ANATOMICAL DIFFERENCES OF ABSCISSION REGIONS AMONG AND WITHIN *ZIZANIA* SPECIES AND IN RELATIONSHIPS TO SEED SHATTERING.

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Introduction

In the wild, seed shattering from plants is an important characteristic for dispersal and survival. However, when plants are grown specifically for their seeds (cultivated) this characteristic causes much seed loss before harvest. The seed from wild rice, *Zizania palustris* L. has long been utilized by Native North Americans for food, but the seed was only collected from wild stands. Wild rice is a recently developed cultivated crop and seed shattering remains in the crop and is a major problem in cultivation. Breeders have selected lines to reduce the tendency of seeds to shatter (abscise), however considerably more shattering resistance is needed to achieve comparable levels of other cultivated cereals such as wheat. Today the main focus in variety improvement continues to be improved seed retention (Hayes et. al, 1988).

The nonshattering characteristic in wild rice is controlled by a small number of recessive genes (Woods and Clark, 1976) and two complementary dominant genes were proposed to explain shattering habit of staminate florets (Elliott and Perlinger, 1977). Hanten et al. (1982) have reported the anatomical changes associated with morphology of the abscission zone of wild rice and correlates those changes with embryo development.

In *Oryza sativa*, the degree of seed shattering is related to the thickness of the cell zone which supports the seed. The zone consists of the vascular channel and the lignified cells around the channel at the separating zone in the variety that has a cracked (cell separation) abscission layer at harvest. In the genus *Oryza*, however, there are many types of abscission regions such as no abscission layer, cracked or noncracked abscission layer, complete or incomplete abscission layer, fully developed or partially developed abscission layer, and dome type or plate type abscission layer that have been observed and several types are closely related with the degree of the seed shattering. The formation of abscission layer is controlled by a dominant gene.

Zizania is a genus of four species and of those species only *Zizania palustris* has been cultivated. This study provides an anatomical description of the abscission zone in wild rice, and the differences among four species belonging to the genus *Zizania* and between shattering and non-shattering types of cultivated wild rice in relation to degree of seed shattering.

Materials and Methods

Germinated seed of *Z. palustris*, cultivar 'Netum', *Z. aquatica*, and crosses between *Z. palustris* and *Z. texana*, obtained from Duvall, National Museum, and *Z. latifolia* collected from Suncheon, Korea were transplanted into flooded soil contained in plastic containers. The plants

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were grown outside at Suncheon, Korea in 1992. In 1993, *Z. texana* obtained from Power, Southeast Texas State University, plants were grown in a growth chamber, 20°C and 12-hour photoperiod, at Minnesota. At panicle emergence and maturity, the pistillate florets of each species were collected.

To observe morphological features of the abscission region about 20 samples, the basal portion of seed and the upper portion of the pedicel, were excised from the upper portion of each panicle and preserved in formalin-acetic acid-alcohol (F.A.A.) for later processing for light microscopy using standard procedures (Sass 1958). Longitudinal serial sections of 10 μm were stained with safranin and fast green.

In this experiment, the strength in grams required to detach a seed from its pedicel or rachilla by a bending force (bending) or by a straight pull force (tensile) as an index of the degree of seed shattering, was measured by an unbonded gauge type transducer (UT: 1 kg) and automatic null balancing recorder, using the same panicle as in the above experiment.

Results and Discussion

Anatomical features of abscission zone observed in this study agrees essentially with the description of Hanten et al. (1980).

At panicle emergence, clearly distinguished abscission layers consisting of one or more layers of parenchyma cells with dense cytoplasm and dark staining nuclei are located in the junction of the spikelet and pedicel surrounding two or more layers of sclerenchyma cells above and below the abscission layer in all of examined lines and cultivars (Fig. 1A through 1F). Parenchyma cells in the abscission layers extend from epidermal cell to central vascular tissue (Fig. 1A through 1F). Therefore, any thicker walled lignified cells between abscission layer and central vascular tissue in the separating zone are not evident as they are in some cultivars of rice, *Oryza sativa*, which have some seed shattering resistance. In wild rice lines and cultivars, except *Z. latifolia*, most abscission layers consist of one or two layers of parenchyma cells and two or more layers adjacent to the vascular channel (Fig. 1A,C-F). In *Z. latifolia* parenchyma cells of the abscission layer are two or three layers at the epidermis, six or eight layers near the cortex and three or four layers adjacent to the vascular channel (Fig. 1B).

Parenchyma cells in the abscission layer are smallest in *Z. aquatica*, and in crosses (open pollinated) between *Z. palustris* and *Z. texana*. The size is similar in *Z. texana, Z. palustris* nonshattering type from Netum, *Z. latifolia* and *Z. palustris* shattering type of cultivar Netum. The number of rows of cells from epidermis to vascular channel is greater in *Z. palustris* both in the nonshattering and shattering types of Netum, and fewer in *Z. aquatica* and *Z. latifolia*. (Table 1.).

In this study, kernels were mature approximately four weeks after panicle emergence as indicated by their dark aleurone layer. Parenchyma cells in the abscission layer were cracked completely at maturity in *Z. aquatica* (Fig. 2A), *Z. palustris* shattering type in Netum, (Fig. 2C), *Z. texana* (Fig. 2E) and crosses between *Z. palustris* and *Z. texana* (Fig. 2F). Cracking of the abscission layers was not evident in *Z. latifolia* or nonshattering type of cultivar Netum, *Z. palustris.* The mass of parenchyma cells in the abscission zone of the former were larger at maturity than at panicle emergence and had dense cytoplasm and enlarged dark staining nuclei (Fig. 1B, 2B and Table 1). However, parenchyma cells of the nonshattering types of *Z. palustris* were smaller at maturity compared to panicle emergence stage of development (Fig. 1D, Fig. 2D and Table 1).

Hanten et al. (1980) concluded that plasmolysis, first anatomical evidence of abscission, occurred prior to separation (cracking) of epidermal cells and parenchyma cells of abscission layer. But any evidence of separation of abscission layer could not be found in *Z. latifolia* or nonshattering types of *Z. palustris*, Netum (Fig 2.B. and D.) examined in this study.

In the abscission layers cracked at maturity, two or three rows of parenchyma cells adjacent to the vascular tissue did not collapse, (Fig. 2A,C,E and F). In *Oryza sativa*, mechanical strength of the lignified cells of the supporting zone and the vascular channel could contribute to the resistance to seed abscission in nonshattering cultivars. The thicker walled lignified cells are more effective for mechanical strength then the vascular channel which consists of thin walled phloem and parenchyma cells and xylem cells without fiber. Sclerenchyma cells between abscission layer and central vascular tissue could not be found in wild rice, genus *Zizania*, which is similar to easily shattered wild and weed type of *Oryza*.

Hanten et al. (1980) concluded that the separation of abscission layer in wild rice, *Z. palustris* is due to dissolution of middle lamella and at completion of seed abscission, wall material appears to be missing or fragmented on the separated surface of the abscission layer. In our study though some parenchyma cells of the abscission layer remained attached to proximal or distal sclerified cells, most of the parenchyma cells in the abscission layer were observed to be eroded with remnants of wall materials attached to proximal or distal surface in all plants having cracked abscission layers (Fig. 2A, C, E and F).

It has been reported that some histological peculiarities are associated with seed shattering in *Oryza*. Histological examination of wild rice in this study showed that histology varies by the species (Table 2.). The diameters of separating zone of *Z. palustris*, both shattering and nonshattering, are large and those of *Z. aquatica* are small among the species of *Zizania*. However, the diameter of the separating zone of the other species appear to be near that of *Z. aquatica*. The diameters of the crosses, between *Z. palustris* and *Z. texana* appear to be near that of *Z. texana*. Also, the diameters of upper most part of the pedicel had a similar tendency as above but not the diameter of vascular channel of the crosses appeared to be near that of *Z. palustris*. *Z. latifolia* has a thicker abscission layer with more layers of parenchyma cells among all the species investigated in this study (Table 2.). The thinnest layers were observed in both *Z. palustris* and *Z. texana* appears to be similar to that of *Z. palustris*.

The abscission zone from numerous wild rice plants from a number of crosses between nonshattering and shattering types of *Z. palustris* were also sampled and will be examined for differences. This will be done by Dr. Jin in Korea and the information should be helpful in the development of more shatter resistant wild rice varieties for Minnesota.

The amount of force necessary to detach a seed by bending or pulling (tensile) is shown in Table 3 for the three species and for shattering and nonshattering types of *Z. palustris*. At heading (panicle emergence) time both bending and tensile forces were greatest for *Z. palustris* than for the other three species and crosses between *Z. palustris* and *Z. texana*. At maturity this was still true except for *Z. latifolia* which had a higher tensile strength than the shattering type of *Z. palustris*. The tensile strength at maturity was highest for the nonshattering type of *Z. palustris* indicating that the degree of seed shattering has been improved in wild rice.

Table 1. Morphological features of parenchyma cells in the abscission layer of four *Zizania* species.

Species	Cell size (μm) at		No. of cells at		Amount of cracking at
	Heading	Maturity	Heading	Maturity	Maturity
Z. aquatica	6.1-6.5	—	14-15	—	Cracking
Z. latifolia	7.5-8.5	9.0-10.0	12-16	12-14	Non-cracking
*Z. palustris**					
shattering types	8.1-8.5	—	24-28	—	Cracking
nonshattering types	7.6-8.0	7.1-7.5	26-30	28-30	Non-cracking
Z. texana	7.5-8.0	—	16-20	—	Cracking
Z. palustris X *Z. texana* crosses**	6.5-7.0	—	20-22	—	Cracking

*Cultivar Netum
**Open pollinated

Table 2. Difference of some histological peculiarities of seed abscission region among four *Zizania* species.

Species	Diameter of abscission region	Diameter of pedicel	Diameter of vascular tissue	Middle cortex thickness	Abscission Layer No. of cell layers at epidermis	at middle cortex	near vascular tissue
	μm						
Z. aquatica	222±17.5	194±14.5	44±2.2	13.3±1.8	1-2	1-2	2-3
Z. latifolia	304±11.4	264±14.6	76±5.3	42.1±6.0	2-3	6-8	3-4
*Z. palustris**							
shattering types	549±50.0	500±50.3	107±8.2	18.6±3.5	1-2	1-2	2-3
nonshattering types	510±39.2	470±40.2	102±11.2	20.1±3.7	1-2	1-2	2-3
Z. texana	305±12.9	295±14.0	66±1.7	11.1±1.0	1-2	1-2	2-3
Z. palustris X *Z. texana* crosses**	326±15.5	310±16.6	91±3.2	16.9±2.1	1-2	1-2	2-3

*Cultivar Netum
**Open pollinated

Table 3. Differences in bending and tensile force required to detach a seed from its pedicel at heading and maturity in four *Zizania* species.

Species	Heading time		Maturity	
	Bending	Tensile	Bending	Tensile
	g			
Z. aquatica	10.4±4.6	63.4±8.4	1.1±0.5	7.4±2.6
Z. latifolia	18.5±5.8	148.1±23.6	6.2±2.8	49.3±14.1
*Z. palustris**				
shattering types	49.8±8.4	244.3±22.2	3.7±1.2	28.9±4.4
nonshattering types	46.4±10.8	217.6±36.6	9.8±2.9	75.2±22.7
Z. texana	16.1±5.3	114.6±19.4	0.2±0.1	1.2±0.4
Z. palustris X *z. texana* crosses**	14.9±5.4	128.4±18.2	2.6±1.2	6.4±2.5

*Cultivar Netum
**Open pollinated

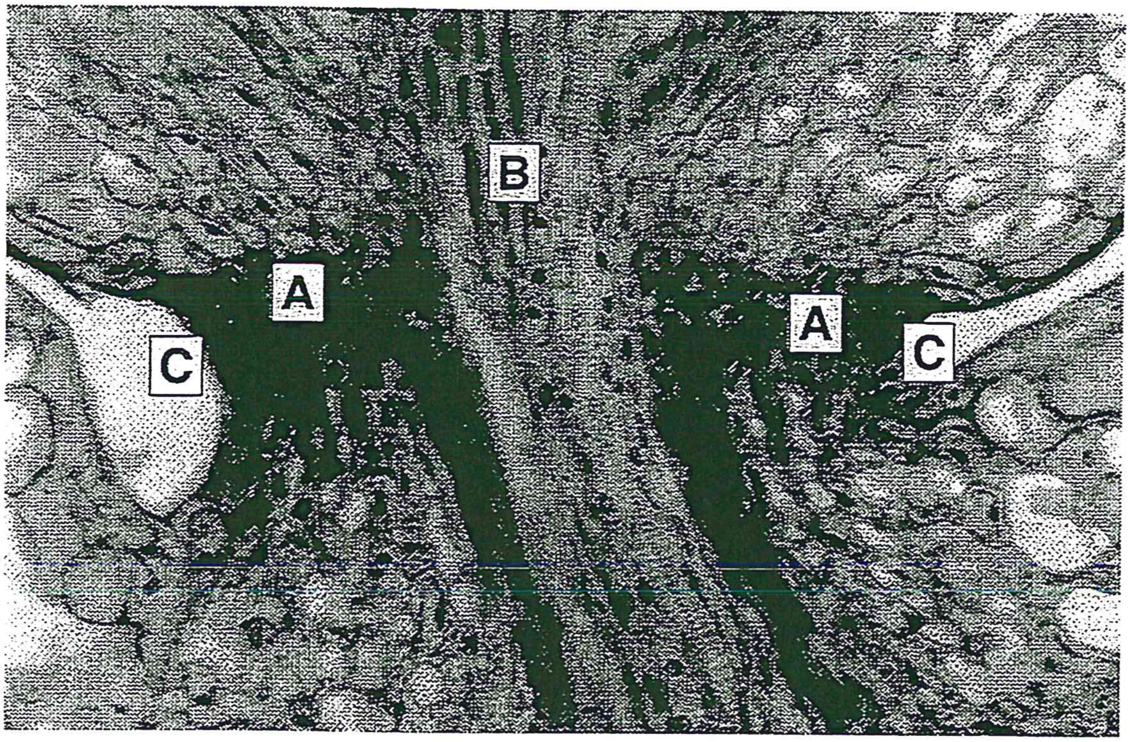

Figure 1A. *Z. aquatica* abscission zone, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

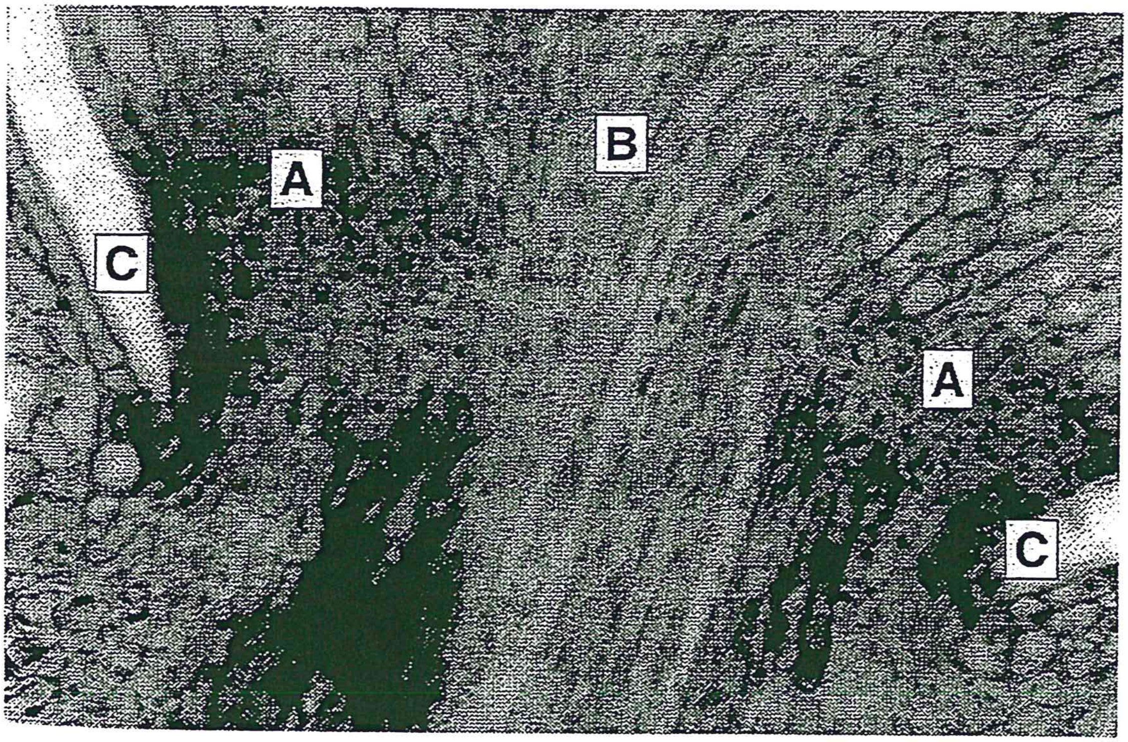

Figure 1B. *Z. latifolia* abscission zone, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

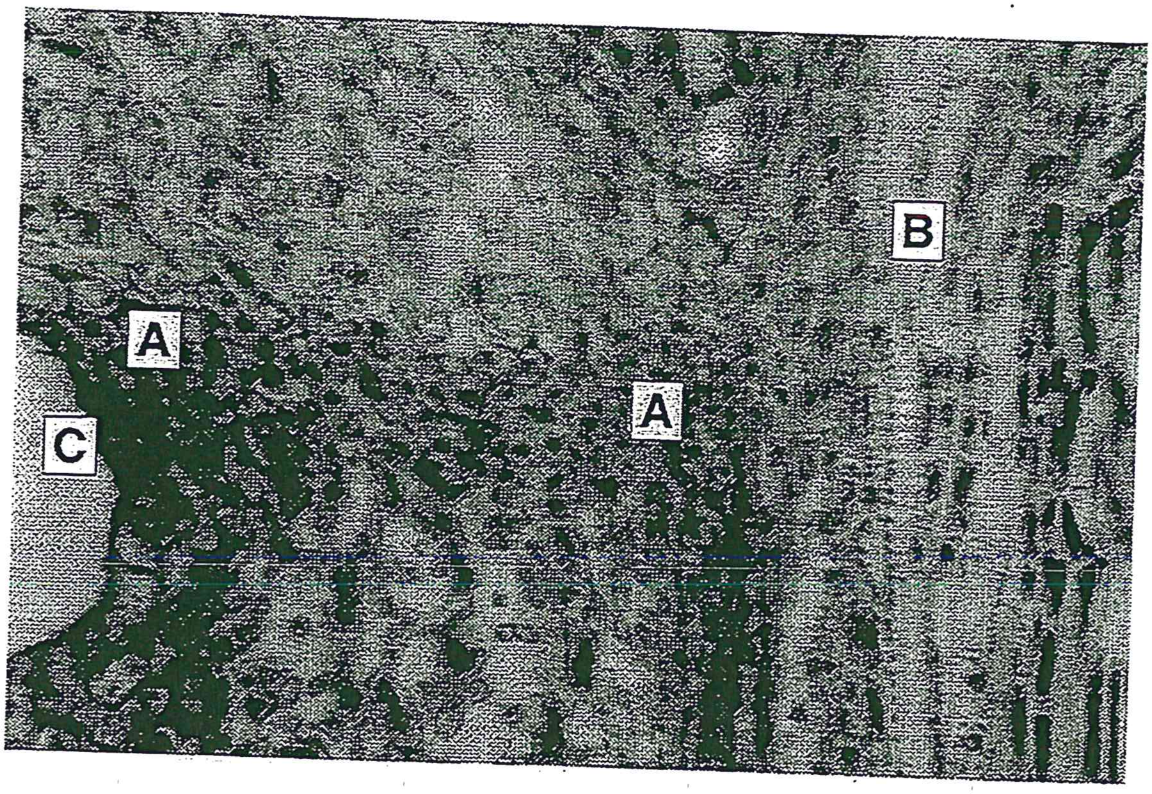

Figure 1C. *Z. alustris* left half of abscission zone, cultivar Netum, shattering type plant, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

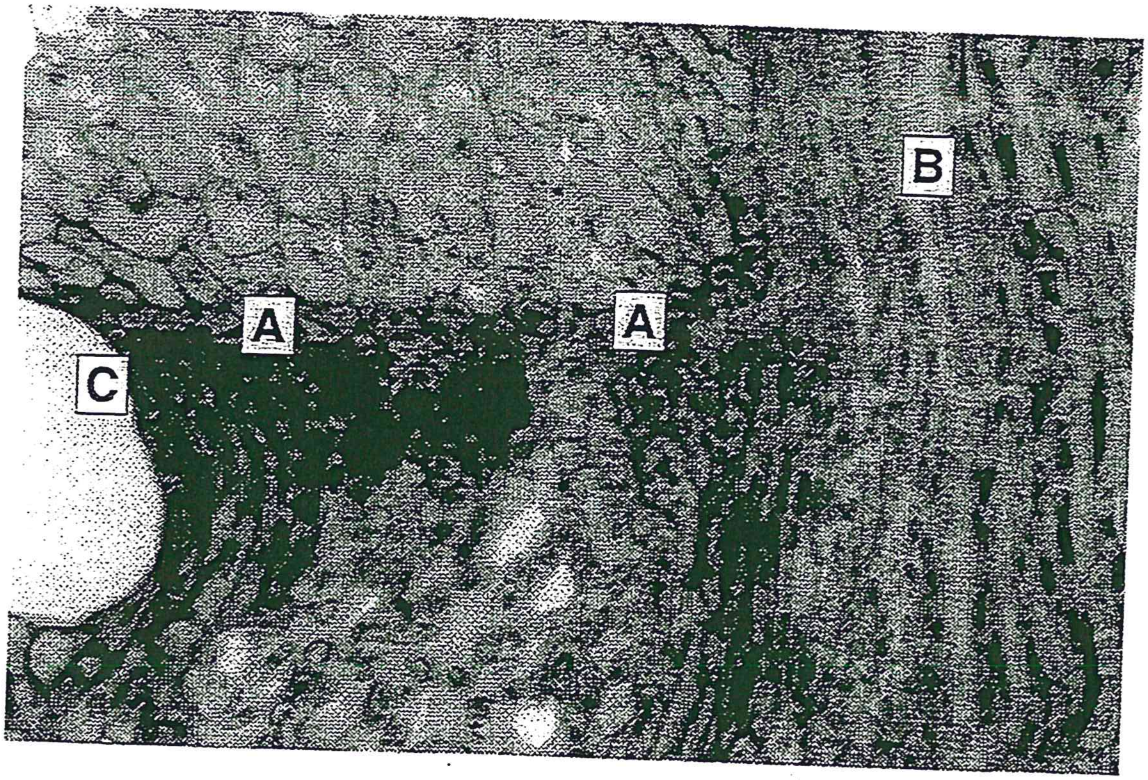

Figure 1D. *Z. palustris* left half of abscission zone, cultivar Netum, nonshattering type plant, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

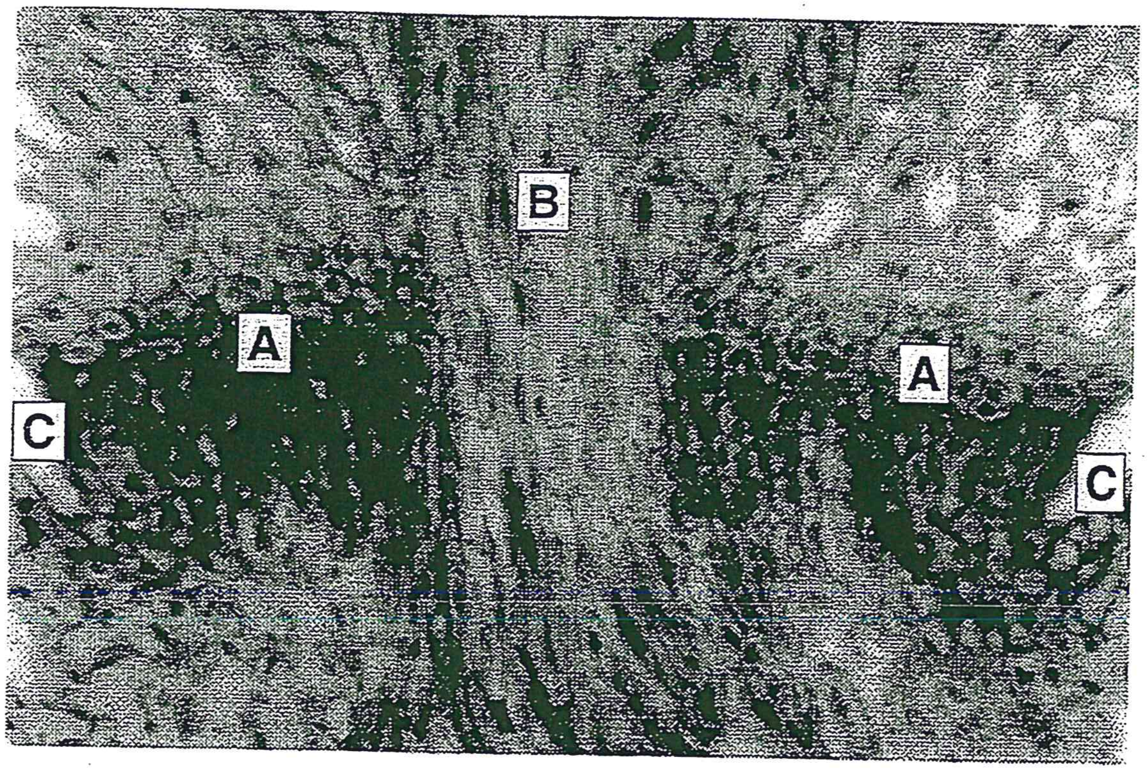

Figure 1E. *Z. texana* abscission zone, immature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

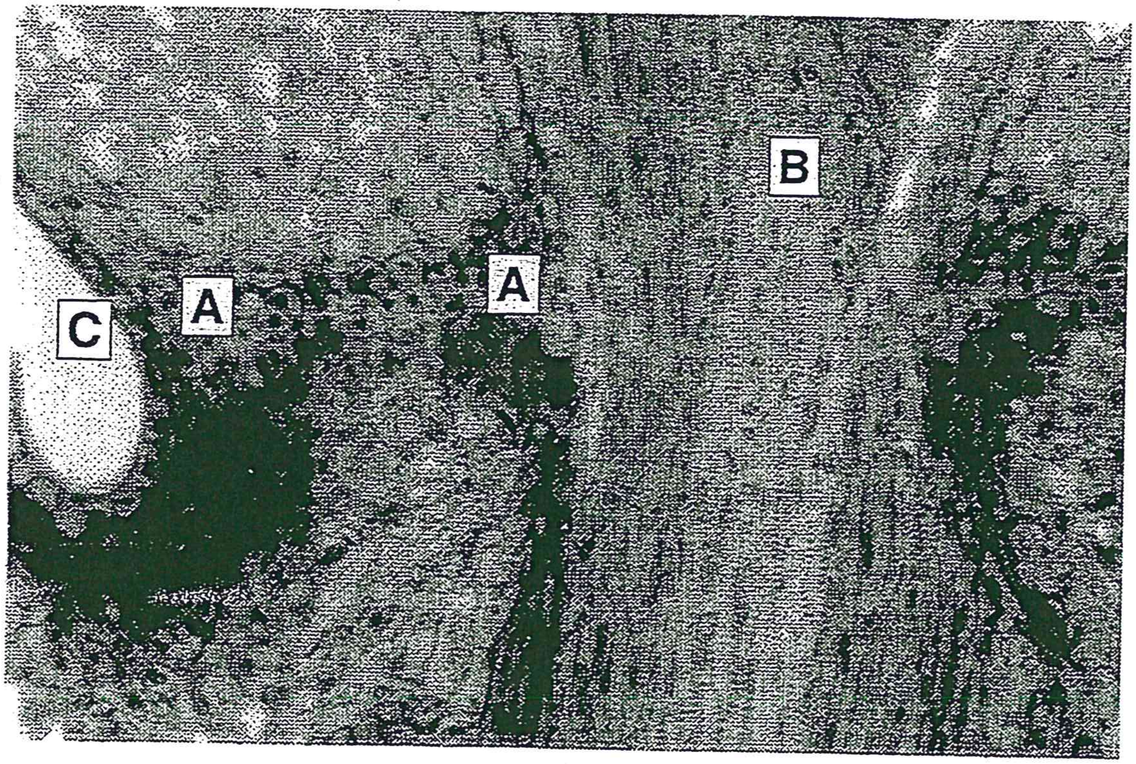

Figure 1F. Left and part of right half abscission zone in plant from cross of *Z. palustris* and *Z. texana*, immature kernal (A = abscission layer; B = central vascular tissue; C = epidermal cells)

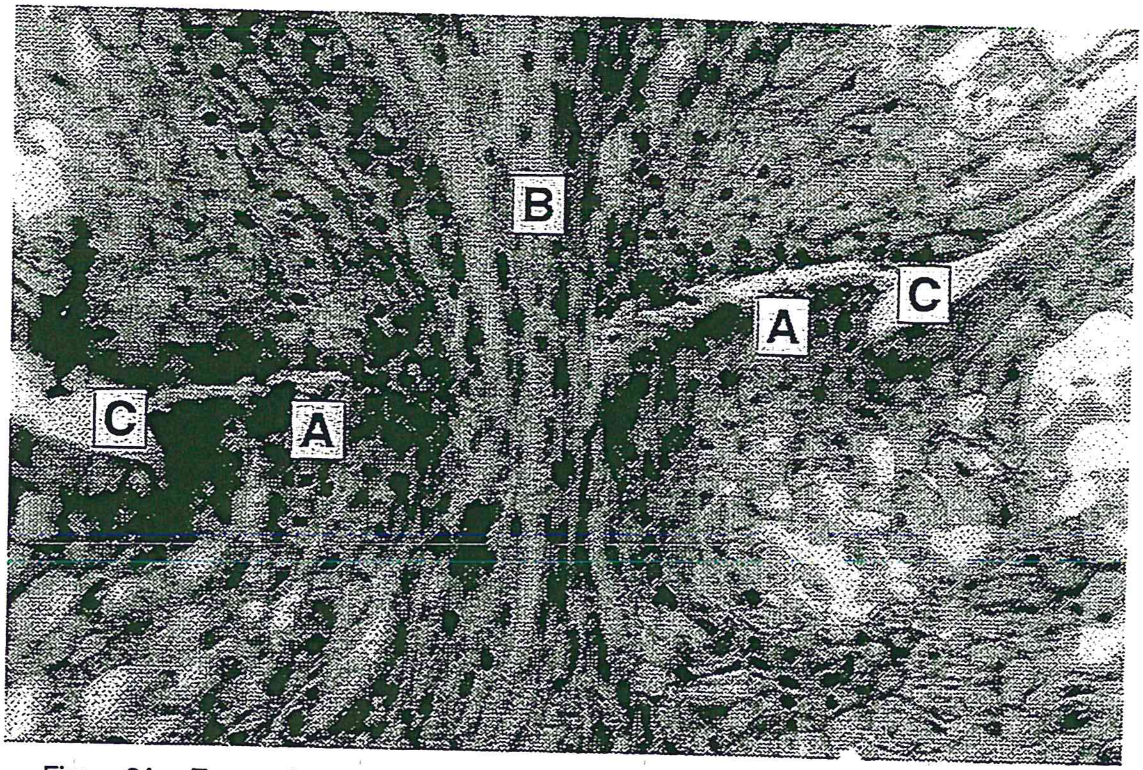

Figure 2A. *Z. aquatica* abscission zone, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

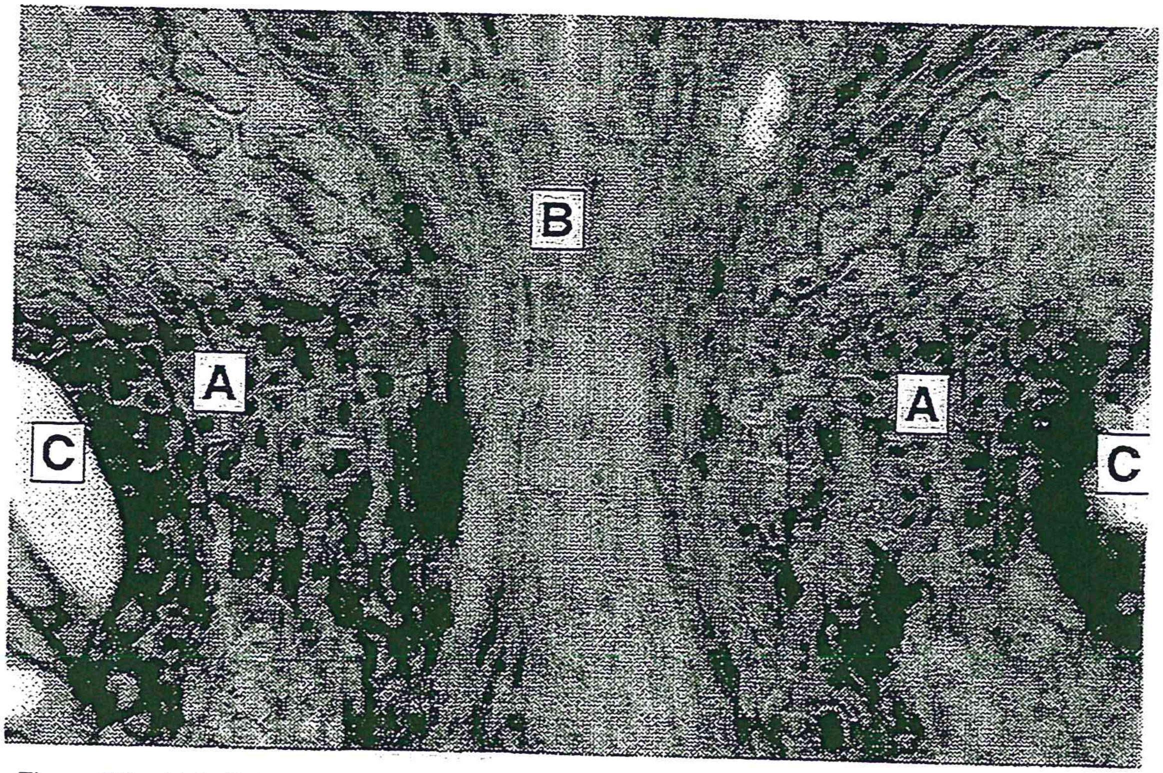

Figure 2B. *Z. latifolia* abscission zone, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

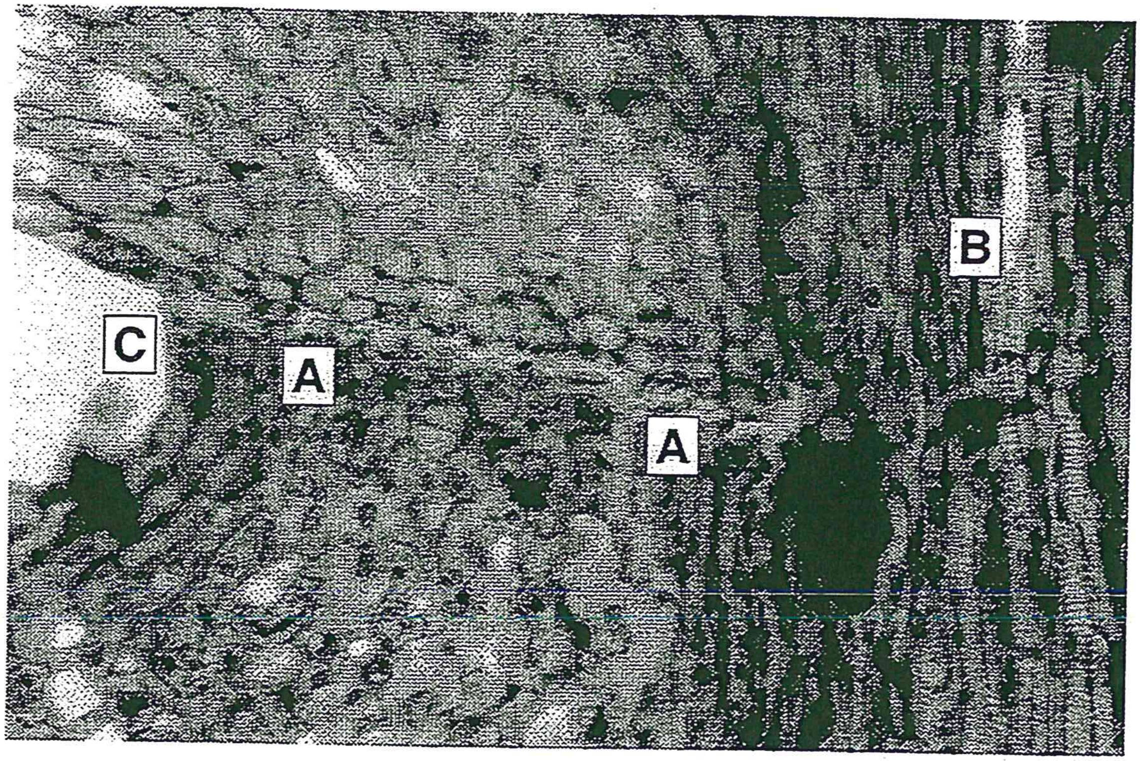

Figure 2C. *Z. palustris* left half of abscission zone, cultivar Netum, shattering type plant, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

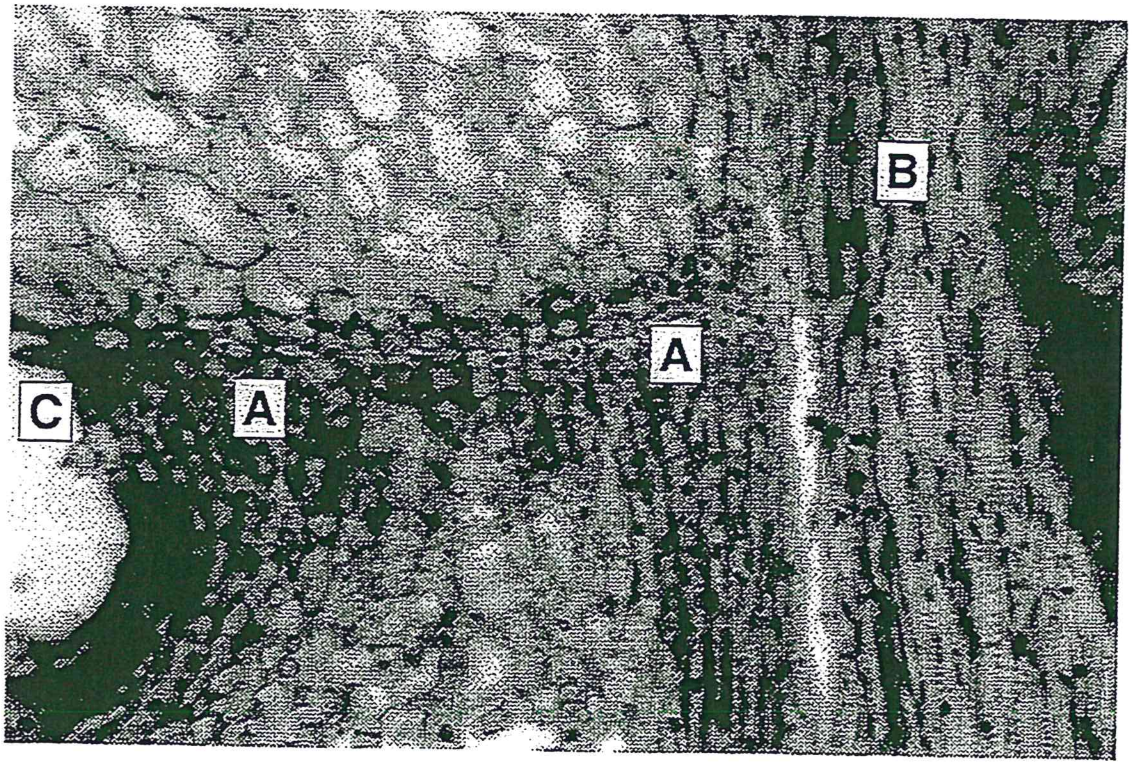

Figure 2D. *Z. palustris* left half of abscission zone, cultivar Netum, nonshattering type plant, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

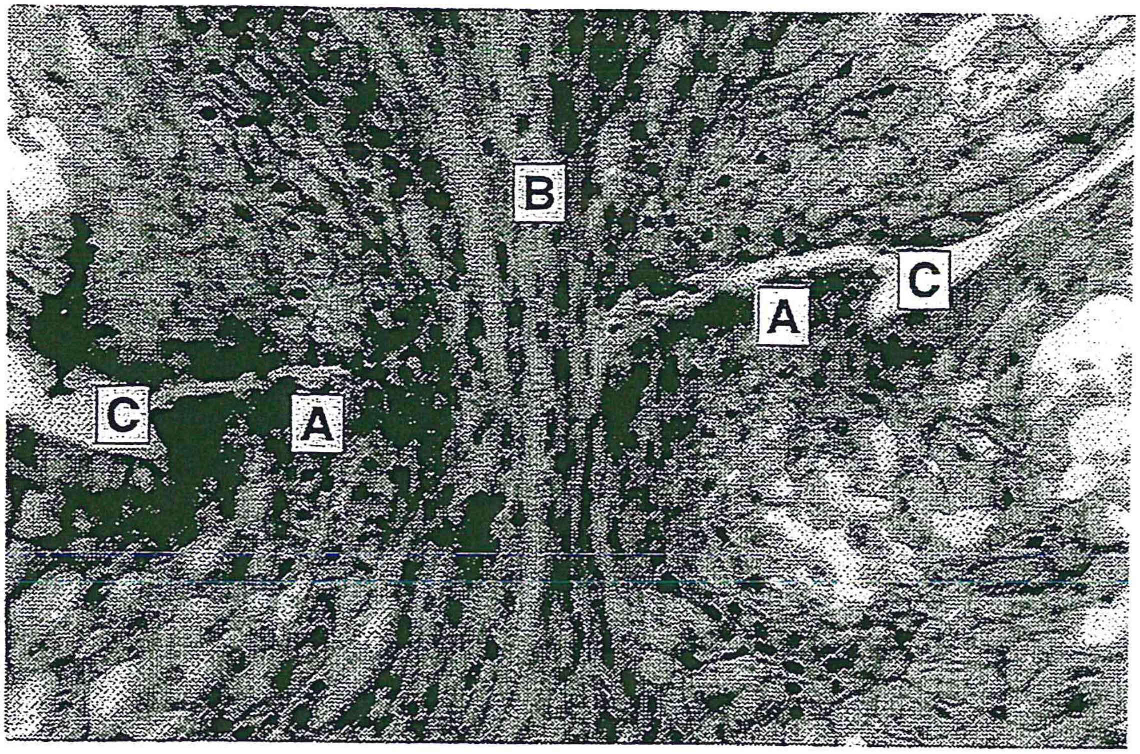

Figure 2E. *Z. texana* abscission zone, mature kernel (A = abscission layer; B = central vascular tissue; C = epidermal cells)

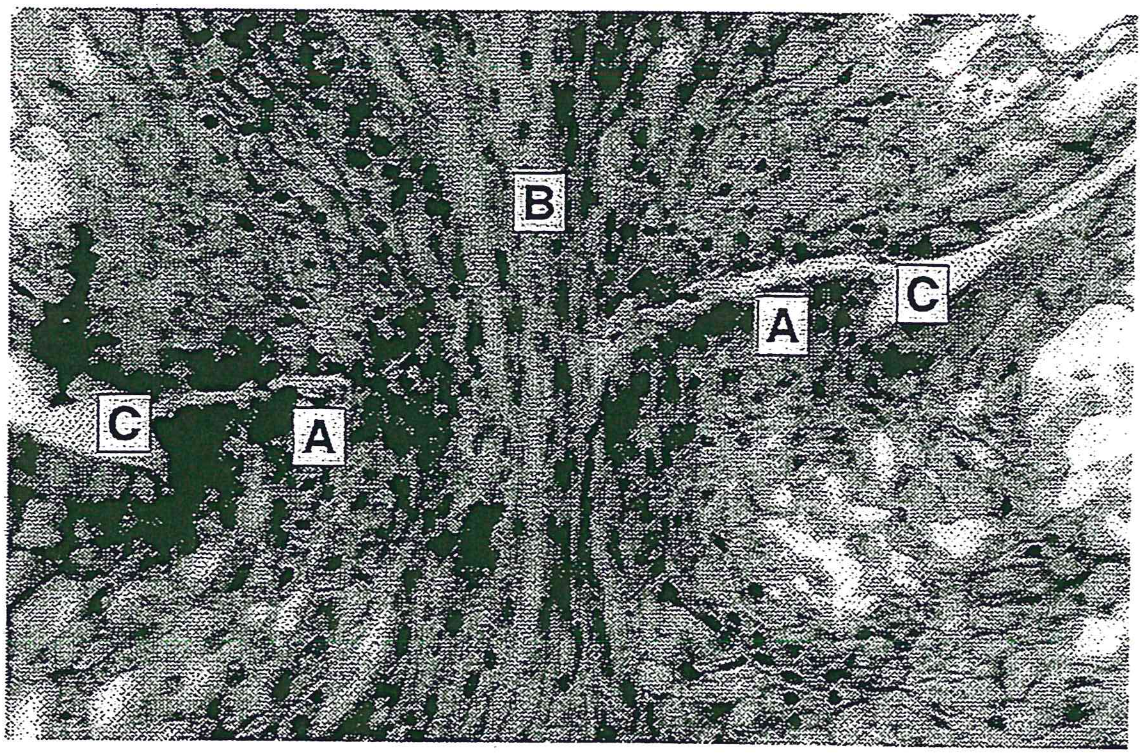

Figure 2F. Left and part of right half abscission zone in plant from cross of *Z. palustris* and *Z. texana*, mature kernal (A = abscission layer; B = central vascular tissue; C = epidermal cells)

References

Elliott, W.A. and G.J. Perlinger. 1977. Inheritance of shattering in wild rice. Agronomy Journal.
17:851-853.

Hanten, H.B., G.E. Ahlgren and J.B. Carlson. 1980. The morphology of grain abscission in *Zizania aquatica*. Canadian Journal of Botany. 58:2269-2273.

Hayes, P.M., R.E. Stucker and G.G. Wandrey. 1988. The domestication of American Wildrice (*Zizania palustris*, Poaceae). Econ. Botany. 43:203-214.

Woods, P.L. and K.W. Clark. 1976. Preliminary observations on the inheritance of non-shattering habit in wild rice. Canadian Journal of Plant Science. 56:197-198.