. 1980. Observations
ants in the male-sterile
21: 63–68.
New independent mu-
Genet. Newsl. 14: 181–

981. Biometry. W. H.

. 1980a. A partially
eans. *Glycine max* (L.)
29: 295–303.
tially male-sterile mu-
max (L.) Merr.: char-
type variation. Euphy-

le development in an-
oybeans. J. Hered. 73:

EIJZER, M. T. M. WIL-
Structure and function
ton during pollen de-
osa (Mill.) H. Duval.

composition and me-
le soybeans. Crop Sci.

ND C. A. BRIM. 1978.
e soybeans. I. Distri-
nd nitrogen during de-
8–841.
ism tests of Shennong
7. Second Assembly
4. No. 053. 241–242.

Amer. J. Bot. 75(1): 157–159. 1988.

517

SHORT COMMUNICATION

ANATOMICAL DISTINCTIONS BETWEEN THE PISTILLATE SPIKELETS OF THE SPECIES OF WILD-RICE (ZIZANIA, POACEAE)[1]

MELVIN R. DUVALL AND DAVID D. BIESBOER

Department of Botany, University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

The most reliable macromorphological characters that can be used to discriminate between the annual species of the genus *Zizania* are found in the pistillate spikelet. One aspect of this morphology is a textural dimorphism. The pistillate lemmas and paleas of *Z. aquatica* are chartaceous (papery) whereas those of *Z. palustris* are coriaceous (leathery). Pistillate lemmas and paleas of the two perennial species, *Z. texana* and *Z. latifolia*, are also chartaceous. To determine the anatomical basis for the nature of this character, pistillate lemmas and paleas were either fixed, treated with hydrofluoric acid, and sectioned; or fresh material was sectioned on a freezing microtome. Those with a chartaceous texture were found to have a single layer of thin-walled, subepidermal fibers whereas those with a coriaceous texture had at least two layers of thick-walled, subepidermal fibers.

THE PISTILLATE SPIKELET in *Zizania* L. exhibits a dimorphism that is of primary importance in the diagnosis of the two annual species. The systematic significance of this character has been extensively discussed elsewhere and spikelet morphology has gained recent wide acceptance as a diagnostic for the two annual species of *Zizania* (Dore, 1969; Dore and McNeill, 1980; Terrell and Wergin, 1981; Gould and Shaw, 1983; Aiken, 1986; Aiken et al., in press). The three varieties of *Z. aquatica* L. have chartaceous (papery) pistillate lemmas and paleas covered uniformly with prickle hairs. Both of the perennial species, *Z. texana* Hitchc., known from only one locality, and *Z. latifolia* (Griesb.) Stapf, the Asiatic wild-rice, also exhibit this morphology. By contrast, the lemmas and paleas of the two varieties of *Z. palustris* L. are coriaceous (leathery) with prickle hairs restricted to rows above the vascular bundles. Previous anatomical and ultrastructural studies of wild-rice anthecia (LaRue and Avery, 1938; Weir and Dale, 1960; Terrell and Robinson, 1974; Emery and Guy, 1979; Terrell and Wergin, 1981; Petrova and Bannikova, 1982) have not explored the basis of this textural dimorphism. We have undertaken an anatomical investigation of this feature because of its systematic importance.

MATERIALS AND METHODS—Plants of all North American taxa (Table 1) were raised under ambient conditions in a common greenhouse environment. Artificial hybridizations were performed in the greenhouse to produce three types of interspecific hybrids: *Z. aquatica* var. *aquatica* × *Z. palustris* var. *palustris*, *Z. aquatica* var. *aquatica* × *Z. palustris* var. *interior*, and *Z. texana* × *Z. palustris* var. *interior*. F_1 hybrids were raised under the same conditions as the stock collections. Field-collected material of *Z. latifolia* was used directly, since this species has not been induced to flower in Minnesota, even in the greenhouse. Five or more fully mature grains (the caryopsis, lemma and palea) of each stock collection were harvested, each from a separate plant in most cases (Table 1). A total of 68 specimens from 31 individuals were examined. Grains were bisected transversely. When possible the caryopsis was removed and discarded. The material was then treated by one of two methods: 1) material was fixed in 100% ethanol : glacial acetic acid (3:1, v/v), digested in 48% hydrofluoric acid for 25 hr, and rinsed in a continuous-drip supply of tap water for 60–72 hr.

Received for publication 26 January 1987; revision accepted 6 July 1987.

This project was funded, in part, by the Carolyn M. Crosby fellowship program, University of Minnesota. The authors thank Dr. Ernst C. Abbe, Eric Johnson, and Sharon Shoemer for technical advice and assistance. The authors also thank Dr. Susan Aiken, National Museums of Canada, Ottawa, Ontario, Canada; Dr. Shou-liang Chen, Nanjing Botanical Garden, Nanjing, Jiangsu, China; Dr. Robert Haynes, Department of Biology, University of Alabama, University, Alabama; and Dr. James Percich, Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, for donating material used in this study.

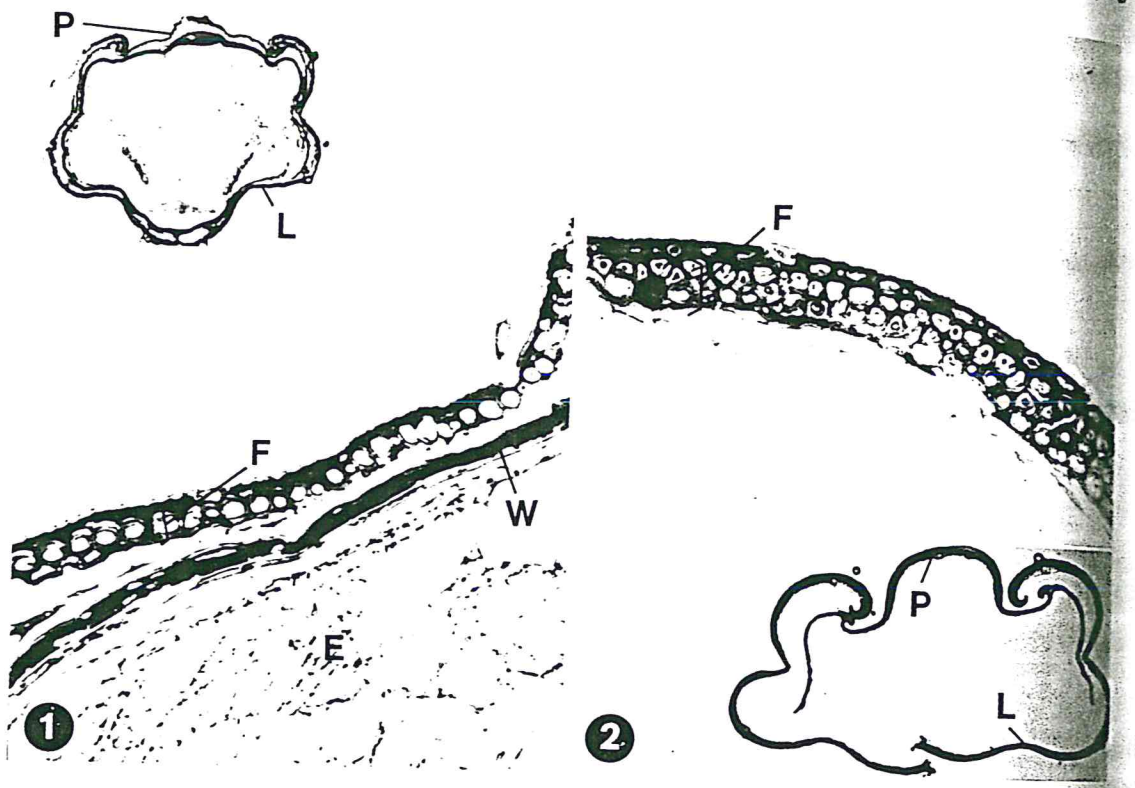

Fig. 1, 2. Light micrographs of typical intercostal regions of lemmas harvested from *Z. aquatica* (1) and *Z. palustris* (2). 1. Subepidermal fibers are found in a single layer. (Note: the enclosed caryopsis is included in this section.) ×367. Inset of entire transverse section. ×42. 2. Subepidermal fibers constitute two or more layers and have comparatively thicker walls. ×367. Inset of entire transverse section (fracture in the lemma is artifactual). ×30. E, endosperm; F, layer(s) of fibers; L, lemma; P, palea; W, pericarp.

The material was then dehydrated, embedded in Paraplast (Sherwood Medical, St. Louis), sectioned transversely at 10 μm, and stained in safranin/fast green. Sections were examined using phase contrast microscopy and photographed. 2) Fresh material was frozen in aqueous 1.5% gelatin and sectioned transversely at 4–8 μm on a cryostat microtome. Sections were mounted in concentrated hydrochloric acid (HCl) which cleared starch grains from the material that otherwise obscured the visual field. The sections were examined using phase contrast microscopy. While this method did not produce whole sections suitable for microphotography, it was adequate for quickly surveying many specimens.

Cell type was confirmed in a preparation of lemmas and paleas that were macerated for 24 hr in 10% chromium trioxide : 10% HCl (1:1) and stained in 1% aqueous safranin.

RESULTS AND DISCUSSION—Conventional paraffin embedding and sectioning of the paleas and lemmas of wild-rice failed to give satisfactory results because the high silica content of these structures seriously hindered sectioning. This difficulty was overcome by digesting the silica with hydrofluoric acid before paraffin infiltration.

Examination of transverse sections of the pistillate lemmas and paleas disclosed a marked anatomical difference between chartaceous and coriaceous forms. Intercostal regions of pistillate lemmas and paleas with a chartaceous morphology (*Z. aquatica, Z. texana,* and *Z. latifolia*) exhibited a single layer of thin-walled, subepidermal fibers (Fig. 1). Those with a coriaceous morphology (*Z. palustris*) typically possessed two or more layers of subepidermal fibers (Fig. 2). The average cell wall thickness of the fibers of the coriaceous forms was approximately 1.5× that of the chartaceous forms.

Three types of interspecific hybrids were also examined. Two types of annual interspecific hybrids, which were produced by crossing *Z. aquatica* var. *aquatica* with each of the two varieties of *Z. palustris,* exhibited a chartaceous morphology and the corresponding anatomy in all cases.

Interspecific hybrids between the perennial

TABLE 1. *Collections of* Zizania. *Five or more fruits were analyzed for each taxon from greenhouse-grown plants. Each [illegible] was known to be harvested from a different maternal stock except for donated collections which are marked [illegible] an asterisk. Note: var.* subbrevis *has been considered to be var.* aquatica *s. l. (Aiken, personal communication)*

Taxon	Collection site(s)
Z. aquatica var. *aquatica**	Mobile River Delta, Baldwin Co., Alabama; Blackberry Creek, Kendall Co., Illinois
Z. aquatica var. *aquatica* "Wading River ecotype"	Wading River, Burlington Co., New Jersey
Z. aquatica var. *brevis*	St. Lawrence River, Compte de Levis, Quebec
Z. aquatica var. *subbrevis**	Bear Brook, Cumberland Co., Ontario
Z. palustris var. *palustris*	Wisconsin River, Lincoln Co., Wisconsin; Cut River, Roscommon Co., Michigan
Z. palustris var. *interior*	Mud Hen Lake, Burnett Co., Wisconsin; Waumandee Creek, Buffalo Co., Wisconsin
*Z. texana**	San Marcos River, Hays Co., Texas
*Z. latifolia**	Jiangsu Province, China

Z. texana and *Z. palustris* var. *interior* displayed the spikelet morphology of the coriaceous parent. On the anatomical level, however, some intermediacy was seen in these perennial hybrids. Intercostal zones in the lemmas and paleas had short regions, 5–20 cells in width, with a single layer of subepidermal fibers. Otherwise these hybrids exhibited the anatomy that corresponds to the coriaceous morphology.

Our investigations disclose the anatomical basis for the observed difference in floral bract texture between species in the genus *Zizania*. The specific anatomical difference, one layer of subepidermal fibers vs. two or more layers, does not permit much intermediacy of this character except as displayed by the perennial hybrids between *Z. texana* and *Z. palustris* var. *interior*. The dimorphic nature of this character is thus confirmed as is its utility in discriminating between the annual species *Z. aquatica* and *Z. palustris*.

LITERATURE CITED

AIKEN, S. G. 1986. The distinct morphology and germination of the grains of wild rice (*Zizania*, Poaceae). Canad. Field-Naturalist 100: 237–240.

———, P. LEE, D. PUNTER, AND J. STEWART. In press. Wild rice in Canada. Ottawa: Agriculture Canada.

DORE, W. G. 1969. Wild-rice. Canada Dept. Agric. Res. Publ. 1393: 1–84.

———, AND J. MCNEILL. 1980. Grasses of Ontario. Agriculture Canada Monograph 26.

EMERY, W. H. P., AND M. N. GUY. 1979. Reproduction and embryo development in Texas wildrice (*Zizania texana* Hitchc.). Bull. Torrey Bot. Club 106: 29–31.

GOULD, F. W., AND R. SHAW. 1983. Grass systematics, 2d ed. College Station: Texas A&M University Press.

LARUE, C. D., AND G. AVERY. 1938. The development of the embryo of *Zizania aquatica* in the seed and in artificial culture. Bull. Torrey Bot. Club 65: 11–21.

PETROVA, L., AND V. BANNIKOVA. 1982. Morphology of the reproductive organs and the anthecology of *Zizania latifolia* (Poaceae). Bot. Zvurn. (Moscow & Leningrad) 67: 26–32.

TERRELL, E. E., AND H. ROBINSON. 1974. Luziolinae, a new subtribe of oryzoid grasses. Bull. Torrey Bot. Club 101: 235–245.

———, AND W. P. WERGIN. 1981. Epidermal features and silica deposition in lemmas and awns of *Zizania* (Gramineae). Amer. J. Bot. 68: 697–707.

WEIR, C. E., AND H. M. DALE. 1960. A developmental study of wild rice, *Zizania aquatica* L. Canad. J. Bot. 38: 719–739.