Eastern Gama Grass. Seed Structure and Protein Quality[1]

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ABSTRACT

Seed structure, starch grains, and protein granules of eastern gama grass, *Tripsacum dactyloides* (L.) L., were examined with scanning electron microscopy, Nomarski interference contrast, and polarizing light microscopy. The embryo and aleurone layer were similar to those of maize (*Zea mays* L.). Starch grains and protein granules were only one-tenth as large as those of maize and ranged from 1.3 to 1.8 μm and from 0.05 to 0.12 μm, respectively. Amino acid analyses were compared to those of normal yellow dent hybrid maize, *opaque-2* maize, and literature reports of maize inbred lines and a line nearly isogenic for the *floury-2* mutant. Basic amino acids and cystine were low and methionine high compared to their levels in maize. Potential uses of gama grass are suggested.

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Eastern gama grass (*Tripsacum dactyloides* (L.) L.) is a widely adapted, wild perennial relative of maize (*Zea mays* L.). It has been studied extensively by taxonomists and geneticists for morphological and cytogenetic clues to the evolution of maize (Mangelsdorf 1974) and for its relationship to other *Tripsacum* species (Newell and DeWet 1974b). Hybrids of maize and *T. dactyloides*, beginning with the successful crosses by Mangelsdorf and Reeves (1931), have been studied with equal intensity for cytogenetic and evolutionary relationships (Chaganti 1965, Newell and DeWet 1974a) and for potential improvement of maize by exploiting the genetic variability transferable from *Tripsacum* (Gutierrez 1974, Johnston 1966).

Paulis and Wall (1977) compared the protein compositions of three maize cultivars, eastern gama grass, and two collections of teosinte (*Zea mexicana* (Schrader) Kuntze) to substantiate evolutionary and biochemical genetic relationships. Others have defined biochemical relationships within the relatives of maize (Rotar et al 1975) and correlated seed protein electrophoretic differences with spike morphological variation (Gray 1975).

Because eastern gama grass is used primarily as fodder or hay and the seed containing rachis segments of these genotypes shatter naturally as a seed-dispersal mechanism, yields of seed are extremely low. However, a nonshattering variant has been found,[4] which is a first step toward domesticating or managing a new perennial crop. This, with the recent discovery of *Zea diploperennis* (Iltis et al 1979), may hasten the advent of perennial crop species to complement the annual field crops of our present agricultural system. Because seed from the nonshattering varient was not available, a fodder type of *T. dactyloides* accession was examined microscopically and chemically to determine its potentially useful traits.

MATERIALS AND METHODS

Sample Identification

Seed of *T. dactyloides* (L.) L. (2n = 36), accession number PMK-24, PI-421612, grown in 1976 was obtained from the Plant Materials Center, Soil Conservation Service, Route 2, Manhattan, KS.

Sample Preparation

Each seed (fruit case plus caryopsis) was cracked with a pair of pliers and the full or broken caryopsis removed by hand. A similar separation of fruit cases from caryopses on a larger scale was attained by coarse corrugated roller milling and sieving.

A small amount of starchy endosperm was scraped from a broken caryopsis and dispersed in distilled water for Nomarski interference contrast and polarizing light examinations with a Reichert Zetopan microscope.

Full undamaged caryopses were cut with a razor blade and mounted on aluminum specimen stubs with Pelco colloidal silver paste for scanning electron microscopy. Samples were vacuum coated with carbon and gold-palladium to approximately 200 Å thick. They were observed at 10 and 20 KV, depending on the desired magnification, on an ETEC Autoscan electron microscope.

A sample of free caryopses was submitted for proximate analysis to the cereal laboratory of Western Star Mill, Salina, KS, for determinations of moisture, protein, fat, fiber, ash, and nitrogen-free extract (NFE).

Free caryopses were ground to a fine powder in a Wig-L-Bug

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[4]J. H. Lovell. 1976. Germination response of *Tripsacum dactyloides* to four seed treatments. Range and wildlife management class term paper No. 5316. Texas Tech University, Lubbock, TX. 17 pp.

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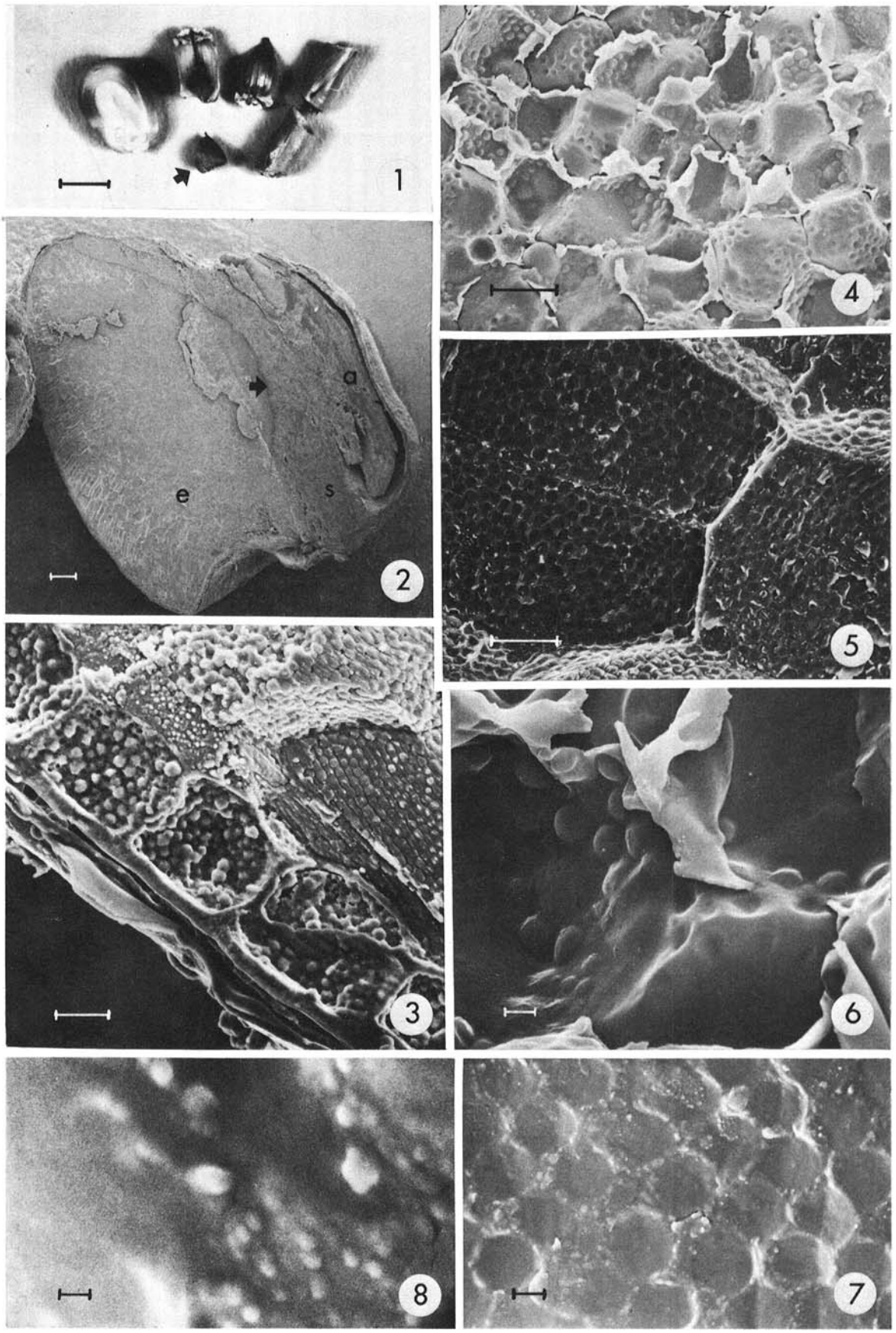
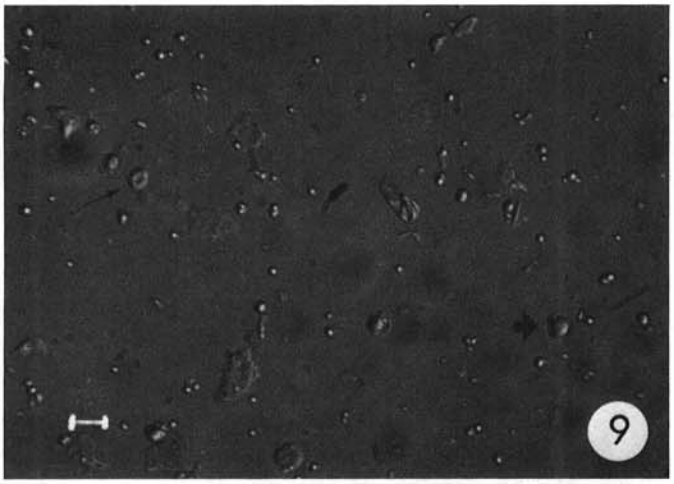


Fig. 1. Free gama grass caryopsis (arrow) compared to intact fruit cases (**right**), opened fruit case (**center**), and miaze (**left**) (bar = ¼in.). **Fig. 2.** Gama grass, longitudinal cross-section of whole caryopsis. **s** = scutellum; **e** = endosperm; **a** = embryonic axis; arrow shows demarcation of embryo from endosperm (bar = 200 μm). **Fig. 3.** Gama grass, close-up of pericarp, aleurone, and starchy endosperm (bar = 10 μm). **Fig. 4.** Maize, starchy endosperm (bar = 10 μm). **Fig. 5.** Gama grass, starchy endosperm (bar = 10 μm). **Fig. 6.** Maize, protein bodies (bar = 1 μm). **Fig. 7.** Gama grass, protein bodies (bar = 1 μm). **Fig. 8.** Gama grass, protein bodies (bar = 0.1 μm).

amalgamator in preparation for amino acid analysis. A portion of the ground sample was defatted with hexane in a Soxhlet extractor. Full fat and defatted samples containing approximately 15 mg of protein were weighed in 16 × 150-mm glass culture tubes. Exactly 10 ml of 3*N* *p*-toluenesulfonic acid containing norleucine internal standard was added, and the tubes were capped with Bacti Capalls. The tubes were placed in an enclosed boiling water bath for 31 hr. The samples were cooled; excess acid was neutralized with 6*N* NaOH; and the hydrolysates were diluted to 1 mg of protein per milliliter of final volume with 0.2*N*, pH 2.2, sodium citrate diluter buffer. Hydrolysates (185-μl aliquots) were analyzed on a Beckman 120C amino acid analyzer according to the 2-hr procedure.



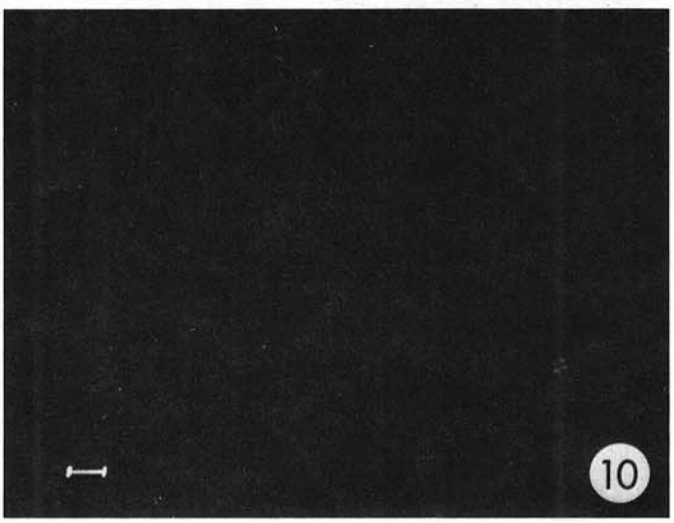


Fig. 9. Gama grass, starch granules, Nomarski interference contrast (bar = 10 μm). **Fig. 10.** Gama grass, starch granules, polarized light (bar = 10 μm).

RESULTS AND DISCUSSION

Seed Structure

Figure 1 compares the relative size of maize to a gama grass caryopsis and its fruit case. The caryopsis is relatively small but has a well-developed embryo with a large scutellum (Fig. 2) similar to that of maize. The aleurone is a single cell layer approximately 23–27 μm thick (Fig. 3). Maize aleurone cell thickness is of the same magnitude, averaging 46.5 ± 6.6 μm in the corresponding top back position of the seed opposite the silk scar (Wolf et al 1952).

Maize starch grains, measured from the scanning electron micrograph (Fig. 4), are 10–12 μm in diameter, which agrees with the average of 10 μm for dent corn reported by Wolf et al (1952). Gama grass starch grains average only 1.3–1.6 μm in diameter (Fig. 5), approximately one-tenth the size of maize starch grains.

An approximate 10-fold size difference is seen also in the protein granules. Figure 6 shows maize protein granules of 1–1.2 μm in diameter, which is in agreement with the reports of Duvick (1961) on normal maize and of Baenziger and Glover (1977) on inbred Oh 43 lines nearly isogenic for several endosperm mutants and the normal counterpart. In contrast, Figs. 7 and 8 show gama grass protein granules ranging from 0.05 to 0.12 μm in diameter.

Relatively few gama grass starch grains exhibited birefringence. Figures 9 and 10 are sequential Nomarski interference contrast and polarized light photographs of the same microscopic field. Only two large starch grains in Fig. 10 out of more than 100 smaller grains in Fig. 9 exhibited any birefringence, and only one showed clearly. Sandstedt et al (1968) reported that irregularly shaped or poorly developed maize starch grains exhibited little birefringence. They also demonstrated that several starch-modifying genes influence birefringence. Keltner et al (1978) reported that even broken maize starch grains were clearly birefringent. Because most gama grass starch grains are small and only the larger grains exhibit birefringence, one can presume the size of the starch grains, and not starch damage or parental plant genotype, is the primary cause of limited birefringence.

TABLE I
Amino Acid Contents of Gama Grass and Maize

	Gama Grass		Maize			
	Full Fat	Defatted	Bulk Normal	Bulk *Opaque-2*	*Floury-2*[a,b]	Range of 114 Inbred Lines[c]
Lysine	1.2	1.0	2.6	4.5	3.2	2.2– 5.2
Histidine	2.0	1.9	2.9	3.0	2.4	1.0– 5.6
Ammonia	3.2	3.2	3.1	3.0	2.8	...
Arginine	2.0	1.6	4.4	6.4	4.7	2.3– 8.1
Aspartic acid	5.4	5.7	6.3	10.3	8.5	4.9–14.5
Threonine	3.1	3.0	3.4	3.2	3.4	2.6– 5.8
Serine	5.3	5.1	4.6	4.3	4.4	0.6– 7.4
Glutamic acid	23.1	23.7	19.3	16.8	20.7	13.8–26.8
Proline	9.7	10.0	9.1	9.5	7.5	3.8–17.3
Half cystine	0.3	0.3	1.4	2.2	1.4	0.5– 3.5
Glycine	2.4	2.3	3.6	4.6	3.6	2.8– 6.4
Alanine	10.3	10.5	7.4	6.0	7.0	2.2–13.7
Valine	2.9	2.8	4.6	4.7	4.5	1.0– 9.1
Methionine	3.7	3.7	1.9	1.8	3.1	0.6– 2.4
Isoleucine	2.3	2.2	3.6	3.4	3.2	1.1– 5.5
Leucine	15.0	14.6	13.0	8.2	10.0	3.5–18.8
Tyrosine	3.6	3.8	3.9	3.7	4.2	0.8– 4.2
Phenylalanine	4.6	4.6	4.9	4.1	5.0	3.0– 9.1

[a] Recalculated to 100%.
[b] From Pfister Associated Growers, Aurora, IL (Nelson and Mertz 1973).
[c] Davis et al 1970.

Protein Quality and Amino Acid Analysis

Proximate analysis of free gama grass caryopses revealed these percentages: moisture, 10.10; protein (N × 6.25), 27.23; fat, 7.39; fiber, 2.50; ash, 1.44; and NFE, 51.34. Moisture and ash levels were similar to those of maize, but protein and fat were higher and fiber and NFE were lower. Departures from maize were expected in view of seed structure differences. For example, fat percentage would be expected to be higher if the embryo-endosperm ratio was proportionately larger in gama grass than in maize (Fig. 2). Embryo-endosperm ratios also influence protein quantity and quality (Bates and Heyne 1980), and smaller seeds generally exhibit higher protein levels with a compensatory drop of NFE.

The basic amino acids lysine, histidine, and arginine are present at only one-half the level found in a normal bulk maize, and, except for histidine, their concentrations fall below the extremes of levels in 114 inbread lines of maize (Davis et al 1970) and are less than one-third the level in bulk *opaque-2* maize (Table I). Glutamic acid, alanine, and leucine levels are higher than in normal maize, although they are within the inbred line range. Amino acid data is generally supported by Paulis and Wall's (1977) data except for the levels of lysine, histidine, arginine, valine, isoleucine, and tyrosine, which are 76% or less than their figures.

These results suggest that gama grass contains more prolamin or prolaminlike protein than does maize, an observation reaffirmed by the concentration of tiny protein granules and a relatively large amount of protein matrix surrounding the small starch grains. Those observations are supported by protein fractionation studies of Paulis and Wall (1977) that show a compensatory shift in the relative size of protein solubility classes, from larger amounts of water/salt-soluble albumins and globulins in normal maize to larger amounts of alcohol-soluble reduced glutelins in *Tripsacum*.

Methionine and cystine levels also fall outside the range of the inbred lines (Table I). However, in this case, their relative amounts are interesting because methionine exceeds the high extreme, whereas cystine falls below the low extreme of the range. Because methionine and cystine are both subject to oxidative losses during hydrolysis, one must be cautious with comparisons involving these two amino acids. We saw no evidence in our samples of methionine sulfoxides, methionine sulfone, or cysteic acid, the respective oxidation products, nor excessive destruction of tyrosine, threonine, and serine. Consequently the results should be valid for comparisons.

The methionine-cystine molar ratios of normal bulk maize and gama grass are approximately 1:1 and 10:1, respectively. *Floury-2* maize gentoypes contain more methionine than does normal maize and more than do some endosperm mutant types (Nelson et al 1965). The methionine-cystine ratio for *floury-2* maize is approximately 2:1, or at most 4:1 (Nelson and Mertz 1973). The normal maize-gama grass molar ratios are approximately 5:1 for cystine and 1:2 for methionine. *Floury-2*-gama grass ratios are 5:1 for cystine and 1:1.2 for methionine. Therefore, gama grass methionine exceeds even the high reported methionine levels of whole-kernel *floury-2* maize by 20%. At the same time, the molar ratios of cystine and methionine within and between the two species suggest that no direct, complete compensatory synthesis is operating between these two sulfur-containing amino acids. The amino acid content of each protein fraction (Paulis and Wall 1977) also reflects the absence of compensatory synthesis.

Protein synthesis in eastern gama grass, as represented by the relative amounts of amino acids in the total protein profile, is apparently a departure from maize protein synthesis, particularly with respect to the methionine-containing proteins. Paulis and Wall (1977) showed that gama grass prolamin fractions differed from maize primarily with respect to methionine. The gama grass genes involved may indeed provide protein nutritional improvement, via rearranged zein protein synthesis, if incorporated into maize. They also form the basis of a *Tripsacum* gene pool from which a new perennial crop species could be selected. In either case, eastern gama grass may provide insights into cereal grain structure and protein synthesis.

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