proteolysis in tris-HCl buffer or in water without protease. Controls showed no evidence of autolysis.

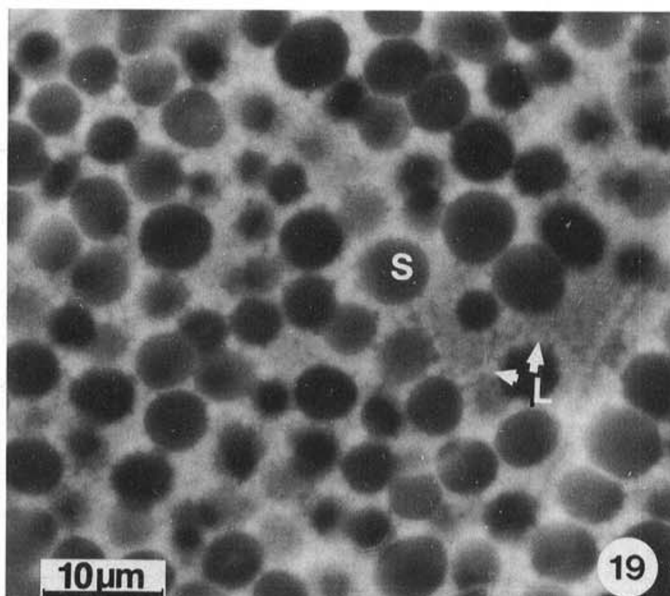
Protein bodies, as defined by acid fuchsin staining and proteolytic digestion, were localized in the scutellum (Fig. 12), coleoptile (Fig. 13), and coleorhiza (Fig. 14). The partial vacuolization of protein bodies (dark centers of protein bodies in Fig. 12) could be the result of autolytic digestion in the dormant caryopsis. Several species of seeds have been reported to contain a number of hydrolytic enzymes in the protein bodies (Rost 1972, Yatsu and Jacks 1968), suggesting that protein bodies may be lysosomal in nature, thus functioning in intracellular digestion (Rost 1972). In *Setaria lutescens*, a member of the gramineae family, Rost (1972) found protein bodies in all stages of digestion in dormant caryopses, indicating that protein body digestion in this species was not inhibited by dormancy. When a protein body was completely digested, all that remained was an empty vacuole (Rost 1972).

Preliminary studies indicated that inclusions were located within some of the protein bodies of *E. turnerana* tissues, which appeared in the smaller, unvacuolated protein bodies (Figs. 12–15) as small areas left unstained by protein dyes. The type of inclusion was not determined, however, with toluidine blue staining (not shown); metachromatic substances located in protein bodies indicated that the inclusions were globoid. Fulcher et al (1981) described phytin globoids found in the aleurone layer of wheat, barley, and oats as crystals of calcium and magnesium double salt of myoinositol hexaphosphate, which stained metachromatically (red) with









Figs. 16, 18. Periodate-Schiff's-treated sections followed by fluorescence viewing of the starchy endosperm/aleurone (**Fig. 16**) and scutellum/aleurone (**Fig. 18**). **Fig. 17.** View of an unstained section of the endosperm/aleurone through polarized light. **Fig. 19.** Nile blue A-treated section of the central endosperm near the embryo of *E. turnerana* showing lipid bodies. Al = Aleurone, End = endosperm, L = lipid body, S = starch granule, Sc = scutellum. Arrows in Fig. 16 indicate starch granules. In Fig. 19, arrows are coming from L, which indicates lipid bodies.

toluidine blue. Globoid inclusions were described elsewhere in cereal grains and legumes (Rost 1972) and were shown to contain a form of stored phosphorus and to be associated with high acid phosphatase activity. Elsewhere, globoid inclusions ("phytate deposits") were reported in protein bodies of wheat scutellum (Swift and O'Brien 1972a) and, in some cases, occupied most of the space within the protein body.

Aleurone transfer cells that occurred in the region of the pigment strand contained deposits that had an affinity for protein stains (Fig. 15). Aleurone transfer cells, ordinarily much larger than normal aleurone cells, were believed to conduct nutrients to the developing caryopsis (Zee and O'Brien 1970, 1971) in other grains.

During histological examination of tissues, we assumed that low molecular weight saccharides were extracted during the tissue preparation; therefore, a positive periodate-Schiff's reaction was regarded as denoting the presence of polysaccharides, mucopolysaccharides, glycoproteins, or glycolipids (Dahlquist et al 1965, Lillie 1954).

The aleurone cells of *E. turnerana* had thickened cell walls (Fig. 16), and carbohydrates appeared to be deposited randomly within the aleurone cytoplasm. The layer of endosperm just beneath the aleurone had smaller and fewer starch granules (Figs. 16, 17) (as expected from the high protein content, Fig. 11) than cells in the center of the kernel. Polarizing optics showed the birefringence of cross walls of the aleurone cells and pericarp cell walls (Fig. 17), which indicated that these walls were composed mostly of cellulose, which has a very regular structure because of the precise arrangement of microfibrils in the cell walls (Esau 1977). Starch granules were also birefringent and demonstrated concentric polarization crosses typical of starch.

The scutellum comprised a large portion of the embryo, as in most grasses. Distinct deposits of carbohydrate in the cytoplasm became visible in the fluorescence microscope upon treatment with periodate-Schiff's (Fig. 18). Viewing through polarized light (not shown) showed that some periodate-Schiff's positive substances in the scutellum were small starch granules, unlike wheat, which was shown to have no starch in the scutellum until germination began (Swift and O'Brien 1972a, 1972b). Periodate-Schiff's positive substances were also found in the coleorhiza and coleoptile. Cell walls of embryonic tissues were all periodate-Schiff's positive, as was found in the scutellum (Fig. 18).

Lipid deposits were localized through staining with Nile blue A of glycol methacrylate sections. Lipids were found in the same locations as protein in the starchy endosperm, suggesting that these were possibly lipoprotein in nature. In a section of endosperm adjacent to the embryo (Fig. 19), the lipid deposits occurred as distinct bodies between starch granules.

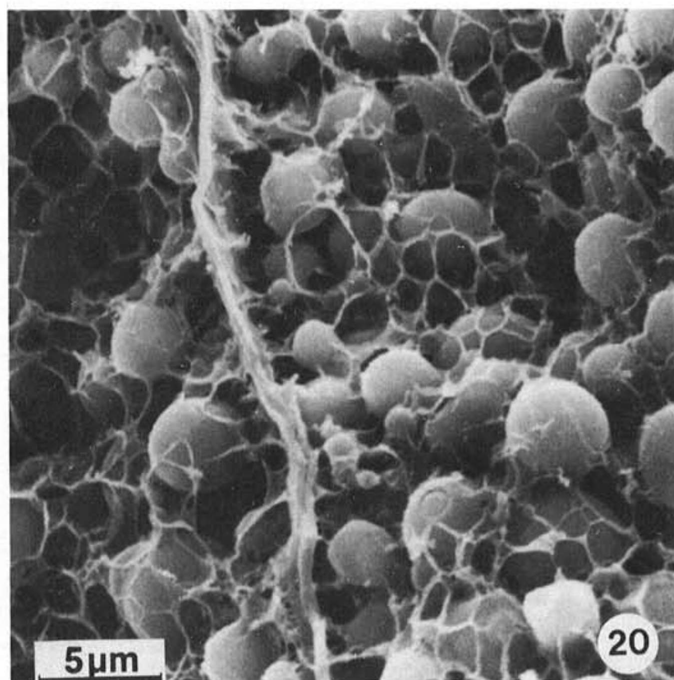
Anatomical relationships were further defined when structures were compared in scanning electron and light microscopes. Preparative techniques for study of *E. turnerana* caryopsis in the scanning electron microscope included fixation, dehydration, and critical-point drying of tissue. Use of this method, rather than observation of unfixed material, provided optimal results, allowing clear definition of protein bodies and other substances in the cells (Irving et al 1981, Sefa-Dedeh and Stanley 1979). Cells of the scutellum as observed in the scanning electron microscope (Fig. 20) contained spherical storage bodies defined as protein, protein-carbohydrate, and starch, as was determined by staining with fluorescent dyes. Observations of the endosperm of *E. turnerana* caryopses in the scanning electron microscope indicated the smooth, nearly spherical starch granules (Fig. 21) with an average size of about 8 μm, as measured in light and scanning electron microscopes. Protein bodies were scattered throughout the endosperm with a greater density near the periphery of the starchy endosperm.

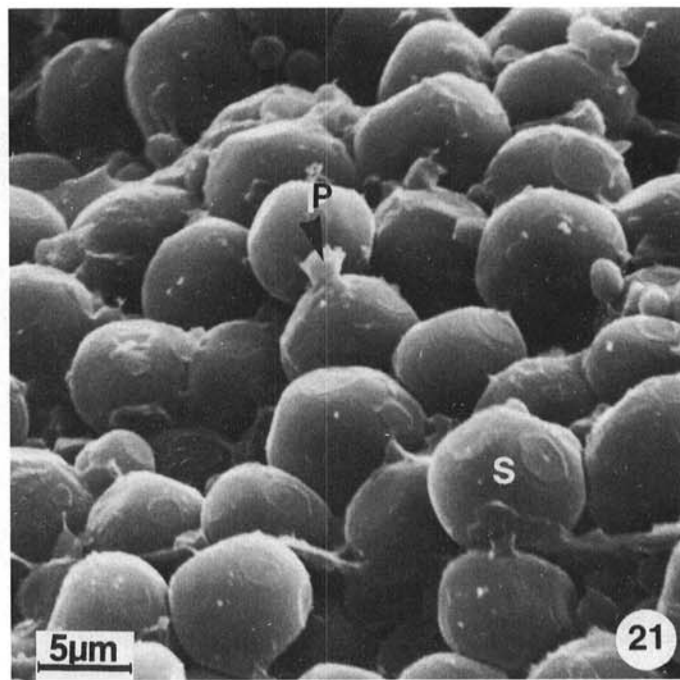
CONCLUSION

E. turnerana showed the major structural characteristics typical of the gramineae and had features similar to those of the major economic grains. Although the *E. turnerana* caryopsis is approximately 15 times smaller (on a weight basis) than that of wheat, some similarities were found in the overall structure of the two grasses. As in wheat and other grains, protein density was greater toward the periphery of the kernel, with starch grains increasing in size toward the center of the caryopsis. Also as in wheat, a single layer of aleurone cells was found. Unlike wheat, *E. turnerana* had no "crease" region and had a more rounded shape typical of millets.

Future plans for *E. turnerana* include study of the compositional and nutritional value both as a forage and as a food and feed grain, and study of its milled fractions as possible additives to baked products.

Breeding efforts will be needed to improve the agronomic value





Figs. 20–21. Scanning electron micrographs of the scutellum (**Fig. 20**) and central endosperm (**Fig. 21**). P = Protein body, S = starch granule.

of *E. turnerana*. It is relatively salt- and drought-tolerant and thus has potential in regions where low rainfall, irrigation expense, and saline soil present problems for the growth of crops.

ACKNOWLEDGMENTS

I thank Antoinette A. Betschart and Robin M. Saunders for their encouragement.

LITERATURE CITED

BECHTEL, D. B., and POMERANZ, Y. 1978a. Ultrastructure of the mature ungerminated rice (*Oryza sativa*) caryopsis. The germ. Am. J. Bot. 65:75.

BECHTEL, D. B., and POMERANZ, Y. 1978b. Ultrastructure of the mature ungerminated rice (*Oryza sativa*) caryopsis. The starchy endosperm. Am. J. Bot. 65:684.

BECHTEL, D. B., and POMERANZ, Y. 1979. Endosperm structure of barley isogenic lines. Cereal Chem. 56:446.

BECHTEL, D. B., and POMERANZ, Y. 1981. Ultrastructure and cytochemistry of mature oat (*Avena sativa* L.) endosperm. The aleurone layer and starchy endosperm. Cereal Chem. 58:61.

BERLYN, G. P., and MIKSCHE, J. P. 1976. Botanical Microtechnique and Cytochemistry. Iowa State University Press, Ames, IA.

DAHLQUIST, A., OLSSON, I., and NORDEN, Å. 1965. The periodate-Schiff reaction: Specificity, kinetics, and reaction products with pure substrates. J. Histochem. Cytochem. 13:423.

ESAU, K. 1977. Anatomy of Seed Plants, 2nd ed. John Wiley & Sons, New York.

FEDER, N., and O'BRIEN, T. P. 1968. Plant microtechnique: Some principles and new methods. Am. J. Bot. 55:123.

FULCHER, R. G., O'BRIEN, T. P., and LEE, J. W. 1972. Studies on the aleurone layer. I. Conventional and fluorescence microscopy of the cell wall with emphasis on phenolcarbohydrate complexes in wheat. Aust. J. Biol. Sci. 25:23.

FULCHER, R. G., O'BRIEN, T. P., and WONG, S. I. 1981. Microchemical detection of niacin, aromatic amine, and phytin reserves in cereal bran. Cereal Chem. 58:130.

FULCHER, R. G., SETTERFIELD, G., McCULLY, M. E., and WOOD, P. J. 1977. Observations on the aleurone layer. II. Fluorescence microscopy of the aleurone-subaleurone junction with emphasis on possible β-1,3-glucan deposits in barley. Aust. J. Plant Physiol. 4:917.

FULCHER, R. G., and WONG, S. I. 1980. Inside cereals—A fluorescence microchemical view. Page 1 in: Cereals for Food and Beverages: Recent Progress in Cereal Chemistry and Technology. G. E. Inglett and L. Munck, eds. Academic Press, New York.

HUMPHREYS, W. J., SPURLOCK, B. O., and JOHNSON, J. 1974. Critical-point drying of ethanol-infiltrated, cryofractured biological specimens for scanning electron microscopy. Page 275 in: Proc. Seventh Annu. SEM Symp. Part I. ITTRI, Chicago, IL.

IRVING, D. W., BETSCHART, A. A., and SAUNDERS, R. M. 1981. Morphological studies on *Amaranthus cruentus*. J. Food Sci. 46:1170.

JENSEN, W. A. 1962. Botanical Histochemistry. W. H. Freeman and Company, San Francisco and London.

LILLIE, R. D. 1954. Histopathologic Technic and Practical Histochemistry. The Blakison Company, Inc., New York.

LULHAM, C. N. 1979. Glycol methacrylate embedding for light microscopy. J. Histotechnology 2:68.

NATIONAL ACADEMY OF SCIENCES. 1975. Underexploited Tropical Plants with Promising Economic Value. Report of an Ad Hoc Panel of the Advisory Committee on Technology Innovation. Washington, DC.

O'BRIEN, T. P. 1967. Observations on the fine structure of the oat coleoptile. I. The epidermal cells of the extreme apex. Protoplasma 63:385.

O'BRIEN, T. P., and THIMANN, K. V. 1967a. Observations on the fine structure of the oat coleoptile. II. The parenchyma cells of the apex. Protoplasma 63:417.

O'BRIEN, T. P., and THIMANN, K. V. 1967b. Observations on the fine structure of the oat coleoptile. III. Correlated light and electron microscopy of the vascular tissues. Protoplasma 63:443.

PEARSE, A. G. E. 1960. Histochemistry Theoretical and Applied, 2nd ed. Little, Brown and Company, Boston.

PEASE, D. C. 1973. Glycol methacrylate copolymerized with glutaraldehyde and urea as an embedment retaining lipids. J. Ultrastruct. Res. 45:124.

ROST, T. L. 1972. The ultrastructure and physiology of protein bodies and lipids from hydrated dormant and nondormant embryos of *Setaria lutescens* (Gramineae). Am. J. Bot. 59:607.

SEFA-DEDEH, S., and STANLEY, D. W. 1979. Microstructure of cowpea variety Adua Ayera. Cereal Chem. 56:367.

SHANNON, M. C., WHEELER, E. L., and SAUNDERS, R. M. 1981. Salt tolerance of Australian channel millet. Agron. J. 73:830.

SMART, M. G., and O'BRIEN, T. P. 1979a. Observations on the scutellum. I. Overall development during germination in four grasses. Aust. J. Bot. 27:391.

SMART, M. G., and O'BRIEN, T. P. 1979b. Observations on the scutellum. II. Histochemistry and autofluorescence of the cell wall in mature grain and during germination of wheat, barley, oats and ryegrass. Aust. J. Bot. 27:403.

SWIFT, J. G., and O'BRIEN, T. P. 1972a. The fine structure of the wheat scutellum before germination. Aust. J. Biol. Sci. 25:9.

SWIFT, J. G., and O'BRIEN, T. P. 1972b. The fine structure of the wheat scutellum during germination. Aust. J. Biol. Sci. 25:469.

WOOD, P. J., and FULCHER, R. G. 1978. Interaction of some dyes with cereal β-glucans. Cereal Chem. 55:952.

YATSU, L. Y., and JACKS, T. J. 1968. Association of lysosomal activity with aleurone grains in plant seeds. Arch. Biochem. Biophys. 124:466.

ZEE, S. Y., and O'BRIEN, T. P. 1970. Studies on the ontogeny of the pigment strand in the caryopsis of wheat. Aust. J. Biol. Sci. 23:1153.

ZEE, S. Y., and O'BRIEN, T. P. 1971. Aleurone transfer cells and other structural features of the spikelet of millet. Short communication. Aust. J. Biol. Sci. 24:391.

[Received July 6, 1982. Accepted October 29, 1982]