Nutrient Distribution in Five Perennial Grain Species Exhibited by Light and Scanning Electron Microscopy

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ABSTRACT

The anatomy and histochemistry of mature caryopses of five perennial grains (*Agropyron intermedium, A. trichophorum, Secale montanum, Hordeum bulbosum, and Tripsacum dactyloides*) were studied by fluorescence and scanning electron microscopy. All five grains contain starch granules and protein in the starchy endosperm. Protein bodies, which often contain phytin inclusions, are abundant in the aleurone and embryo. Lipid deposits are primarily located in the embryo and aleurone. Caryopses of *A. intermedium, A. trichophorum, S. montanum, and H. bulbosum* are similar morphologically, having two populations of starch granules as well as continuous protein matrix in starchy endosperm tissue. The aleurone of *Agropyron* spp., *T. dactyloides,* and *S. montanum* are single celled in thickness. *H. bulbosum* has a multiple-layered aleurone (usually three cells thick). The caryopsis of *T. dactyloides* is nearly spherical, and the starchy endosperm contains spherical starch granules embedded in a matrix of small protein bodies. The embryo of *T. dactyloides* contains starch granules.

Perennial grains provide an alternative to traditional cereal production. Conventional cereal crops are grown annually and require replanting, cultivation, and fertilization, which often results in detrimental effects to the soil. Additionally, conventional agriculture has limited the genetic diversity of crops due to the few plant species grown for economic purposes.

The inclusion of unconventional practices in our food production may limit detrimental effects to the environment. Such unconventional practices would include the introduction of new plants such as perennial grains in areas that are not prime agricultural land and the harvesting of the grain only, leaving the rest of the plant to regenerate. Perennial grains also provide new germ plasm (Suneson et al 1969, Jain 1977), which could be incorporated into breeding programs for disease resistance, stress tolerance, or other desirable characteristics.

Agropyron spp. are grown as forage crops (Knowles 1977, Wilson and Smoliak 1977, White and Currie 1980, Karn and Berdahl 1984) and have been shown to prevent soil erosion (Hanson 1972). Interest in the plant as a food has arisen, and information on nutrition, composition (Becker et al 1986), agronomy (Knowles 1977, Wagoner 1988), and acceptability as an additive to baked products (Wagoner 1988) has been reported. Leaf waxes have been examined for composition and show potential as a source of commercial wax (Tullock and Hoffman 1976).

Tripsacum dactyloides (Eastern gamagrass), a wild relative of corn (Paulis and Wall 1977), has been studied as a forage crop and found to be digestible (Horner et al 1985) and palatable to range animals (Dewald and Louthan 1979). Inadequate seed production and inferior seed quality have prevented much interest in the grain (Dewald and Louthan 1979, Dewald and Sims 1981), although new lines have been developed that have higher seed yield than the wild cultivars (Dewald and Dayton 1985a,b).

Less information is available on *Secale montanum* (Khush 1962, Suneson et al 1969, Gustafson and Lukaszewski 1985) and *Hordeum bulbosum* (Leshem 1971, Leshem and Nir 1972, Baum and Bailey 1985), but the potential of each to be developed as a forage crop, a grain crop, or a source of germ plasm warrants further investigation.

A factor contributing to the value of a new cereal crop is processing potential, which requires an understanding of the morphology and nutrient distribution within the caryopsis. It is also useful to understand how new crops compare with each other as well as with conventional crops.

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This study addresses comparative anatomy and nutrient distribution in caryopses of *Agropyron intermedium* (Host) P. Beauv., *A. trichophorum* (Link) K. Richt., *H. bulbosum* L., *S. montanum* Guss., and *T. dactyloides* (L.) L. and has provided basic information on possible uses of the grains of these plants.

MATERIALS AND METHODS

Samples

The samples consisted of caryopses of plants from four genera, five species, and included *A. intermedium, A. trichophorum, H. bulbosum, S. montanum*, and *T. dactyloides. Agropyron* spp., *H. bulbosum*, and *S. montanum* were supplied by Peggy Wagoner, Rodale Press, Emmaus, PA. *T. dactyloides* was provided by Chester Dewald, U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Range Research Station, Woodward, OK.

Instrumentation

Two compound fluorescence microscopes were employed. Both microscopes contain similar exciter-barrier filter combinations. The Zeiss Universal Research microscope is equipped with a 100-W mercury illuminator (Osram HBO 100W, Berlin) and two exciter-barrier filter combinations consisting of FC B (excitation filter, 450–490; beam splitter, FT 510; barrier filter, LP 520), and FC G (exciter filter, BP 546; beam splitter, FT 580; barrier, LP 590). The Nikon Fluophot microscope has a 200-W mercury illuminator (Osram HBO 200W, Berlin) and the following two filter combinations: FC U (excitation filter, UV330–380; dichroic mirror, DM400; barrier filter, 420K) and FC B (excitation filter IF420–490, auxiliary filter, 460; dichroic mirror, DM505; barrier filter, 520W). In the above, FC = filter combination, U = UV excitation, B = blue excitation, and G = green excitation. Note that the same designation is given for FC G on both microscopes. Minimal visual differences were apparent between results obtained for the two microscopes. Therefore, for simplicity of presentation, only the excitation color is given in further descriptions.

Sample Preparation: Fluorescence Microscopy

Fixation and embedding. Caryopses were cut transectionally into pieces approximately 1.5 mm thick and placed into fixative. Tissues were fixed in 2–3% glutaraldehyde with or without the addition of 2% formaldehyde (from paraformaldehyde) in phosphate buffer for preservation of general morphological structure and such tissue components as protein and starch. Calcium dichloride ($CaCl_2$) was added to some preparations in an effort to retain lipids in tissues, as calcium reduces extraction of cellular components (Hayat 1981). Where calcium was added to fixatives, cacodylate buffer was used instead of phosphate buffer to avoid the formation of insoluble Ca_2PO_4.

Specifically, tissues were fixed overnight at 0–4°C in one of

the following fixatives: 1) a mixture of 2% glutaraldehyde and 2% formaldehyde in 0.05*M* Na_2HPO_4/NaH_2PO_4 buffer, pH 7.0; 2) 3% glutaraldehyde in 0.05*M* Na_2HPO_4/NaH_2PO_4 buffer, pH 7.0; 3) 2.5% glutaraldehyde and 2% formaldehyde in 0.1*M* cacodylate buffer (Glauert 1974) with 2.5 m*M* $CaCl_2$, pH 7.2; or 4) 1% glutaraldehyde in 0.05*M* cacodylate buffer with 2.5 m*M* $CaCl_2$. Additionally (fixative 5), samples were fixed in fixative 4 at 23°C for 3.5 hr. Tissues were rinsed in the same buffer, then dehydrated in an ethanol-butanol graded series (Jensen 1962) with 10–20 min per exchange. Samples prepared using fixatives 1–3 were infiltrated for three to five days and embedded in JB-4 (Polysciences, Warrington, PA) glycol methacrylate (Feder and O'Brien 1968) using heat polymerization (40–50°C for two days). Samples prepared using fixation schedules 4 and 5 were infiltrated for three to five days and embedded at 23°C using Historesin glycol methacrylate (LKB, Bromma Sweden).

Embedded samples were sectioned at 2–4 μm on a Sorvall Porter-Blum MT-2 ultramicrotome, mounted onto glass slides, and stained.

Staining procedures. Several stains were employed to demonstrate storage components and morphology in the grains.

1. Calcofluor. Sections on slides were briefly flooded with 0.01% aqueous calcofluor, rinsed, air dried, and mounted in immersion oil. Sections were then viewed using FC U and photographed (Fulcher and Wong 1980). Cell walls fluoresce blue with calcofluor. Note that autofluorescence of proteins also occurs at this wavelength and appears as a lesser intense blue than the cell walls.

2. Safranin O. Sections were placed in a Coplin staining jar containing 0.5% aqueous Safranin O and stained for 15 min (Revilla et al 1986). Slides were removed, rinsed under running tap water, air dried, and mounted in immersion oil or Eukitt mounting medium. Sections were viewed and photographed using FC B. Under these conditions, starch fluoresces yellow-green, proteins yellow to pink, and nuclei red.

3. Acid fuchsin. Slides containing sections were flooded with 0.01% acid fuchsin in 1.0% acetic acid, allowed to stain for approximately 2 min, rinsed in running water, air dried, and mounted in immersion oil. Sections were viewed and photographed using FC G (Fulcher and Wong 1980); storage proteins fluoresce red under these conditions.

4. Nile blue A. Sections prepared specifically to preserve lipids were mounted in 0.01% aqueous Nile blue A. As a check, hand-sections were also observed following staining with Nile blue A. Lipid droplets appeared bright yellow when viewed using FC B (Fulcher and Wong 1980).

5. Acriflavine HCl (Yiu et al 1982). Slides were placed in a Coplin staining jar containing 0.01% acriflavine HCl in Walpole sodium acetate buffer (Pearse, 1960), pH 4.3, and stained for 15 min. Sections were rinsed briefly in 95% ethanol, air dried, and mounted in immersion oil. Sections were viewed and photographed using FC G. Phytic acid inclusions appear red under these conditions.

6. Periodic acid/Schiff's (PAS). Sections were treated in a saturated solution of dinitrophenylhydrazine in 15% acetic acid to block preexisting aldehyde groups (Feder and O'Brien, 1968). The sections were then treated in 1.0% periodic acid, rinsed and stained in Schiff's reagent (PAS kit, Sigma Diagnostics, St. Louis, MO). Sections were air dried, mounted in immersion oil, and observed using conventional brightfield optics. Carbohydrate groups containing vicinal hydroxyl groups, such as starch and cell walls, appeared pink to red (Fulcher and Wong 1980).

7. Iodine potassium iodide (IKI, Jensen 1962). Sections were stained briefly with a drop of IKI solution, rinsed, and mounted in water. The sections were viewed and photographed using either conventional brightfield conditions or Hoffman modulation contrast (similar to Nomarski DIC), in which starch granules appear black.

Starch Isolation

Starch was isolated from whole grains by washing ground material repeatedly with water using a modified technique of Knight and Olsen (1984). Caryopses (2–10 g) were ground using a mortar and pestle and sieved successively through 20-mesh and 60-mesh screens. The fine particles that passed through the 60-mesh screen were retrieved and mixed with water, stirred, and allowed to stand 20 min. More water was added to the suspension, and the mixture was stirred for 10 min. The suspension was then sieved through an 80-mesh screen, and the portion retained on the screen was washed and resieved. The resulting suspension was sieved successively through 150- and 270-mesh screens and allowed to settle for approximately 1 hr in a centrifuge tube. Floating debris and supernatant were removed and discarded. The precipitate was washed repeatedly by resuspending and centrifuging at 2,000 × *g* until it was completely white, which indicated that the isolate was relatively pure starch. The starch was allowed to dry at 23°C and was observed in a scanning electron microscope.

Scanning Electron Microscopy

Caryopses of *T. dactyloides* were cut into pieces (approximately 1 mm^3), fixed overnight in buffered 2% glutaraldehyde, rinsed in buffer, and fixed 2 hr in buffered 1% OsO_4. Samples were then dehydrated in a graded series of ethanol, cryofractured (Humphreys et al 1974), and critical-point dried using CO_2 in a Polaron E3000 critical-point dryer. The dried samples were affixed to aluminum specimen stubs using carbon paint and were coated with gold in a Polaron E3100 sputter-coating unit. Samples were viewed and photographed in a Hitachi S530 scanning electron microscope (SEM) at 10 kV. Starch samples were air-dried, affixed to aluminum specimen stubs using double-stick tape, gold coated, viewed, and photographed at 4 kV in the SEM.

Measurements of Components

Starch granules and protein bodies (of *T. dactyloides*) were measured from scanning electron micrographs and in the light microscope. Starch granules from *H. bulbosum, Agropyron* spp., and *S. montanum* were found to vary considerably in size and shape. Thus, reported sizes and shapes are approximate. Protein bodies and starch granules of *T. dactyloides* were less variable, and reported sizes and shapes are more representative of that species. Values reported are from scanning electron micrographs taken from samples that were broken, mounted (as is) with epoxy onto SEM stubs. Samples were then photographed in the SEM at several magnifications, and measurements of components were taken with a ruler. Means and standard deviations were calculated and are presented here in parentheses after an estimated, rounded value, along with the number of measurements. In all cases, the numbers obtained from SEM micrographs agree with those taken directly from sections in the light microscope.

RESULTS

Figure 1 shows representative drawings of the four species.

***Agropyron* Species**

A. trichophorum and *A. intermedium* were nearly identical

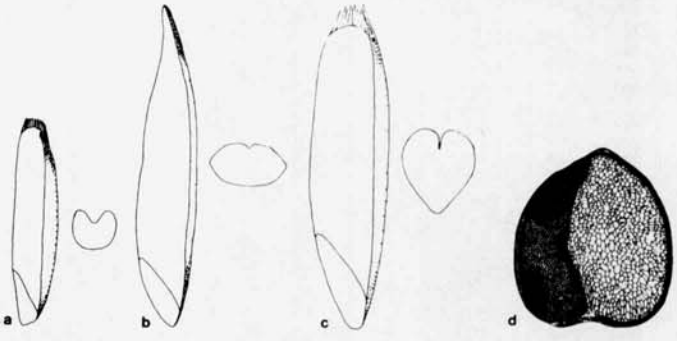
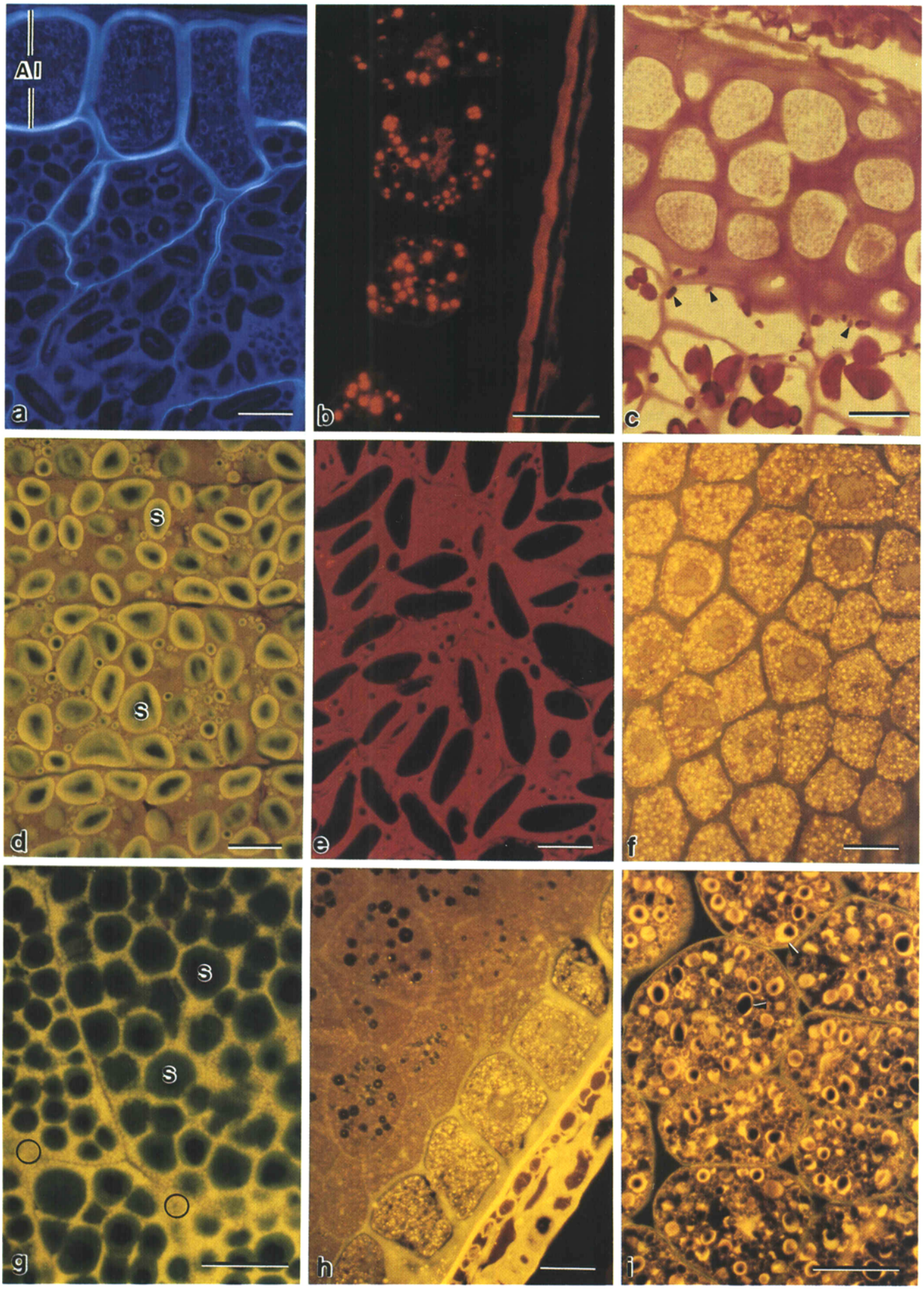


Fig. 1. Illustrations of medium longitudinal (a–d) and cross (a–c) sections of *Agropyron* sp. (a), *Hordeum bulbosum* (b), *Secale montanum* (c), and *Tripsacum dactyloides* (d).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morphologically and histochemically. *Agropyron* spp. exhibited a single layer of aleurone cells (Fig. 2a and b), which surrounds the starchy endosperm. Cell walls of the endosperm and aleurone exhibited blue fluorescence when stained with calcofluor and observed with FC U (Fig. 2a). Aleurone cells contained numerous protein bodies with phytin (a cationic salt of myoinositol hexaphosphoric acid) inclusions (globoids), which were evident using FC G following treatment with acriflavine HCl (Fig. 2b). Aleurone cells also contained lipid droplets (not shown, similar to those in *T. dactyloides,* Fig. 2h). Lipid droplets and protein bodies with phytin inclusions were also abundant in the embryo (germ).

The embryo appeared similar to that of other grasses. The scutellum, the largest structure in the embryo, was adjacent to the starchy endosperm and contained protein bodies (not shown; scutellum similar to Fig. 2i). Phytic acid inclusions were evident within protein bodies after staining with acriflavine HCl. Protein bodies in the coleoptile were much smaller than in the scutellum and not as abundant. Lipid droplets were located throughout the embryo (not shown, similar to *S. montanum*, Fig. 2f). Neither lipid droplets nor protein bodies were found in cells of the starchy endosperm. The material between starch granules appeared as a smooth matrix when viewed in the light microscope following staining with protein-specific dyes. Protein also autofluoresces in the near UV; thus, the proteinaceous material is visible in Fig. 2a between dark starch granules.

The main storage components observed in the starchy endosperm were starch granules. Two distinct populations of starch granules were present in the starchy endosperm of both *Agropyron* spp. (Fig. 3a). Small starch granules were approximately 5 μm (5.24 ± 1.67, $n = 236$) in diameter and were roughly spherical in shape. Large starch granules were approximately 9 μm (9.14 ± 2.33, $n = 56$) $\times$ 20 μm (20.63 ± 4.42, $n = 56$) in the largest diameter and were disk-shaped. A continuous mass of proteinaceous material between starch granules in the starchy endosperm was evident in the light microscope. (Figures 2d and e, although taken of other genera, are representative of *Agropyron* spp.; protein appears as a pinkish background in Fig. 2d and as red in Fig. 2e.)

Hordeum bulbosum

H. bulbosum had a multiple aleurone layer that was usually three cells thick over the starchy endosperm (Fig. 2c) and one cell thick adjacent to the embryo. Protein bodies, most of which contained phytin inclusions, were located in aleurone and embryo cells (especially abundant in cells of the scutellum, similar to *T. dactyloides*, Fig. 2i). Lipid droplets were located throughout the aleurone (not shown, similar to Fig. 2h) and embryo cells (not shown, similar to Fig. 2f). Starch granules, located only in the starchy endosperm (Fig. 2c), consisted of small spherical granules of about 4 μm (3.64 ± 1.40, $n = 88$) in diameter and larger ellipsoid-shaped granules of approximately 13 μm (12.64 ± 1.99, $n = 22$) $\times$ 18 μm (18.22 ± 3.31, $n = 22$) in diameter.

The protein matrix in the starchy endosperm was continuous, as in *Agropyron* spp. and *S. montanum*, but more of a protein gradient was evident (Fig. 2c, unstained portion in subaleurone represents protein). Protein was more abundant in the cells at the periphery, especially in the small cells adjacent to the aleurone layer, where starch granules were usually smaller and less abundant (Fig. 2c).

Secale montanum

S. montanum was similar in structure to *Agropyron* spp. A single layer of aleurone cells was generally found, although occasionally a double row was evident. Aleurone cells contained protein bodies with phytin inclusions (similar to Fig. 2b) and lipid droplets (similar to Fig. 2h). The starchy endosperm contained two populations of starch granule, smaller spherical granules approximately 5 μm (5.37 ± 1.87, $n = 79$) in diameter and larger disk-shaped granules approximately 16 μm (16.04 ± 6.37, $n = 7$) $\times$ 30 μm (30.23 ± 4.16, $n = 7$) in diameter (Fig. 2e).

The proteinaceous matrix between starch granules was evenly distributed throughout the starchy endosperm (Fig. 2e); no protein gradient was evident (i.e., more protein at the periphery). The embryo, especially the scutellum, contained protein bodies, most of which had phytin inclusions. Lipid deposits were abundant throughout the embryo and are shown in Fig. 2f for the scutellum.

Tripsacum dactyloides

The *T. dactyloides* caryopsis was located inside hardened glumes; this arrangement was also noted in *Echinochloa turnerana* (channel millet, Irving 1983) and also occurs in rice (Juliano and Bechtel 1985). The seed, a relative of corn, is more spherically shaped than caryopses of other grains presented in this study (Fig. 1), but it had many similar features, including starch as the major storage component of the seed.

Starch granules in the starchy endosperm were roughly spherical, consisting of a single population of approximately 9 μm (8.94 ± 1.2, $n = 66$) in diameter (Figs. 2g and 3b) and exhibited birefringence through crossed polarizers (Fig. 3c). Small protein bodies, approximately 1 μm (1.15 ± 0.25, $n = 68$) in diameter (Figs. 2g and 3b), were located throughout the starchy endosperm and did not contain phytin inclusions. Protein bodies were numerous and tightly packed in the subaleurone. A gradient of proteinaceous material was evident, being greatest at the periphery (adjacent to the embryo and aleurone) and diminishing toward the central starchy endosperm.

Protein bodies, containing phytin inclusions, were abundant in aleurone cells (similar to Fig. 2h). The aleurone layer consisted of a single layer of cells (Fig. 2h), which were smaller and had thinner cell walls than *Agropyron* spp., *S. montanum*, or *H. bulbo-*

Fig. 2. Light micrographs of perennial grains depicting storge components within caryopses. Part **c** is a brightfield micrograph; all others are fluorescence micrographs. **a,** *Agropyron intermedium* section stained with calcofluor and photographed using filter combination (FC) UV (365-nm excitation). Section shows a single aleurone cell layer (Al) and blue-white fluorescence of cell walls. Continuous light blue matrix material inside cells of endosperm is autofluorescence of protein. Note the absence of discrete bodies. Bar = 20 μm. **b,** Section of *A. trichophorum* showing phytin inclusions (red bodies) in the aleurone layer. Section was stained with acriflavine HCl to demonstrate phytin inclusions and photographed using FC green (G) (546-nm excitation). Bar = 20 μm. **c,** Periodic acid/Schiff's stained section of *Hordeum bulbosum*. The micrograph was recorded under brightfield conditions and exhibits the multilayered aleurone typical of *Hordeum*. Note the small starch granules located just beneath the aleurone (arrows). Unstained areas in the subaleurone are filled with protein; when stained with acid fuchsin for protein (for example), they appear as in section e. Bar = 20 μm. **d,** *H. bulbosum* section that was stained with safranin O and photographed using FC blue (B) (420- to 490-nm excitation) to demonstrate starch (S) and protein. Starch granules are the greenish yellow bodies; protein is the pink background. Note the absence of discrete protein bodies. Bar = 20 μm. **e,** Section of the central portion of the endosperm of *Secale montanum* stained with acid fuchsin and photographed using FC G (546-nm excitation) to show protein. Protein occurs as the reddish-stained background material (no discrete protein bodies); starch is unstained and appears black. Bar = 20 μm. **f,** Nile blue stained section of the scutellum of *S. montanum*, showing lipid bodies. The section was photographed with FC B (450- to 490-nm excitation). Lipids appear as the bright yellow bodies located throughout the cells. Bar = 20 μm. **g,** Section of *Tripsacum dactyloides* stained with Safranin O and photographed using FC B (450- to 490-nm excitation), showing starch (green bodies with black centers (S) and protein bodies (small yellow bodies, circles) in the starchy endosperm. Note that the other grains do not have discrete protein bodies in starchy endosperm tissue, similar to wheat. Bar = 20 μm. **h,** Nile blue stained section of *T. dactyloides*, showing lipid droplets (bright yellow bodies) located in the aleurone cells. The section was photographed with FC B (450- to 490-nm excitation). This micrograph is representative of the other grains, in which lipids also occur in small bodies. Bar = 20 μm. **i,** Section of the scutellum of *T. dactyloides* stained with acid fuchsin and photographed using FC B (450- to 490-nm excitation) to show protein bodies (arrows). The structures that appear to be holes or voids within the protein bodies are unstained phytin inclusions. Bar = 20 μm.

sum. Small lipid deposits, which appeared as brilliant yellow dots visible when viewed with fluorescence illumination after staining with Nile blue, were also abundant in the aleurone (Fig. 2h).

As in aleurone cells, embryo cells contained lipid deposits and protein bodies (Fig. 2i) with phytin inclusions (unstained, appearing to be dark holes, Fig. 2i). Starch granules ranging from 1 to 3 μm in diameter were located throughout the embryo (Fig. 3d).

DISCUSSION

The histochemical and morphological structure of the four genera (*Agropyron, Hordeum, Secale*, and *Tripsacum*) share similar features and exhibit typical Gramineae structure. Starchy endosperm, which consists of starch granules embedded in a proteinaceous matrix, is the major storage tissue in all grasses. Aleurone cells contain lipid and protein bodies with phytate inclusions. The embryo has numerous protein bodies of various sizes, some of which contain phytic acid. Small lipid droplets are located throughout the grass embryo and are especially abundant in the scutellum.

Agropyron spp., *S. montanum*, and *H. bulbosum* have continuous matrices of protein in starchy endosperm tissues—as does wheat (Parker 1980, Simmonds and O'Brien 1981). In the mature wheat caryopsis, a gradient in the staining pattern for protein is evident, with the staining heaviest at the periphery of the starchy endosperm (subaleurone and adjacent to the scutellum) and decreasing toward the center. This staining pattern was also evident in *H. bulbosum*, although the staining gradient was less than that of wheat. *Agropyron* spp. exhibited less of a gradient than *H. bulbosum*, and a protein staining gradient was not evident in *S. montanum*. A protein gradient was noted in *T. dactyloides*; however, the protein in the endosperm occurred as protein bodies.

Protein bodies of *T. dactyloides* endosperm were approximately 1 μm in diameter. Protein bodies have also been found in the endosperm of other Gramineae such as corn (*personal obser-*

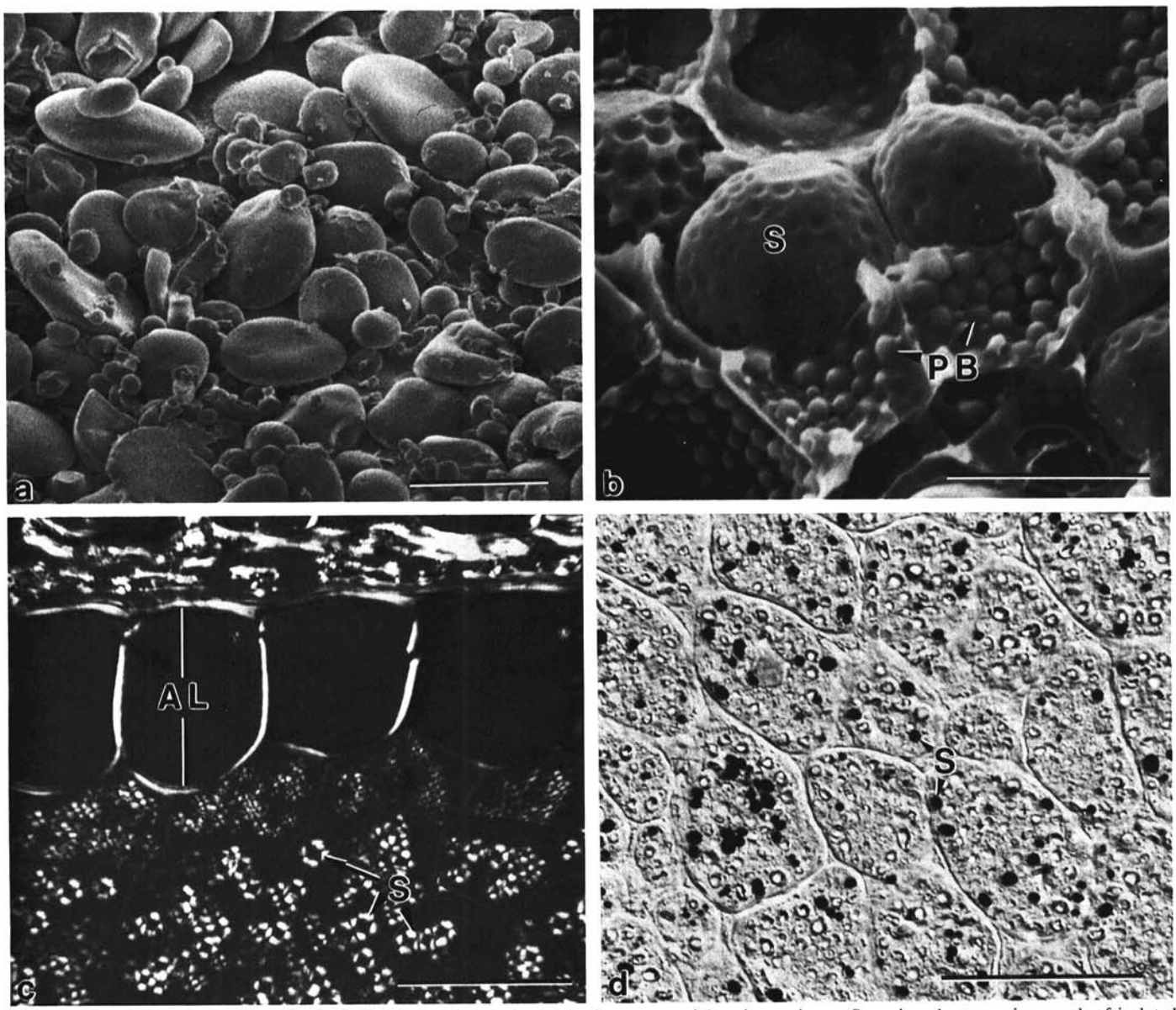

Fig. 3. Scanning electron micrographs (**a,b**) and light micrographs (**c,d**) of two perennial grain species. **a,** Scanning electron micrograph of isolated starch of *Agropyron intermedium*, showing the disk-shaped large granules and spherical small granules. Isolated starch, such as in this micrograph, gives a clear indication of the shape of starch granules. Bar = 20 μm. **b,** Scanning electron micrograph of starchy endosperm cells of *Tripsacum dactyloides*, showing spherical starch granules (S) and protein bodies (PB). Identification of cellular components was made by staining sections for light microscopy before SEM observation. Bar = 20 μm. **c,** Light micrograph of an unstained section of the starchy endosperm and aleurone (AL) of *T. dactyloides* through crossed polarizers, showing birefringence of starch (S). Starch granules appear as the bright structures. Bar = 50 μm. **d,** Light micrograph of a section of the scutellum of *T. dactyloides* stained with iodine potassium iodide and photographed using Hoffman modulation contrast (brightfield) optics, showing darkly stained starch granules (S). Bar = 50 μm.

vation, Duvick 1961), rice (*personal observation*, Bechtel and Pomeranz 1978, Juliano and Bechtel 1985), oats (Fulcher 1986), and channel millet (Irving 1983), where protein bodies are more abundant at the periphery. As in all grains, protein is a minor portion of the endosperm, whereas starch is abundant.

Starch granules were positively identified by their characteristic staining with IKI and PAS, as well as the birefringence they exhibited when viewed through crossed polarizers (Fig. 3c). Bates et al (1981) did not see birefringence in isolated starch preparations of *T. dactyloides* and suggested that the lack of birefringence is perhaps due to the small size of the starch granules. However, in previous studies on *Amaranthus*, which has polyhedral starch granules approximately 1 μm in diameter (Irving et al 1981, Irving and Becker 1985), starch was noted to exhibit birefringence (*unpublished data*). Bates et al (1981) reported that, in *T. dactyloides*, starch was approximately 1 μm in diameter. However, in this study, starch granules of *T. dactyloides* were found to be about 10 μm in diameter, similar to those of corn (*personal observation*, Wolf et al 1952). A few smaller granules (about 5 μm in diameter) were located in the subaleurone. Perhaps Bates et al (1981) were measuring protein bodies, since they reported a lack of birefringence with polarized light and a diameter of 1 μm. Protein bodies, identified by staining reactions in this study, fit the description of the "starch granules" reported by Bates et al (1981).

H. bulbosum has a multiple aleurone layer, usually three cells thick, similar to cultivated barley (Jones 1969, Jacobsen et al 1971). Rice also has a multiple aleurone layer (*personal observation*, Juliano and Bechtel 1985). *Agropyron* spp., *S. montanum*, and *T. dactyloides* exhibited a single aleurone layer around much of the caryopsis similar to those in wheat and oat (Fulcher 1986) caryopses.

Protein bodies were found in aleurone and embryo cells of all five species (similar to Fig. 2i), and these protein bodies served as a storage location for phytin inclusions (Fig. 2b and 2i). Phytin inclusions are common in other cereal grains (Fulcher 1986) and other seeds (Lott and Buttrose 1977). They were identified both by birefringence when viewed through crossed polarizers (Fulcher et al 1981) and by staining with acriflavine HCl (Yiu et al 1982), as shown in aleurone cells of *A. trichophorum* (Fig. 2b). Figure 2i, although stained for protein, clearly shows where phytin inclusions are located inside protein bodies; they were previously identified by positive staining with acriflavine HCl and show in Figure 2i as lack of staining (dark areas).

In addition to an abundance of protein bodies, the embryo of *T. dactyloides* contained a few starch granules (Fig. 2d), especially in the scutellum. This was also noted for corn (*personal observation*). Starch granules were not found in the embryos of the four other species, which is similar to wheat (Swift and O'Brien 1972).

Another similarity to wheat was noted in the amino acid profile of the protein of *Agropyron* spp. (see the amino acid table in Lockhart and Hurt 1986). However, the protein content was reported to be higher than that of wheat, about 17% for *A. trichophorum* and 15% for *A. intermedium* versus 14% for wheat (Becker et al 1986). Perhaps the higher protein content of the caryopses of *Agropyron* spp. is due to the proportionately larger embryo and aleurone tissues compared to those of wheat, since protein is more highly concentrated in these tissues. The wheat kernel has been bred to have a large caryopsis, much of which is endosperm storage tissue, and thus a relatively small embryo. Embryo and aleurone tissues in the gramineae typically have a greater percentage of protein than the endosperm.

The protein content of *T. dactyloides* was almost triple that of normal corn, 29.3% for *T. dactyloides* versus 10.9% for normal corn, and approximately the same protein content as that in high-protein corn, 26.2% (Paulis and Wall 1977). Amino acid profiles show that *T. dactyloides* is low in lysine (containing about half that of conventional grains), high in methionine (containing about twice that of conventional grains) (Lockhart and Hurt 1986; Bates et al 1981), and high in leucine, similar to corn (Paulis and Wall 1977).

Compositional data on *S. montanum* and *H. bulbosum* was not available, however. Becker et al (1986) have reported compositional data on a *S. cereale* × *S. montanum* hybrid (Michael's rye), as 7.7% protein (N × 5.85) versus 13.4% protein for *S. cereale*. It is likely that the lower protein content of *S. cereale* × *S. montanum* is a consequence of the genetic complement of *S. montanum*, since *S. cereale* has twice as much protein as the hybrid.

CONCLUSION

Agropyron spp. and *T. dactyloides* show potential for development as perennial grain crops. Their favorable nutritional and morphological aspects, similarity to conventional grain crops, and perennial habit seem favorable to increase genetic diversity either as a crop or as germ plasm for hybridization with conventional crops. *S. montanum* and *H. bulbosum* also exhibit perennial habit and have morphological and histochemical features in common with conventional grain crops. Further studies are necessary to ascertain the value of using *S. montanum* or *H. bulbosum* as a crop species. However, the increase of genetic diversity through hybridization with conventional crops would be of value.

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