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Microstructure and Composition of *Digitaria exilis* Stapf (acha): A Potential Crop

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ABSTRACT

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Microstructure of the mature caryopsis of *Digitaria exilis* Stapf was studied by light and scanning electron microscopy and compared to chemical composition. The general structure of the caryopsis was similar to that of other grains, notably the millets. Thin bracts (the palea and lemma) and two glumes encased the caryopsis which consists of the thin, compressed layers of pericarp, testa, and cuticle surrounding the endosperm and embryonic tissues. The endosperm consisted of a single layer of aleurone cells and the starchy endosperm. The aleurone layer was thin over most of the starchy endosperm and thicker at the junction of the embryo and starchy endosperm. Aleurone cells contained lipid

droplets and protein bodies. The cell contents of the starchy endosperm consisted of simple, polyhedral starch granules, lipid droplets, and protein bodies. Protein bodies were more abundant toward the periphery, and diminished toward the central portion of the starchy endosperm. Cells in certain regions of the embryo contained few, small, spherical starch granules and an abundance of protein bodies. Protein bodies containing phytic acid inclusions were located in the scutellum of the embryo. Compositional analyses revealed that the grain contained 8.2% protein, 2.1% fat, 0.48% fiber, and 1.4% ash.

Digitaria exilis Stapf has the common names of acha, pene, fonio, petit mil, fundi, and hungry rice (Porteres 1976). It is one of the smallest cereals known. The grain is smooth and usually yellow. The grain weight varies from 0.4 to 0.5 mg (Jideani and Akingbala 1993) compared to 7–17 mg for millets (Simmonds 1978). *D. exilis* is a tropical African plant grown in a region extending from Cape Verde to Lake Chad (Annegers 1973). Unlike conventional cereal crops such as wheat, rice, or corn, *D. exilis* can grow on poor, sandy soil. Good yields are normally considered to be 500–800 kg per hectare, but more than 1,000 kg per hectare have been reported (Jideani 1990).

Like sorghum and millets, *D. exilis* has been cultivated in West Africa since ancient times (Wendorf et al 1992). It is an important food crop (Jideani 1990) that is palatable and highly digestible (Stapf 1915, Carbiener et al 1960). The protein has been characterized (Jideani et al 1994a,b) and has a higher methionine content than that of conventional cereals (FAO 1970; Yannai and Zimmermann 1971; de Lumen et al 1986, 1993), which are limiting in this essential amino acid. The starch has also been characterized (Jideani et al 1996).

Understanding the microstructure and chemical composition of the caryopsis of *D. exilis* is important in the exploitation of the plant as a crop for marginal regions. The distribution and state of major storage constituents are important in determining grain characteristics and processing parameters. This article is concerned with determining the microstructure and distribution of the major storage constituents in the kernel of *D. exilis* and provides chemical data.

MATERIALS AND METHODS

Both hulled and intact *D. exilis* florets were collected from farms in Jos, Nigeria, during the harvest season. The samples were cleaned of debris before subsequent handling.

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Compositional Analyses

Wholemeal flour used for analyses was obtained by grinding the grain to pass through a 72-mesh (212 μ m) sieve using a coffee mill; it was analyzed for nitrogen, fat, crude fiber, ash, and minerals (AACC 1995). Values are reported on a moisture-free basis.

Light Microscopy

Caryopses were cut in half and fixed for 24 hr at 22°C in 2% glutaraldehyde in 0.05M sodium phosphate buffer, pH 6.9. The tissues were then dehydrated in an ethanol-butanol graded series and infiltrated and embedded in glycol methacrylate (GMA, JB-4, Polysciences, Warrington, PA). Sections 2–4 μ m thick were prepared on a Sorvall-Porter Blum MT-2 ultramicrotome equipped with a glass knife and collected on glass slides (Irving et al 1990).

A double stain was employed as a general staining protocol to provide contrast to longitudinal sections of the embryo. Plastic sections 4 μ m thick were first submerged in a solution of mercury bromphenol blue (1.0% HgCl₂ and 0.05% bromphenol blue in 2% aqueous acetic acid) for 2 hr and rinsed for 5 min in 0.5% acetic acid (Pearse 1960). The sections were then treated for ~5 min with 0.05% toluidine blue O in citrate buffer, pH 4.5 and rinsed for ~1 min in running tap water (Feder and O'Brien 1968). Sections were dried and mounted in immersion oil.

Both starch and proteins were visualized in sections simultaneously by treating sections for 15 min in a Coplin staining jar containing 0.5% aqueous Safranin O (Revilla et al 1986). Following staining, slides were removed, rinsed, air-dried, and mounted in immersion oil. Sections were viewed and photographed using the 450–490 (FT510, LP520) fluorescence filter set. Under the previous conditions, starch produced yellow-green fluorescence, proteins appeared yellow to pink, and nuclei appeared red. As a second verification for proteins, sections were stained for 2 min in 0.01% acid fuchsin in 1.0% acetic acid, rinsed in running tap water, air-dried, and mounted in immersion oil (Fulcher and Wong 1980). Proteins appear red when viewed using the BP546 (FT580, LP590) fluorescence filter set. Carbohydrates, particularly starch, were localized in sections by using the periodic acid–Schiff's reaction. Sections were first treated in 1% periodic acid and then rinsed and stained in Schiff's reagent for 15 min for bright field or 1 min for fluorescence microscopy (Fulcher and Wong 1980). Sections were observed using standard bright field optics or the BP546 filter set for fluorescence. To localize phytic acid (inclusions of myoinositol hexaphosphate), sections were treated

for 15 min in aqueous 0.01% acriflavine HCl, buffered at pH 4.3. Sections were rinsed briefly in 95% EtOH, air-dried, and mounted in immersion oil. Sections were observed and photographed using the BP546 filter set for fluorescence microscopy (Yiu et al 1982).

Cryosections were prepared from fixed caryopses as an alternative to plastic sections, allowing the preservation of lipid. Sections 10 μm thick were cut (Precision Microtome-Cryostat, Lab-Tek Instruments Co., Westmont, IL) and collected on warm, poly-lysine coated slides. The slides were placed on a warming tray overnight to ensure that the sections stuck to the slides. Sections were mounted directly in Nile blue A (Fulcher and Wong 1980) and photographed under fluorescence illumination using the 450–490 fluorescence filter set.

Sections were viewed and photographed in a Zeiss Universal Research Microscope equipped with a 100W Hg burner (Osram HBO 100W, Berlin) using Kodak Tri-X pan film.

Scanning Electron Microscopy

Intact florets or caryopses were mounted directly onto aluminum specimen stubs. Alternatively, caryopses were fixed in 3% glutaraldehyde in 0.05M sodium phosphate buffer, pH 6.9, dehydrated, cut, and critical-point-dried (Polaron E3000 Critical Point Drier) and then mounted onto stubs. Specimens were sputter

coated with gold in a Polaron E5000 sputter coating unit before observation and photography using Kodak Tri-X Pan 4x5 sheet film in a Hitachi S-530 scanning electron microscope.

RESULTS

The floret of *D. exilis* (the intact caryopsis with adherent bracts and glumes) was ~1.5 mm long (Fig. 1A). The caryopsis (grain) of *D. exilis* was ~1.0 mm long and 0.75 mm wide (Fig. 1B). The

TABLE I
Chemical Composition of *Digitaria exilis*^a

Component	Percent	Component	mg/100 g
Nitrogen	1.31 \pm 0.05	Potassium	260 \pm 30
Protein ^b	8.2 \pm 0.3	Phosphorus	120 \pm 20
Fat	2.1 \pm 0.2	Magnesium	70 \pm 20
Crude fiber	0.48 \pm 0.02	Sodium	30 \pm 10
Ash	1.40 \pm 0.01	Calcium	30 \pm 10
Carbohydrate ^c	87.8 \pm 2.0	Iron	12 \pm 2.0
		Zinc	3.9 \pm 0.1

^a Average of triplicate analyses; results on dry basis.

^b Protein = 6.25 \times Kjeldahl nitrogen.

^c Carbohydrate by difference.

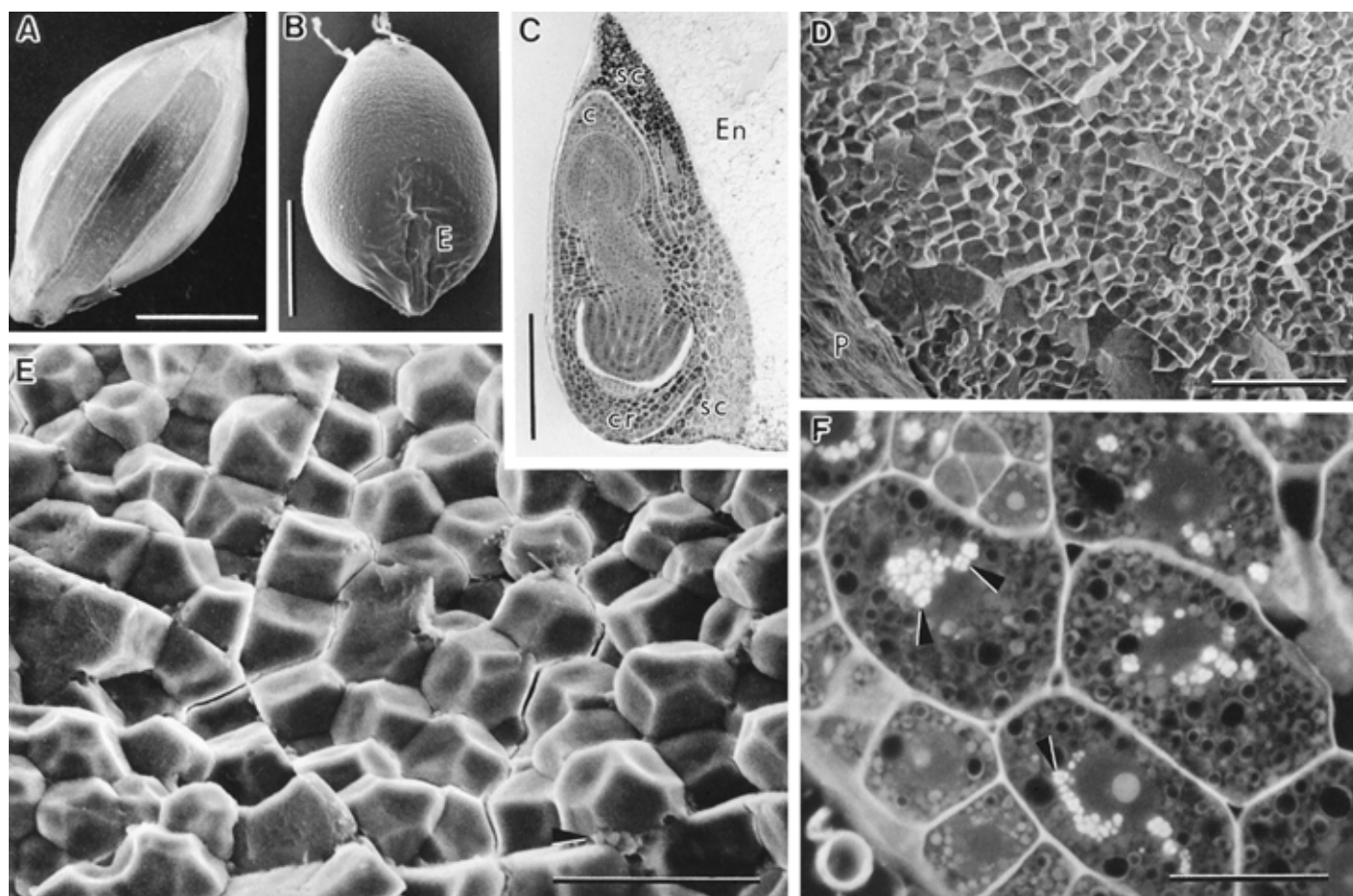
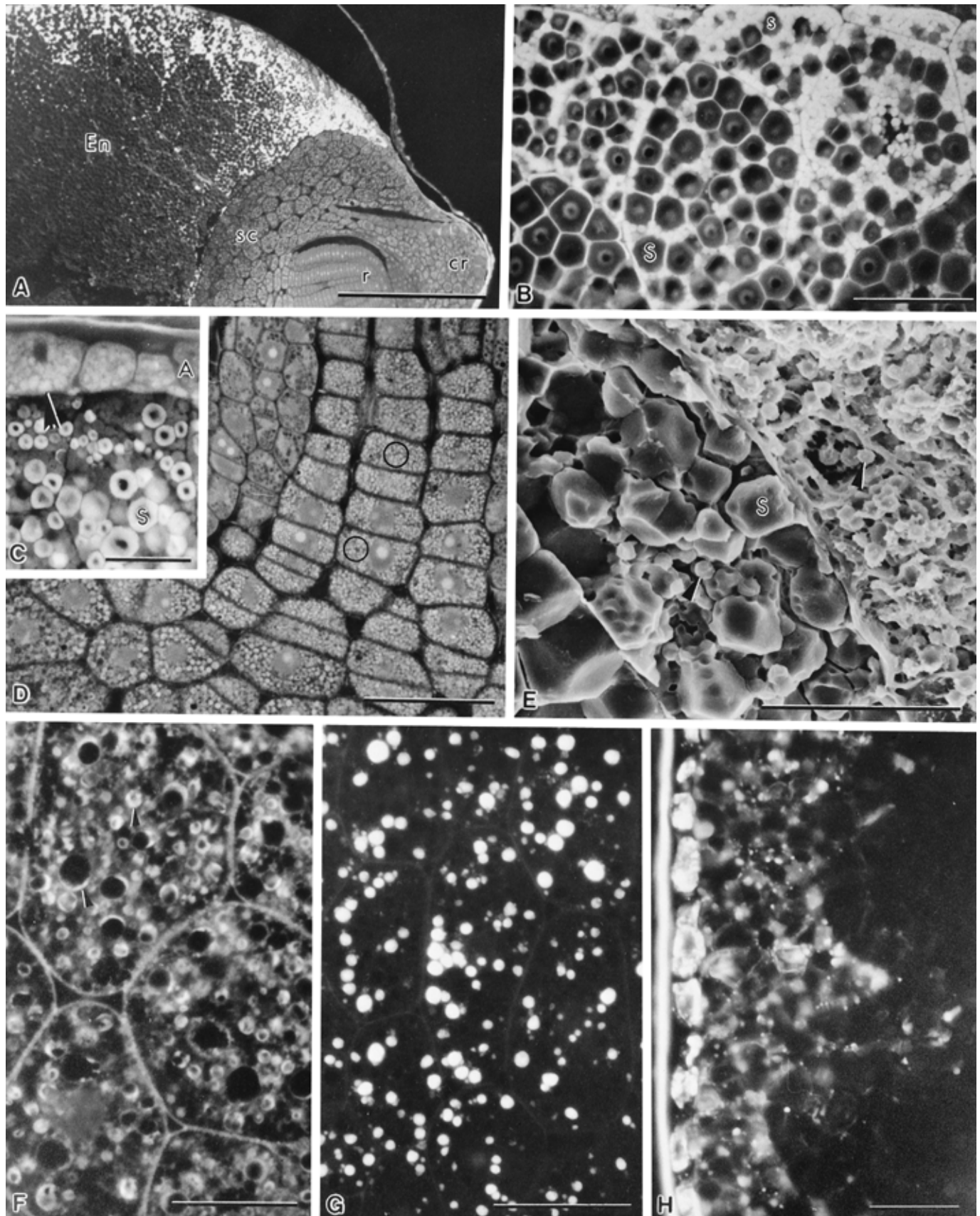


Fig. 1. Scanning electron micrographs (SEM) showing general morphology and starch distribution in *Digitaria exilis*. **A.** *D. exilis* floret with the lemma facing up. Magnification bar = 500 μm . **B.** SEM of the caryopsis with the embryo (E) facing up. Magnification bar = 500 μm . **C.** Longitudinal section through an approximately mid-sagittal region of the embryo shown under bright field conditions after staining with mercury bromophenol blue (protein stain) and toluidine blue O (a general stain). The scutellum begins toward the top of the micrograph (sc) and extends on the outside of the embryo on the right side (sc). The radicle (root) is toward the bottom with the cells forming the root cap just after the gap and the coleorhiza (cr) which encases the root and occurs at the very bottom. The shoot apex is toward the top of the micrograph adjacent to the encasing coleoptile (c). The starchy endosperm (En) occurs on the right side of the micrograph but has been left nearly unstained. Magnification bar = 250 μm . **D.** SEM of a portion of the starchy endosperm showing the large cells filled with polyhedral starch granules. A portion of the outside of the pericarp (P) is also visible. Magnification bar = 50 μm . **E.** SEM of the interior of a part of a single cell of the starchy endosperm showing the polyhedral starch granules and protein bodies (arrow), toward the bottom of the micrograph. Magnification bar = 20 μm . **F.** Plastic-embedded section through the scutellum of the embryo showing clusters of starch granules (arrow) stained with periodate-Schiff's reagent and photographed using the BP546 fluorescence filter set. Magnification bar = 20 μm .

caryopsis consisted of the endosperm, the embryo, and the associated layers of the pericarp and cuticle. The embryo, which constituted about a third of the total caryopsis, is shown in longitudinal section in Fig. 1C. The embryo had typical Poaceae structure and was larger in proportion to the caryopsis than that of

conventional cereal grains. The endosperm, which constituted the bulk of the caryopsis, consisted of both the aleurone layer and the starchy endosperm.

Starchy endosperm cells of *D. exilis* (Fig. 1D) contained the major storage reserve of the caryopsis, which was starch occurring



as simple, polyhedral granules ~10 μm in diameter (Fig. 1E). These starch granules of the starchy endosperm stained blue with aqueous iodine potassium iodide solution (not shown) indicating that they were high in amylose. Clusters of spherical starch granules (Fig. 1F) occurred as relatively minor components in the scutellum (the sheath covering the rest of the embryo) (Fig. 1C).

Another important storage component of the *D. exilis* caryopsis was protein occurring as protein bodies and found in all tissues of the grain (Fig. 2A). Protein bodies in the starchy endosperm were most abundant at its periphery (Fig. 2A) just below the single layer of aleurone cells (Fig. 2B,C). The spherical protein bodies of the starchy endosperm formed a tightly packed matrix between the starch granules. Aleurone cells also contained protein bodies (Fig. 2C), but the difference in staining intensity as compared to those in the starchy endosperm suggested that the aleurone protein bodies were of different chemical composition. Aleurone cells were small and the cell contents did not stain well with any dye other than that for lipid.

Protein bodies in the embryo occurred as small bodies, composed mostly of protein (without inclusions) in the coleorhiza and coleoptile (Fig. 2D). All protein bodies, including the protein bodies in the scutellum, appeared to be homogenous structures in the scanning electron microscope (Fig. 2E). However, sections of the scutellum viewed by light microscopy, followed by staining with a protein stain, revealed that the protein bodies contained structures that were left unstained (Fig. 2F) and, in many cases, the protein stain occurred only as a sliver at the periphery of the protein body. The unstained structures stained positively for phytic acid and were in the form of globoids (inclusions of myoinositol hexaphosphoric acid) (Fig. 2G). The globoids probably accounted for most of the phosphorus in the grain (0.12%) (Table I).

The third storage component of the caryopsis of *D. exilis* was lipid, which constituted ~2% by weight of the caryopsis (Table I). Lipid is difficult to preserve by standard embedding methods for light microscopy. Thus, cryosections were prepared to observe the general distribution of the storage lipid in the caryopsis. A cryosection of *D. exilis* (Fig. 2H) reveals the distribution of lipid in the aleurone and periphery of the starchy endosperm. Although the lipids actually occur as small droplets, they appear larger due to the thickness of the section as well as their observed tendency to coalesce upon preparation. Lipids were also generally distributed throughout the embryo tissues (not shown).

The chemical composition (dwb) of *D. exilis* is reported in Table I as: protein 8.2%, fat 2.1%, fiber 0.48%, and ash 1.4%. Total carbohydrate was calculated by difference from the total weight and was assumed to be 88%.



D. exilis is in the tribe Paniceae of the Poaceae family and shares similar features to other Paniceae such as *Echinochloa turnerana* (Australian channel millet) (Irving 1983). Similarities between the two species include the general shape and ratio of tissues, as well as the distribution of protein and starch. In *E. turnerana*, as in *D. exilis*, there is an unequal distribution of protein in the kernel. Protein bodies are abundant at the periphery of the starchy endosperm and decrease toward the center of the grain. The unequal protein distribution pattern is typical of conventional cereal grains (Adams et al 1976, Bechtel et al 1980). Protein in the starchy endosperm of mature kernels may occur as protein bodies in cereals such as oats, rice, and corn, or it may occur as a continuous matrix as it does in wheat and rye. Thus, *D. exilis* shares features of oat, rice, and corn in regard to the presence of protein bodies in the mature kernel.

There is a clear distinction between the protein bodies of endosperm cells and those of the aleurone layer. These two cell types differ in composition, structure, and function even though they are both classified as part of the endosperm. The cells of the starchy endosperm have storage function exclusively, and are, in fact, nonliving, whereas the cells of the aleurone layer possess synthetic and secretory functions as well (Simmonds and O'Brien 1981). Protein bodies in the aleurone layer of cereals are common and have been reported, for example, the aleurone grains of the wheat aleurone layer (Simmonds and O'Brien 1981). Generally, protein bodies of aleurone cells in cereals contain inclusions (Morrison et al 1975, Fulcher and Wong 1980) such as globoids, also known as phytic acid inclusions. Globoids have been found in the aleurone layer of wheat, barley, and oats and were determined to be crystals of calcium and magnesium double salt of myoinositol hexaphosphate (Fulcher et al 1981). Globoid inclusions were described elsewhere in cereal grains and legumes, and were shown to contain a form of stored phosphorus and to be associated with high acid phosphatase activity (Rost 1972). Globoid inclusions were not found in the aleurone cells of *D. exilis* but were within certain protein bodies in the embryo.

As in conventional cereal grains, starch is the major storage reserve found in the caryopsis of *D. exilis*. Starch morphology varies considerably in the cereal grains. For example, oat has compound starch granules, whereas wheat has simple granules with a bimodal distribution, i.e., two sizes and shapes of starch granules. The simple, polyhedral starch granules of *D. exilis* are similar in shape but larger than those of amaranth (Irving et al 1981, Zhao and Whistler 1994) but unlike the composition of amaranth starch, which is high in amylopectin. Starch granule size, shape, and relative ratios of amylose to amylopectin have a significant impact on the utilization of the starch, in both food and nonfood applications.

Fig. 2. Scanning electron micrographs (SEM) of *Digitaria exilis*. **A.** Longitudinal plastic section in the opposite plane of that in Fig 1C. The section was stained with acid fuchsin and photographed using the BP546 fluorescence filter set and shows protein distribution at the periphery of the endosperm (EN) (white areas) as well as in the embryo (not as easily seen). Scutellum (sc), radicle (r), coleorhiza (cr). Magnification bar = 250 μm . **B.** Plastic section of the starchy endosperm stained with acid fuchsin and photographed using the BP546 filter set showing protein bodies (small, white bodies which are tightly packed) at the periphery as well as starch granules (S). Note that the starch granules become larger toward the center of the starchy endosperm. The aleurone layer is just out of view at the top of the micrograph. Magnification bar = 20 μm . **C.** Plastic section stained with safranin O and photographed using the 450–490 fluorescence filter set to emphasize the starch granules in the starchy endosperm which appear as white bodies (with unstained centers) as well as the protein bodies in the aleurone cells (arrow). Note that the staining intensity of the protein bodies in the aleurone is much different than those of the starchy endosperm, which are barely visible. Magnification bar = 20 μm . **D.** Section through the coleoptile of the embryo stained with acid fuchsin and photographed using the BP546 fluorescence filter set showing small protein bodies (circles). Magnification bar = 50 μm . **E.** SEM of the junction of the starchy endosperm and the scutellum showing starch granules (S) and protein bodies (arrows). Magnification bar = 25 μm . **F.** Section of the scutellum stained with acid fuchsin (BP546) showing protein bodies (arrows). Note that virtually all of the protein bodies have centers that do not stain. The larger protein bodies only stain at the periphery, sometimes only on one side. Magnification bar = 20 μm . **G.** Section through the scutellum, stained with acriflavine HCl and photographed using the BP546 fluorescence filter set showing globoid (myoinositol hexaphosphoric acid) inclusions inside protein bodies in the scutellum. The ghost-like cell walls are visible in this micrograph. Magnification bar = 50 μm . **H.** Cryosection of the outer periphery of the endosperm stained with Nile blue A and photographed using the 450–490 fluorescence filter set to show lipid distribution (bright areas). Note that the aleurone cells contain a large quantity of lipid whereas, the starchy endosperm has much less. Magnification bar = 25 μm .

The composition of *D. exilis* grain is within the range of conventional cereals. Some values are lower and others are higher. Proximate composition of *D. exilis* was reported by Temple and Bassa (1991) for grain that was purchased directly from the market. The values for lipid (or fat) of 2.1% and nitrogen-free extract (or carbohydrate) of 87.5% agree with the findings presented in this report. However, values for protein (8.2 vs. 6.96%), ash (1.4 vs. 2.4%), potassium (260 vs. 109 mg/100 g) and magnesium (70 vs. 84.9 mg/100 g) disagree with our findings. Reasons for the discrepancies are probably the result of growing conditions, regions, or year in which the crops were grown. These types of discrepancies are within the normal range of what is to be expected from cereal crops.

Similar to *E. turnerana*, which was recognized as a promising grain crop for arid regions (NAS 1975), *D. exilis* is relatively drought-tolerant. Thus, *D. exilis* has potential in regions where low rainfall and irrigation expense present problems for the growth of crops. The low-yielding grain is in high demand in regions where it is grown and therefore commands a high price. *D. exilis*, or acha, as it is commonly known, is traditionally consumed as a whole grain after removal, usually done by winnowing, of the outer bracts and glumes. The small size and location of constituents in the grain suggest that minimal processing is the best option. Thus, removal of the outer bran layer by milling to make the grain more palatable is not advisable. However, as with conventional cereals, the advantage of a minimally processed grain is that the nutritional value is higher and the crop is less labor-intensive. Another positive attribute, perhaps due to its small size as well as its loss of moisture during storage (3.8%) (Temple and Bassa 1991), is that *D. exilis* is less susceptible to attack by organisms causing storage diseases. Thus, a crop such as *D. exilis* may be advantageous in the tropics and subtropics where a huge percent of a cereal crop is lost during storage.

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