High-Throughput Phenotyping of Soybean Seed Morphology

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github.com/njwitthoeft/BeanCounter

Abstract

Seed morphology of soybeans can be variable, with sources of variation being cultivar, environment, and position on plant. Growers of food-type soybeans seek circular seeds, and to minimize variation in seed shape, to produce a more consistent crop.

Measuring dimensions of seeds manually is time-consuming for some traits and impossible for others. It is known that average soybean seed shape of a cultivar has a genetic basis, less is known about within-cultivar uniformity.

In order to validate the image-based method we developed, we screened 40 varieties grown under pennycress intercropping. After validating the method using this data, we investigated heritability of variation in soybeans with the same accessions.

Development of Software

The quantity of seed measurements necessary for statistical analysis required the development of custom software, BeanCounter, to automate many of the measurement processes.

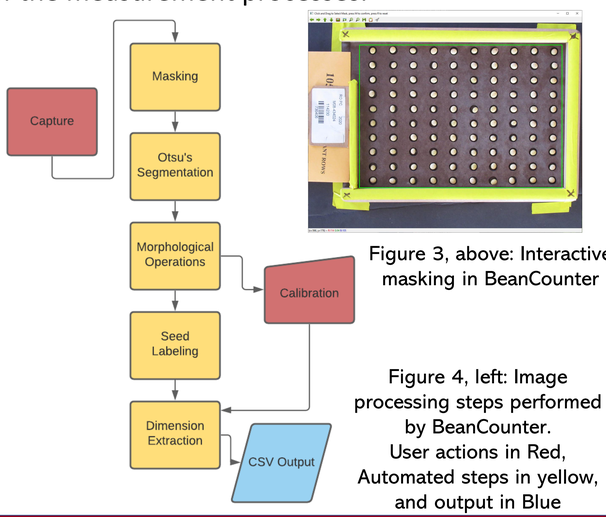

Figure 3, above: Interactive masking in BeanCounter

Figure 4, left: Image processing steps performed by BeanCounter. User actions in Red, Automated steps in yellow, and output in Blue

Acknowledgement

We would like to thank Sam Kuralle for his efforts threshing.

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Image Collection

Figure 1: Overhead tripod rig with Raspberry-Pi tethering system.

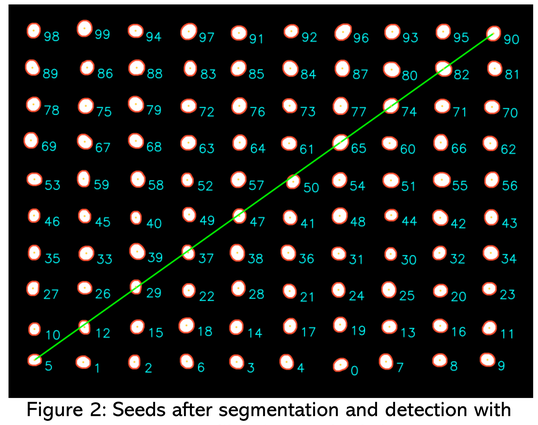

Figure 2: Seeds after segmentation and detection with automatic calibration method shown.

Our need to efficiently collect 240 images prompted the development of an additional device, SojaBoy. SojaBoy is a hardware device based around a 10" touchscreen and a Raspberry-Pi single-board-computer. The software developed for SojaBoy allows user control over file naming while taking images. Alternately, the images can be automatically named using barcode-detection. Seed-counting trays were modified to allow easy segmentation by software.

Application and Validation

We examined the effect of pennycress intercropping on soybean seed morphology. Forty varieties -- consisting of GRIN accessions, advanced breeding lines, and commercial varieties – were evaluated. The effect of environmental conditions on soybean seed morphology were successfully quantified. Surface area, length, and width were all decreased by pennycress intercropping.

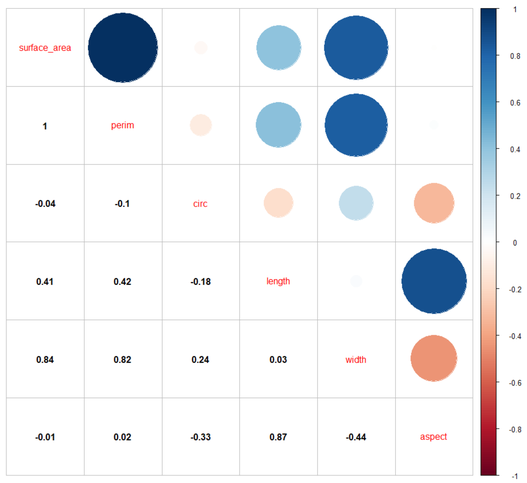

Figure 4: Correlation matrix of measured traits

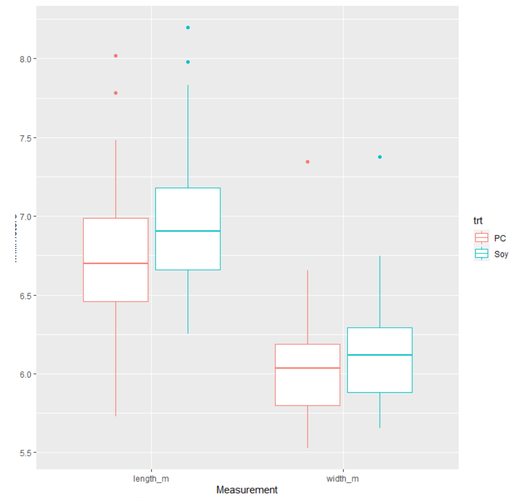

Figure 5: Length and Width by Treatment

Future Work

Heritability estimates for each seed characteristic suggest a strong genetic component to both the mean shape values, and the variation in those values. Future studies should focus on developing new varieties and discovering genes that produce uniformly circular seeds.

Conclusions

Image-based seed phenotyping proved to be a valid method for characterizing variation in seed size and in seed circularity. Heritability of seed shape parameters was quantified, showing both the mean values and within-variety variances have a strong genetic basis.

Image-based phenotyping relies on two-dimensional images. Stronger relationships may be seen if a phenotyping method is developed using 3-dimensional seed measurements.

Soybean seed morphology and its uniformity may influence early season vigor and is a desirable trait for food-type soybeans. The techniques developed here should allow the integration of phenotyping of seed morphology into a breeding program.

Application and Validation

Figure 6: Significance of Contributing Factors to Measures of Seed Shape

	Length	Len. Var.	Width	Wid. Var.	Aspect	Asp. Var.
Treatment	***	***	***	***	***	***
Block	***	*	***	***	*	*
Pedigree	***	***	***	***	***	***
Trt by Ped				*		
	S. Area	**S. A. Var.**	**Perimeter**	**Perim. Var.**	**Circularity**	**Circ. Var.**
Treatment	***	***	***	***	***	***
Block	***	***	***	**	**	
Pedigree	***	***	***	***	***	***
Trt by Ped						

p < 0.001	***
p < 0.01	**
p < 0.05	*
p > 0.05	None

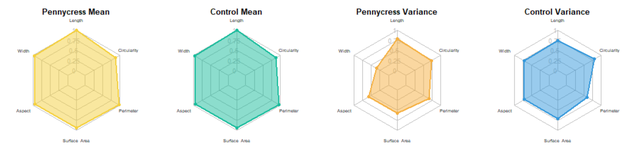

Figure 7: Heritability (H2) estimates of each seed shape parameter by treatment and statistical summary.

IPREFER Integrated Pennycress Research Enabling Farm & Energy Resilience