GENETIC VARIATION FOR SEED RETENTION IN ACCESSIONS AND GENOTYPIC LINES OF PERENNIAL RYEGRASS

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Introduction

Many grasses naturally shed their seeds at maturity as a means of dissemination. This process of seed shedding is known as shattering. Shattering reduces the seed yield of grasses grown for seed crops and is an economic constraint on the profitability of seed-production enterprises. Therefore, retention of seed is an essential trait for improving seed yield in grasses.

Perennial ryegrass (*Lolium perenne* L.) is a cool-season turf and forage grass for which most genetic improvement efforts have focused on end-use quality characteristics, such as dry matter forage yield or turf quality, rather than on traits associated with seed yield, such as seed retention (Stewart and Hayes, 2011; McDonagh et al., 2016). Seed yield in perennial ryegrass is the product of two components: seed number and seed weight. Genetic improvement in seed retention may increase seed yield by significantly increasing the seed number harvested.

There may be viable sources of high seed retention present within germplasm collections of perennial ryegrasses available to plant breeders. Genetic variation for traits in natural populations and cultivars of perennial ryegrass is high, and nonuniformity in these plant materials is the rule rather than the exception. The objectives of this study were to determine whether genetic variation for seed retention was present in readily available perennial ryegrass accessions and to ascertain whether there was variation within these accessions.

Materials and Methods

Perennial ryegrass plants derived from 40 diverse global accessions were grown for 2 years in field trials at Oregon State University's Hyslop Crop Science Field Research Laboratory, near Corvallis, OR. The plant accessions were sourced from seeds acquired from the USDA Western Regional Plant Introduction (PI) Station in Pullman, WA, from two commercial U.S. cultivars, and from an experimental line from the United States.

Seeds from each accession were planted in the greenhouse and grown into plants robust enough (multiple tillers) for cloning. Each accession was represented by four plants derived from four different seeds within the accession in order to characterize the variation within each accession. These plants were chosen as the progenitors for creating vegetative clones of the four genotypic lines within each accession or cultivar. Each genotypic line was cloned 4 times to produce a total of 640 transplants for the field trials.

The Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale was used to assess plant maturity for determination of seed retention. Seed retention was determined on three spikes chosen at random from each plant at BBCH 80 (dough development) or greater. Spikes were placed on an aluminum base plate, and a standardized steel bar (0.5 kg) was rolled by hand (about 24 Newtons) over each spike five times (three from tip to base and two from base to tip) in order to subject each spike to a consistent external force. Seeds that were dislodged from the spike in a tray were determined to be viable (caryopsis at least one-half the length of the palea) and were counted and weighed. The seeds retained on the spike were hand stripped from the spike, counted, and weighed.

Measurements of spike length and spikelet number per spike were made by using two-dimensional photogrammetry. Images of the three spikes were captured with a Nikon D5000 camera, and spike images were analyzed for length and spikelet number using Fiji (ImageJ) software. R studio (Version 1.3.1093) was used for computations and data analysis. Years, accessions, and genotypic lines nested within accessions were considered fixed effects in the analysis of variance. Replications were considered random effects.

Results and Discussion

Only 21 of the 40 accessions survived in sufficient numbers (3 of the 4 genotypic lines representing each accession) across both years for robust statistical analysis (data not shown). None of the plants survived in two of the accessions (231605 and 220179), and another 14 accessions exhibited more than 50% mortality among plants across years. One commercial cultivar did not vernalize the first year; its data are not included in the analysis. This result suggests that not all of the accessions were adapted to the conditions prevalent at the field site or that some had innately short life spans. The results presented in this report are for the 21 best adapted and longer-lived accessions.

The analysis of variance revealed that there was significant variation among accessions in seed retention and other characteristics of the spike. A large range in seed retention values was observed among accessions. The 21 accessions are ranked from high to low seed retention values in Table 1. The mean seed retention for the 21 accessions was 44.2%, meaning that most of the potential seed would not have been harvested by the seed grower. The seed retention observed in the commercial cultivar 'Cutter' was near the mean of the accessions. Some of the accessions evaluated had superior seed retention compared to that found in 'Cutter' and the experimental cultivar line PR 12.1206. This result is evidence that sources of higher seed retention than that of commercial perennial ryegrass cultivars are available from these accessions and could be used in the breeding of shatter-resistant cultivars.

All spike characteristics measured, including spikelets per spike, seed number per spike, spike length, and seed weight, exhibited high levels of variation among accessions (Table 1). Field studies have indicated that seed yield in perennial ryegrass is most related to the size of the spike and that plant breeders should concentrate on increasing the number of spikelets per spike in order to improve seed yield (Abel et al., 2017).

Some significant differences between years ($P = 0.007$) were evident and were attributed to environmental factors. However, these differences did not differentially affect seed retention or other characteristics, so values were combined across years.

There is variability in seed retention both among accessions and among the genotypic lines within accessions (Figure 1). This variation among the genotypic lines and within accessions is responsible for much of the large standard deviations in seed retention observed for the accessions in Table 1. One practical consideration for genetic improvement is that this high

Table 1. Mean and standard deviation values for spike characteristics and seed retention in perennial ryegrass accessions in 2018 and 2019.[1]

PI number	Country of origin	Spikelets	Seeds	Seed weight	Spike length	Seed retention
		(no./spike)	(no./spike)	(mg)	(cm)	(%)
231581	Algeria	21.7 ± 4.9	67.5 ± 32.5	1.2 ± 0.6	13.3 ± 2.5	78.7 ± 16.8
231594	Algeria	26.0 ± 5.5	89.4 ± 32.9	1.6 ± 0.4	19.5 ± 4.6	65.6 ± 20.0
598916	Tunisia	22.9 ± 3.6	116.5 ± 58.3	1.6 ± 0.5	18.1 ± 3.6	62.5 ± 17.7
231575	Algeria	22.7 ± 5.1	69.9 ± 25.2	1.5 ± 0.4	18.2 ± 8.0	60.5 ± 16.3
231579	Algeria	22.3 ± 3.7	87.8 ± 42.6	1.3 ± 0.6	17.6 ± 4.8	58.4 ± 19.7
231620	Iran	26.4 ± 4.6	72.7 ± 40.4	1.9 ± 0.8	21.0 ± 3.0	55.3 ± 16.1
231587	Algeria	21.4 ± 4.5	77.6 ± 37.1	1.9 ± 0.7	19.3 ± 4.7	44.0 ± 20.2
Cutter	USA	23.8 ± 5.0	75.4 ± 30.5	2.0 ± 0.3	19.6 ± 2.5	42.4 ± 14.6
418708	Romania	28.1 ± 3.6	81.9 ± 33.1	2.2 ± 0.8	21.2 ± 4.7	41.0 ± 25.2
231584	Algeria	27.6 ± 3.9	71.4 ± 37.2	2.0 ± 0.3	19.2 ± 4.7	40.0 ± 16.5
384479	Poland	25.0 ± 3.3	71.0 ± 33.3	1.9 ± 0.5	23.3 ± 3.4	38.3 ± 16.7
PR 12.1206	USA	22.4 ± 3.0	67.8 ± 24.3	1.8 ± 0.6	15.8 ± 2.8	38.1 ± 18.1
418705	Italy	24.2 ± 2.9	77.0 ± 35.9	2.0 ± 0.5	20.2 ± 2.5	37.0 ± 13.5
231619	Iran	21.6 ± 3.0	83.2 ± 21.5	1.5 ± 0.7	20.1 ± 4.4	36.9 ± 21.7
234779	Germany	21.5 ± 3.5	79.9 ± 46.0	2.0 ± 0.5	20.0 ± 3.4	35.5 ± 15.9
418707	Romania	22.3 ± 2.8	56.8 ± 17.1	2.6 ± 0.6	19.3 ± 3.8	34.2 ± 15.1
220878	Ireland	24.6 ± 3.3	52.6 ± 21.7	1.8 ± 0.7	17.1 ± 3.4	34.1 ± 13.7
238938	New Zealand	21.4 ± 3.0	62.8 ± 31.4	2.4 ± 0.8	22.4 ± 4.1	33.5 ± 13.2
376878	New Zealand	19.3 ± 2.7	61.7 ± 30.4	2.2 ± 0.8	21.4 ± 3.9	33.5 ± 15.9
371952	Bulgaria	22.1 ± 3.9	64.1 ± 28.8	2.1 ± 0.8	18.8 ± 4.9	32.1 ± 14.5
231580	Algeria	25.9 ± 3.7	54.8 ± 27.1	2.0 ± 0.7	21.4 ± 4.0	27.1 ± 14.8

[1]Accessions are denoted by plant introduction (PI) number and country of origin.

variability within accessions means that not all source plants will have the desired level of seed retention. Variation within accessions was also observed for seed weight but not to the same extent as for seed retention (data not shown).

Accession PI 231581 had the highest average seed retention (78.7%) but also had the shortest spike length (13.3 cm). Regression analysis shows that there is a significant relationship between spike length and seed retention for the accessions (Figure 2). Shorter, more compact spikes might be superior in seed retention over longer, more open spikes by physically holding the seed in the spike. Spike length is clearly not the only factor contributing to seed retention, however.

There was a relationship of seed retention with seed weight (Figure 2). Seed weight is an important factor in seed retention, as heavy seed have a greater propensity to be lost in shattering than light seed; accessions tending to produce heavy seed showed greater seed shattering losses. The collective weight of seeds within a spikelet might increase breaking of abscission layers, thereby reducing seed retention (Elgersma et al., 1988). Heavy seeds might be more readily dislodged by forces in the environment, such as wind, than light seeds. Selection of small-seeded types of perennial ryegrass to increase seed retention might not be desirable, however, as some of the accessions had seeds that were lighter than cultivars currently in the marketplace. The

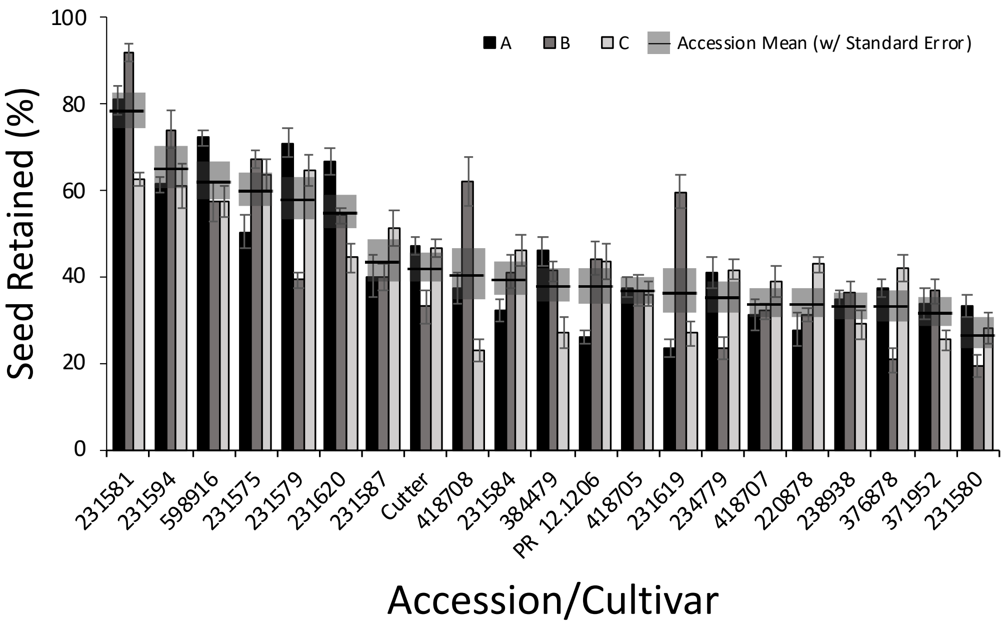

Figure 1. Seed retention among and within accessions or cultivars of perennial ryegrass in 2018 and 2019. Three genotypic lines within accessions or cultivars are denoted A–C.

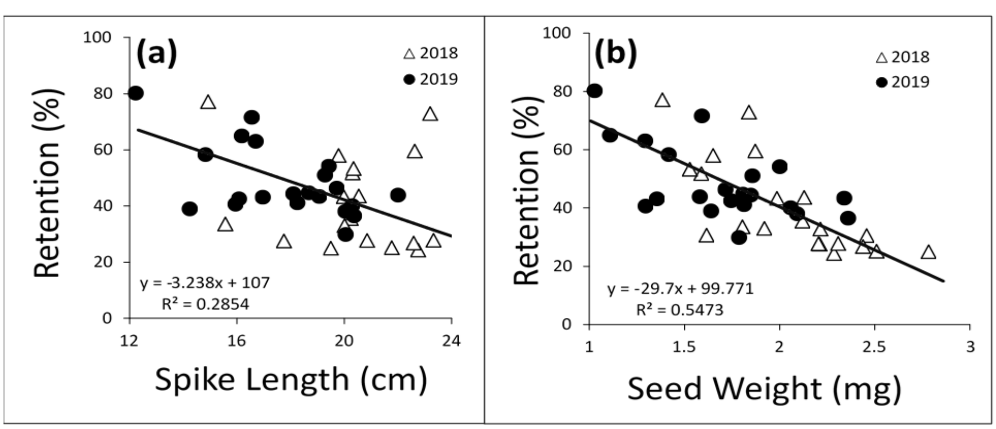

Figure 2. Effect of spike length on seed retention (a) and seed weight on seed retention (b) in accessions or cultivars of perennial ryegrass in 2018 and 2019.

analysis also indicated that seed weight was related to spike length, with long spikes tending to have higher individual seed weight (data not shown). Seed retention was independent of the number of seeds produced per spike or number of spikelets per spike.

This study demonstrates that beneficial variation in seed retention is present in genetic materials accessible to plant breeders and that some plant characteristics influence seed retention in perennial ryegrass. Our work using 3-D topometric imaging techniques for phenotyping has been aimed at further refining our understanding and identification of characteristics related to seed retention (Tubbs and Chastain, 2020). Morphological characteristics of the spike considered in the topometric image analysis included spike architecture, distance between spikelets along the rachis, angle of spikelet attachment to the rachis, and spikelet size. The results of that work will be presented in future reports.

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