DEVELOPMENT OF 3-D TOPOMETRIC IMAGING METHODS FOR HIGH-THROUGHPUT PHENOTYPING OF SEED RETENTION TRAITS IN PERENNIAL RYEGRASS

T.B. Tubbs and T.G. Chastain

Introduction

Shattering is a widespread natural phenomenon in plants and serves as a mechanism for dispersal of seed to favorable environments. However, in agriculture the loss of seeds resulting from shattering prior to and during harvest can be an important constraint to seed yield. In perennial ryegrass, shattering was observed to cause seed yield losses as great as 700 lb/acre in Oregon (Anderson et al., 2019). Seed lost during shattering is the source of crop volunteers, a major weed problem in perennial ryegrass seed production.

Results from a recent study show some promise in identifying the genes that play a critical role in seed shattering of perennial ryegrass using a comparative genomics strategy (Fu et al., 2018). Identification of phenotypic characteristics associated with shattering is likely to play a key role in reducing seed losses (Elgersma et al., 1988). Methods for field evaluation of plant traits in grasses have long relied on laborious and time-consuming measurements. Moreover, human ratings of plants can be subjective and inconsistent. Since data collection on these characteristics is slow, the quantity of data gathered is generally limited (low-throughput) on these traits (phenotypes).

Current technology has increased the adoption of high-throughput phenotyping (HTP) methodologies in crop physiology and breeding programs, replacing much of the slow and labor-consuming human-based data collection with a variety of sensing devices (Vázquez-Arellano et al., 2016). Advances in topometric scanning and imaging technologies have enabled three-dimensional (3-D) modeling of the physical world. Is it possible to use 3-D topometric imaging methods to provide rapid and repeatable estimates of crop phenotypic characteristics (HTP) under field conditions?

Our objectives were to develop 3-D topometric imaging methods for HTP in perennial ryegrass and to determine the relationship of measurable phenotypic characteristics of spikes and seed shattering.

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. The plant accessions were sourced from seeds acquired from the USDA Western Regional Plant Introduction Station in Pullman, WA, and from commercial cultivars. Each accession was represented by four plants derived from four seeds within the accession in order to characterize the variation within each accession. These plants were used to create vegetative clones of the four genotypic lines (denoted A–D) within each accession or cultivar.

Seeds from each accession were planted in the greenhouse and grown into plants robust enough (multiple tillers) for cloning. Each genotypic line was cloned 4 times to produce a total of 640 transplants for field trials. Transplants were planted in 32 rows with 20 plants per row. Rows were separated by 2-foot-wide access walkways of tall fescue. The experimental design was a randomized block design with four replications, and treatments were the 40 accessions and the 4 genotypic lines within each accession. Standard practices for culture of perennial ryegrass seed crops were employed in the study, with the exception that no plant growth regulators were applied to control lodging.

A portable, hand-held scanner (Artec 3-D Spider) was used to capture 3-D topometric images of perennial ryegrass spikes in the field. The scanner uses blue LED light and has 3-D resolution to 0.1 mm. Unlike a photograph, the 3-D topometric image captured by the scanner can be rotated by the user to reveal all sides.

The Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale was used to assess crop maturity. Representative spikes from the 40 accessions and genotypic lines within accessions were scanned with the 3-D optical scanner at intervals from spike emergence (BBCH 50) to seed maturity (BBCH 80–89). Morphological characteristics of the spike considered in the topometric image analysis included spike length and architecture, number of spikelets/spike, distance between spikelets along the rachis, angle of spikelet attachment to the rachis, and spikelet size.

Once the image of the spike is captured by scanning and saved as a stereolithography (STL) file, the data must undergo several steps before they are ready for topometric image analysis. The optical imaging system had not been used previously in topometry of plant tissues, so considerable work to develop methods for postprocessing of data was needed to adapt the scanning device. One promising data postprocessing method is known as mesh skeletonization; this approach has the potential to save time and labor needed for data analysis. This process enables the automated measurement of the characteristics found on each spike. The 3-D data from the scan is cropped out of the image, and the surface 3-D polygon mesh is exported to a more usable file format.

The STL files were uploaded into the Slicer program, where 3-D models were incrementally sliced into hundreds of individual "stacked" images. Sliced 3-D images were analyzed by Imagej (image analysis software) for calculation of center points. These center point images were then restacked so that all of the center points were connected by an individual meshed surface (Figure 1).

Field-based sampling for the 40 accessions was done to validate the 3-D scanning methodology. Each scanned spike was collected along with two other representative spikes from the accession. Accessions and cultivars were tested for shatter resistance (seed retention) by placing spikes on an aluminum base plate and rolling a standardized steel bar (0.5 kg) by hand (approximately 24 Newtons) over each spike five times (three times from tip to base and two times 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 competent (caryopsis at least one-half the length of the palea) and were counted and weighed. The seeds retained on the spike were then hand-stripped from the spike, counted, and weighed. Each spike was photographed with a camera. The number of spikelets per spike and the length of the spike were measured per established methods (Chastain et al., 2014).

Results and Discussion

Perennial ryegrass spikes taxed the resolution threshold for the scanner, but it was able to record the diversity of characteristics and architecture of spike morphology observed among accessions. The scanner was not able to consistently distinguish individual florets on the spike, but other structural components of the spike were distinguishable at the device's resolution. The work to date indicates that multiple measurements can be made in the field at sequential stages of development and that the scanner has the ability to capture a 3-D representation of the spike and store that data for subsequent topometric analysis.

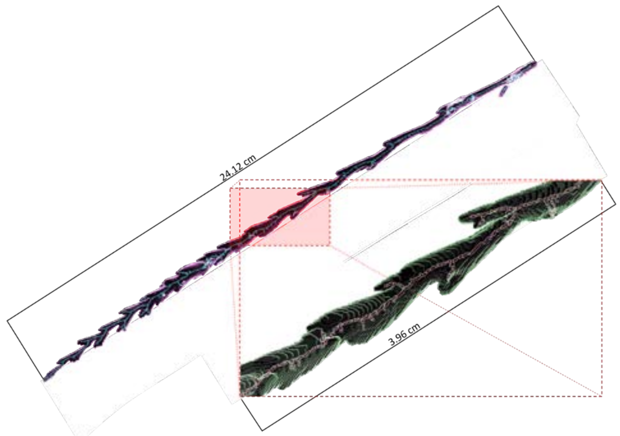

Figure 1. Artec Studio x-ray representation of a scan of a perennial ryegrass spike. This representation depicts two models created from one object and superimposed in Artec Studio. The inner model is the smoothed chordal axis that was created with the Slicer program to convert the 3-D mesh into a stacked image. This stacked image was skeletonized using Imagej software.

The 40 accessions and genotypic lines within accessions were found to differ greatly in plant mortality, crop architecture, maturity, spike size, and seed shattering. Some of the accessions and genotypic lines were observed to be short lived as perennials and did not survive more than 1 year. Since the mortality was generally consistent across replications within accessions, the poor survivorship was likely the result of lack of adaptation to the environment by the accession or innate short life span.

There was evidence of large differences in seed retention in the spike among accessions, as shown in Table 1. An accession from Tunisia (PI 598916) showed excellent seed retention (low shattering) compared to a commercial cultivar ('Cutter') and a shattering-susceptible accession from Poland (PI 384480). These accessions also differed in spikelets/spike, seed number/spike, and seed weight.

There was considerable variation in seed retention, both among accessions and cultivars and within accessions (genotypic lines A–D) and cultivars (Figure 2). This was expected because of the genetic nature of perennial ryegrass; phenotypic and genotypic variation among plants within accessions or cultivars is common and can be quite large. Nevertheless, some accessions had much higher seed retention than others and in general had greater seed retention than that found in commercial cultivars such as 'Accent'. This is evidence that sources of higher seed retention are available from these accessions and could be used in the breeding of shattering-resistant cultivars.

Our investigations suggest that the topometric scanner has promise as an HTP tool, potentially replacing slow and laborious human-based data collection in studies of the inflorescence in grasses. With this device, data can be collected quickly, and measurements can be made multiple times on live plants in the field at sequential stages of development without destructive removal. The 3-D images are available after the user leaves the field for additional examination and future comparisons. Nevertheless, requirements for postprocessing currently delay the availability of estimates of crop phenotypic characteristics under field conditions using 3-D topometric imaging.

References

Anderson, N.P, M. Goussard, B.C. Donovan, C.J. Garbacik, and T.G. Chastain. 2019. Seed yield and seed shattering with different windrowers in Oregon grass seed crops. In *Proceedings of the 10th International Herbage Seed Conference* 10:59–64.

Chastain, T.G., W.C. Young III, T.B. Silberstein, and C.J. Garbacik. 2014. Performance of trinexapac-ethyl on seed yield of *Lolium perenne* in diverse lodging environments. Field Crops Res. 157:65–70.

Elgersma, A., J.E. Leewangh, and H.J. Wilms. 1988. Abscission and seed shattering in perennial ryegrass (*Lolium perenne* L.). Euphytica 5:51–57.

Fu, Z., J. Song., J. Zhao, and P. Jameson. 2018. Identification and expression of genes associated with the abscission layer controlling seed shattering in *Lolium perenne*. AoB PLANTS 11:ply076.

Vázquez-Arellano, M., H. Griepentrog, D. Reiser, and D. Paraforos. 2016. 3-D imaging systems for agricultural applications—a review. Sensors 16(5):618.

Acknowledgments

The authors wish to thank the Oregon Tall Fescue Commission and the Agricultural Research Foundation for support of this work.

Table 1. Spike characteristics and seed retention in perennial ryegrass accessions or cultivars.

	Spikelets	Seeds	Seed weight	Seed retention
Accession or cultivar	(no./spike)	(no./spike)	(mg)	(%)
PI 598916	21.7	46	1.50	76.1
Cutter	20.9	30	1.49	32.0
PI 384480	31.4	57	2.24	23.9

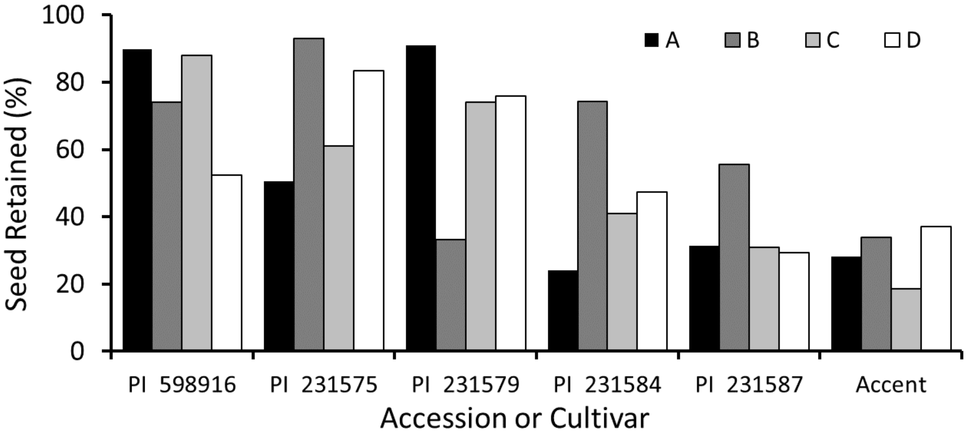

Figure 2. Seed retention among and within accessions or cultivars of perennial ryegrass. The four genotypic lines within accessions or cultivars are denoted A–D.