Seed abscission and retention in Indian ricegrass

R.D.B. WHALLEY, T.A. JONES, D.C. NIELSON, AND R.J. MUELLER

Abstract

Each spikelet of Indian ricegrass [*Oryzopsis hymenoides* (Roem. and Schult.) Ricker] consists of a floret enclosed in a pair of glumes. As each seed matures, (1) the glumes open, (2) the lemma hairs reflex outward, and (3) the abscission layer across the rachilla fractures. This study concerned the relationship of these 3 processes to seed shattering. PI 478833 (Yellowstone Co., Mont.), had a more acute angle between the opened glumes (glume pair angle) and lower seed weight, both of which may contribute to seed retention, than 'Paloma' (Pueblo Co., Colo.). In Paloma and PI 478833, glume pair angle was not greater with a floret in the spikelet than without, thus the influence of lemma hairs on opening the glumes is probably minimal. The abscission (separation) layer between the floret and rachilla of Paloma consists primarily of cells with cellulosic walls, is 1 to 2 cells thick, and lies diagonally across the rachilla. The abscission layer is distal to several layers of sclerenchyma cells with heavily lignified walls (protective layer). The abscission layer is well developed before anthesis, and it is unlikely that any genotypes lack this layer. Since lemma hairs are not related to seed retention and the abscission layer is well developed long before abscission, selection for acute glume pair angle at seed maturity may improve seed retention in Indian ricegrass, increasing harvestable seed yield.

Key Words: abscission layer, *Oryzopsis hymenoides*, seed shattering

Indian ricegrass [*Oryzopsis hymenoides* (Roem and Schult.) Ricker] is a common bunchgrass found mostly on sandy soils in the Intermountain West from Mexico to Canada (Robertson 1976, Cronquist et al. 1977). Flowering is indeterminate and continues through spring and summer (Zemetra and Cuany 1984), provided growing conditions are favorable. Therefore, panicles of different maturities are present on a plant at any particular time during the growing season. Spikelets have a single floret and mature from the top of the panicle down. The abaxial (outer) surface of the lemmas is covered with dense white hairs, which facilitate wind dispersal of the seeds (caryopses enclosed in a lemma and palea). The 2 glumes open as the spikelet matures, and the floret disarticulates, so the proportion of glume pairs containing mature seed is never high (Robertson 1976). Empty florets, however, remain in the spikelet. Early harvest results in a higher yield of poorer quality seed in *Phalaris aquatica* L., in which shattering is also a problem, whereas delaying harvest results in a lower yield of better quality seed (McWilliam 1980). Increasing the length of time in which the abscissed Indian ricegrass floret is held in the glumes could reduce shattering losses and increase yields of quality seed.

This study investigated the process of seed shattering in Indian ricegrass to provide a basis for selection for shattering resistance. We studied variation in glume pair angle and seed weight of 2 genotypes, importance of lemma hairs in glume opening, and development of the abscission layer at the base of the rachilla.

Authors are USDA-ARS foreign research associate and Quinney Visiting Fellow, Utah State University-College of Natural Resources; research geneticist and entomologist, USDA-ARS, Forage and Range Research Laboratory, Logan, Utah 84322-6300; and associate professor, Biology Dept., Utah State University, Logan 84322-5305. Senior author's permanent address is Botany Dept., The University of New England, Armidale, N.S.W. 2351, Australia. Contribution of Utah Agr. Exp. Sta., Journal Paper 3845, and USDA-ARS, Forage and Range Research Laboratory.

The senior author wishes to thank the J.P. Quinney Foundation for financial support while at Logan. The assistance of Camille Mathys and Scott Parker is also gratefully acknowledged.

Manuscript accepted 19 October 1989.

Materials and Methods

Floret Removal and Glume Pair Angle-Experiment 1

Paired inflorescences at approximately the same stage of maturity were selected from the large-seeded cultivar Paloma (Pueblo Co., Colo.) in the greenhouse and from small-seeded PI 478833 (Yellowstone Co., Mont.) in the field at North Logan, Utah. Three pairs of inflorescences were selected from the greenhouse material and 2 pairs from the field because of the larger number of spikelets per inflorescence in the field material. Immature florets were carefully removed from all spikelets, leaving only the glumes, on 1 inflorescence of each pair. Spikelets without a developing caryopsis, indicated by a light-colored lemma, were removed from all inflorescences and were not included in the experiment. The panicle pairs were harvested when all seeds were mature and stored at room temperature until data collection. The rachilla of each spikelet was cut about 5 mm below the lower glume. Glume pairs were magnified by an overhead projector to measure the angle between glume apices and the base of the floret, referred to here as glume pair angle.

Seed Weight and Glume Pair Angle-Experiment 2

Six replications of 20 plants (5 rows of 4) each of Paloma and PI 478833 (entries) were planted in a split-plot design at North Logan, Utah, on 18 Aug. 1987. Whole plots were entries and split plots were harvest treatments. Plant spacing was 1 m. The experiment was bordered by PI 478833. When the first mature seeds shattered at the end of June, 1988, 5 plants (1 randomly selected from each row) from each split plot were assigned to 1 of 4 treatments. Treatments were numbered based on harvest date and designated U (unbagged) or B (bagged). Treatment 1U, 2U, and 3U plants were allowed to shatter without bags and were harvested at 2-wk intervals on 28 June, 12 July, and 26 July, respectively. Treatment 4B plants were enclosed with a paper bag 28 June 1988 to collect all mature seeds as they shattered, although some seed had already fallen. Plants were harvested 3 August 1988. Treatment 1U plants were bagged 3 August 1988 before seed shattering commenced in regrowth. These were harvested 30 August 1988 as Treatment 5B. Inflorescences were stored at room temperature for at least 2 weeks to dry.

Inflorescences were threshed by rubbing between corrugated rubber mats. Seed was cleaned with a South Dakota seed blower and separated into "light" and "heavy" fractions by shaking in a 2 drop^{-1} aqueous solution of liquid detergent. Seed from all 5 plants in the split plot was bulked. Light and heavy fractions were dried and weighed. Seed weight was estimated from 1,000-seed samples from each split plot. Seed number was estimated from seed yield and seed weight data. Percentage heavy seed was calculated and an arcsine transformation was applied to percentage data before analysis. Five glume pairs were removed at random from 2 mature inflorescences from each plant of Treatment 1. Glume pair angle was measured as described above.

Anatomy of the Abscission Layer and Glume Bases

Greenhouse-grown Paloma spikelets, harvested between pre-

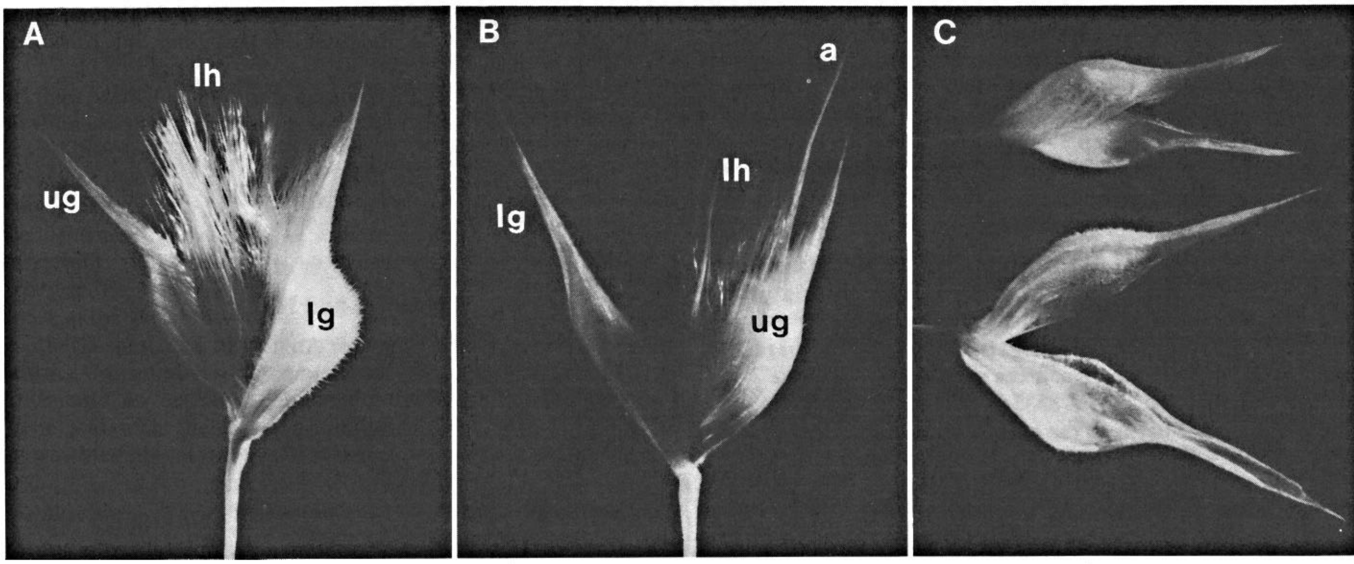

Fig. 1. (A) Mature Paloma spikelet showing opened glumes and reflexed lemma hairs. Glume pair angle is 53°. (B) Opened glumes of a Paloma spikelet with an undeveloped caryopsis. Glume pair angle is 52°. (C) Comparison of glume pair of PI 478833 (upper) and Paloma (lower) after seed shattering. Glume pair angles are 21° and 48°, respectively. a=awn, lg=lower glume, lh=lemma hairs, ug=upper glume.

anthesis and seed maturity, were fixed in 3% glutaraldehyde in Sorenson's phosphate buffer. The glumes and the floret were cut off 1 to 2 mm from their bases to allow penetration of the fixative. The palea was retained to allow orientation of the structures during sectioning. The truncated spikelets were then dehydrated in alcohol, embedded in glycol methacrylate, and sectioned at 5 um with a glass knife. Mounted sections were stained in aqueous 0.05% toluidine blue 0 and examined with a light microscope.

Results and Discussion

Glume pair angles in Experiment 1 (from which the florets had and had not been removed) were 69° and 60° ($P>0.05$) for Paloma and 34° and 22° ($P>0.05$) for PI 478833, respectively (Fig. 1a, 1b). Therefore, glume pair angle is not increased by the force of a floret or any of its parts. This indicates that lemma hairs function in seed dispersal after shattering (Robertson 1976), but not in shattering itself.

In Treatment 1 of Experiment 2, mean glume pair angle of Paloma was 70% greater ($P<0.01$) than that of PI 478833 (48° vs. 28°) (Fig. 1c), which indicates that glume pair angle of dried mature florets is to some extent genetically determined. However, angles among glume pairs of spikelets of the same panicle were quite variable. Variation between entries, among glume pairs on the same panicle, between panicles, and among plants of an entry accounted for 60.7, 32.7, 3.9, and 2.7%, respectively, of the total variation in glume pair angle in the field.

Glume base anatomy did not clearly indicate why glumes opened as they dried out or why the glume pair angles for the 2 entries differed. A small notch of unknown function was present on either margin of the upper glume of Paloma (Fig. 2a) but absent in PI 478833. Glume pairs of Paloma closed when soaked in water and opened up again when subsequently dried. Therefore, the opening process is reversible.

Light seeds contained caryopses ranging from vestigial to fully developed, but unlike heavy seed, did not completely fill the space available. Lemma color varied from green (no caryopsis) to straw colored, brown, or black. The few caryopses threshed free of the lemma and palea during the cleaning processes were included in the heavy fraction. Lemma hairs were removed from most seeds by the seed cleaning operation and their presence did not influence classification as heavy or light.

Table 1. Yield of heavy and light seed fractions of 2 Indian ricegrass entries harvested at 4 different times during the summer, 1988 at North Logan, Utah.

Treat-ment[1]	Paloma		PI 478833		Paloma vs. PI 478833	
	Heavy	Light	Heavy	Light	Heavy	Light
	------------ kg/ha ------------					
1U	8.2c	nc[2]	22.0c	nc	**	nc
2U	16.7bc	9.7b	35.4b	6.6b	*	*
3U	22.8ab	15.4a	40.4ab	9.8a	NS	*
4B	32.8a	10.9b	51.0a	10.2a	NS	NS
5B	18.4bc	4.8c	6.5d	0.6c	**	*

Means in a column followed by different letters are significantly different by the Waller-Duncan k-ratio t test at k ratio = 100.
*,**Contrast significant at $P<0.05$, and 0.01, respectively.
[1]Unbagged treatments 1U, 2U, and 3U were harvested 28 June, 12 July, and 26 July 1988, respectively. Treatment 4B was bagged 28 June and harvested 3 August 1988. Treatment 5B was bagged 3 August and harvested 30 August 1988.
[2]Data not collected.

Heavy seed yield per plant was greatest for bagged Treatment 4B and least for unbagged Treatment 1U (Paloma) or bagged Treatment 5B (PI 478833) (Table 1). For unbagged Treatments 1U and 2U heavy seed yield per plant of PI 478833 was greater ($P<0.05$) than that of Paloma. For bagged Treatment 4B and unbagged Treatment 3U, differences between the entries were nonsignificant ($P>0.05$). For bagged Treatment 5B, heavy seed yield of Paloma was nearly 3 times ($P<0.01$) that of PI 478833. This can be attributed to Paloma's superior summer reproductive regrowth.

Plants of Treatments 1U and 4B were harvested and bagged, respectively, 2 days after a severe thunderstorm which caused considerable shattering of mature seeds. Heavy seed yield of Paloma for unbagged Treatment 1U was only about 37% ($P<0.01$) that of PI 478833 (Table 1). This difference must have largely resulted from greater shattering of Paloma than PI 478833. The percentage difference in heavy seed yield between the 2 entries decreased with the 2 subsequent unbagged harvests and by the harvest time of Treatment 3U, this difference was not significant ($P>0.05$). Likewise, Treatment 4B bags of the 2 entries, harvested 5 weeks after the storm, were not different ($P>0.05$).

Table 2. **Mean seed weight (mg) of heavy and light seed fractions of 2 Indian ricegrass entries harvested at different times during the summer, 1988 at North Logan, Utah.**

Treat-ment[1]	Paloma		PI 478833		Paloma vs. PI 478833	
	Heavy	Light	Heavy	Light	Heavy	Light
	------------ mg ------------					
1U	4.98b	nc[2]	3.22b	nc	**	nc
2U	4.83b	2.44bc	3.20b	1.72b	**	**
3U	4.98b	2.26c	3.27ab	1.74b	**	**
4B	5.50a	2.65b	3.40ab	1.82b	**	**
5B	5.89a	3.93a	3.51a	2.44a	**	**

Means in a column followed by different letters are significantly different by the Waller-Duncan k-ratio t test at k ratio = 100.
*,**Contrast significant at $P<0.05$, and 0.01, respectively.
[1]Unbagged treatments 1U, 2U, and 3U were harvested 28 June, 12 July, and 26 July 1988, respectively. Treatment 4B was bagged 28 June and harvested 3 August 1988. Treatment 5B was bagged 3 August and harvested 30 August 1988.
[2]Data not collected.

Mean seed weight was much more stable than seed yield per plant (Table 2). Heavy Paloma seed was 51-68% heavier ($P<0.01$) than heavy PI 478833 seed. Paloma heavy seed weight was greater for bagged Treatments 4B and 5B than for unbagged Treatments 1U, 2U, and 3U. PI 478833 heavy seed weight was greatest for bagged Treatment 5B and least for unbagged Treatment 2U. Bagging increased mean harvested seed weight, probably because the heaviest seeds shattered first and were lost in unbagged treatments.

Heavy seeds shattered readily, but light seeds, largely immature, aborted, or empty, did not abscise. Thus, percentage heavy seed reflects seed set in bagged treatments, but reflects both seed set and seed retention in unbagged treatments. The final harvest (Treatment 5B) had the highest percentage heavy seed (Table 3). Seeds of some very late Paloma inflorescences were still immature at the Treatment 5B harvest date, accounting for the lower proportion of heavy seed relative to PI 478833. Unlike Treatment 4B, bagging of Treatment 5B occurred before any seed had shattered. In the unbagged Treatments 2U and 3U, PI 478833's percentage of heavy seed was greater ($P<0.01$) than Paloma's, and this difference most likely reflects a difference in the degree of seed shattering between the 2 entries. Higher seed set of PI 478833 relative to Paloma could also have contributed to the difference. However, if this was strictly the case, PI 478833 should have had higher percentage heavy seed than Paloma in bagged Treatment 4B, where there was less seed lost because of shattering. Percentage heavy seed was higher in PI 478833 than Paloma in Treatment 4B, but not significantly so. The difference in percentage heavy seed between PI 478833 and Paloma

Table 3. **Percentage heavy seed of 2 Indian ricegrass entries harvested at 4 different times during the summer, 1988 at North Logan, Utah.**

Treatment[1]	Paloma	PI 478833	Paloma vs PI 478833
	------------ % ------------		
1U	nc[2]	nc	nc
2U	45c	74b	**
3U	39c	68b	**
4B	58b	72b	NS
5B	73a	89a	**

Means in a column followed by different letters are significantly different by the Waller-Duncan k-ratio t test at k ratio = 100.
*,**Contrast significant at $P<0.05$, and 0.01, respectively.
[1]Unbagged treatments 1U, 2U, and 3U were harvested 28 June, 12 July, and 26 July 1988, respectively. Treatment 4B was bagged 28 June and harvested 3 August 1988. Treatment 5B was bagged 3 August and harvested 30 August 1988.
[2]Data not collected.

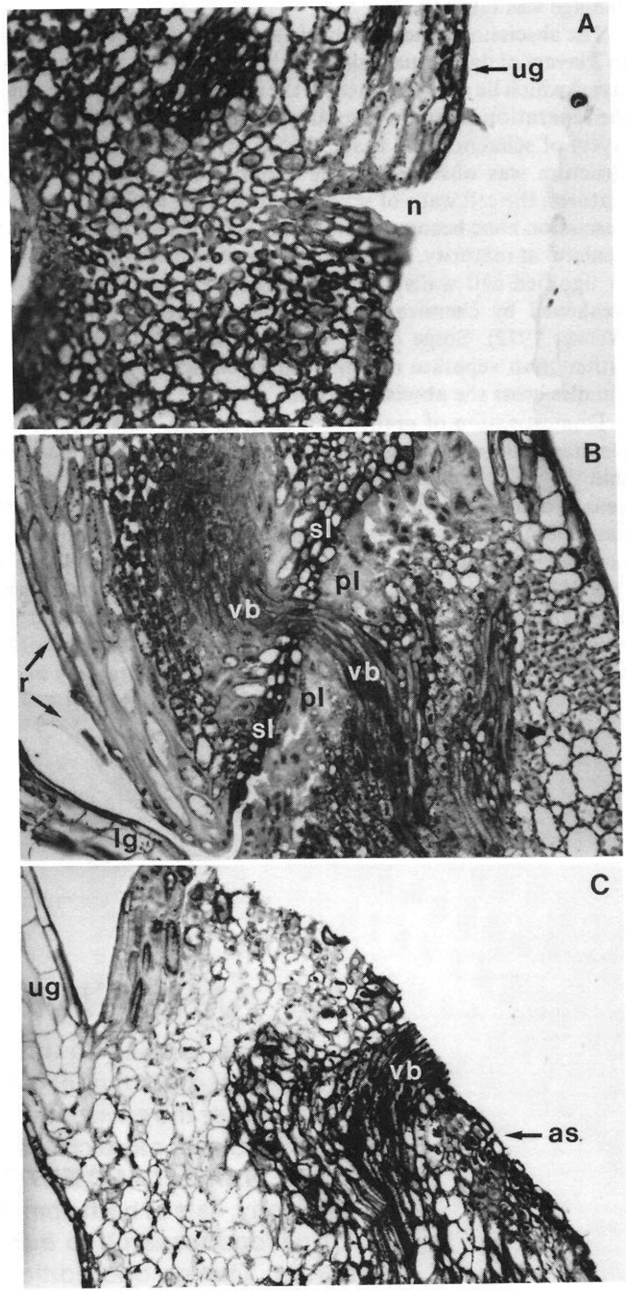

Fig. 2. (A) Tangential longitudinal section of a Paloma spikelet showing the notch on the margin of the upper glume. (B) Abscission zone across the rachilla of a Paloma spikelet prior to anthesis. (C) Proximal side of the abscission zone across the rachilla of a Paloma spikelet after seed shattering. as=abscission scar consisting of fractured and separated cells of the abscission zone, lg=lower glume, n=notch, pl=protective layer, r=rachilla, sl=separation layer, ug=upper glume, vb=vascular bundle.

was twice as great in unbagged Treatments 2U and 3U ($P<0.01$) as in bagged Treatment 4B ($P>0.05$).

Although seed shattering was not measured directly, higher seed retention by PI 478833 best explains these data. Higher seed retention of PI 478833 may be related to its more acute glume pair angle and lower seed weight. Percentage heavy seed was lower in unbagged harvests (Treatments 2U and 3U) than their bagged control (Treatment 4B) in Paloma, but not in PI 478833. It was necessary to remove the seeds with fine forceps in order to get a clear image when measuring angles. In many cases, the PI 478833 glumes held the seeds so firmly that their removal without glume damage was difficult.

The abscission zone, clearly visible before anthesis, consists of 1 to 2 layers of dark-blue staining cellulosic-walled cells (separation layer) which lie obliquely across the rachilla (Fig. 2b). Proximal to the separation layer are several layers of lignified cells (protective layer) of sclerenchyma tissue. Little variation in abscission zone structure was observed in the material examined. As the seed matures, the cell walls of sclerenchyma in the proximal part of the abscission zone become heavily lignified. When the rachilla disarticulates at maturity, actual separation may occur in the cellulosic or lignified cell walls (Fig. 2c). These cell walls were probably weakened by chemical changes during seed ripening (Fahn & Werker 1972). Some of the sclerenchyma cells appear to break rather than separate during seed ripening. Four to 5 vascular bundles cross the abscission zone.

Domestication of grain crops such as rice, oats, and sorghum resulted in the elimination of abscission layers. Studies have shown that the presence or absence of abscission layers in these crops is usually associated with 1 or 2 recessive or dominant genes (McWilliam 1980). Unless such mutations can be found in Indian ricegrass, improvement of seed retention and therefore harvestable seed quantity and quality must be achieved by some other mechanism. The evaluation of a large number of accessions is the best initial strategy for the general improvement of Indian ricegrass (Jones and Nielson 1989) and glume pair angles can be rapidly and precisely measured to estimate within and between-accession variability. In this study, PI 478833 exhibited a more acute glume pair angle than Paloma. It therefore appears that an important criterion for evaluating accessions for seed retention should be glume pair angle. If acute glume pair angles increase seed retention, harvestable seed yields may be expected to increase. Lowering seed weight may also increase seed retention, but this may be a less desirable approach.

References

Cronquist, A., A.H. Holmgren, N.H. Holmgren, J.L. Reveal, and P.K. Holmgren. 1977. Intermountain flora—vascular plants of the Intermountain West, U.S.A. Vol. 6. The Monocotyledons. Columbia University Press.

Fahn, A., and E. Werker. 1972. Anatomical mechanisms of seed dispersal. p. 151-221. *In:* T.T. Kozlowsi (ed.) Seed biology. Academic Press, New York.

Jones, T.A., and D.C. Nielson. 1989. Self-compatibility in 'Paloma' Indian ricegrass. J. Range Manage. 42:187-190.

McWilliam, J.R. 1980. The development and significance of seed retention in grasses. p. 51-60. *In:* P.D. Hebblethwaite (ed.) Seed production. Butterworths, London.

Robertson, J.H. 1976. The autecology of *Oryzopsis hymenoides.* Mentzelia 2:18-21, 25-27.

Zemetra, R.S., and R.L. Cuany. 1984. Variation in lemma thickness in Indian ricegrass: implications for dormancy, scarification, and breeding. Crop Sci. 24:1082-1084.