ADAPTATION OF HIGH-THROUGHPUT SEED-BASED DNA EXTRACTION PROTOCOLS FOR WHEAT-MARKER ASSISTED BREEDING

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Hypothesis

A high throughput seed based DNA extraction protocol may be optimized to allow screening of a large number of genotypes for markers of interest to the UMN wheat breeding program at reduced cost and time compared to current methods

Introduction

The UMN wheat breeding program uses Marker Assisted Selection (MAS) in its breeding programs. MAS allows selection of plant genes based on genotype instead of phenotype, reducing or eliminating the need for growing thousands of seedlings. MAS speeds up the breeding cycle, identifies recessive alleles and is not affected by environmental conditions. To conduct MAS we need to extract DNA.
Limitations of the current methods of DNA include

- High Cost
- Labor Intensive
- Cause Bottleneck in getting genetic information

Methods Used

Bio-sprint (Current Method)	**Quick Extract Protocol**
• Grinding and lysis • Magnetic particles capture DNA • Robotic process purifies DNA • Clean DNA eluted for use	• Split seeds into embryo and endosperm • Crush and grind seed tissues with metal beads • QuickExtract seed extract solution • Heat at 65°C • Stop process at 95°C • Chill Samples on Ice • Centrifuge and remove supernatant

Quantification and Quality Assessment

- Use UV Plate reader to analyze the quantity of DNA
- Use Agarose Gel to asses the quality of the DNA

DNA Quality and Quantity Assessment

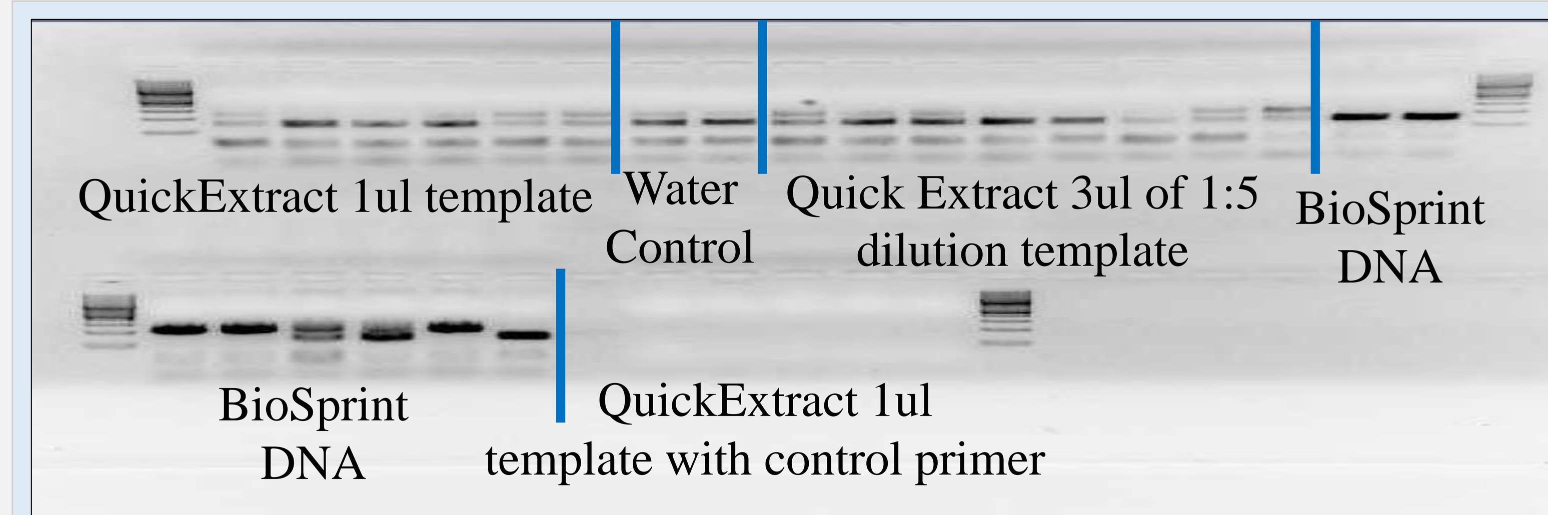

Figure 1) PCR of QuickExtract and BioSprint extracted DNA used for PCR with Lr34 marker and a second control primer.

Results of Germination Tests

Examination of split seeds and half seeds because it is needed to know if split seeds can germinate as well as whole seeds.

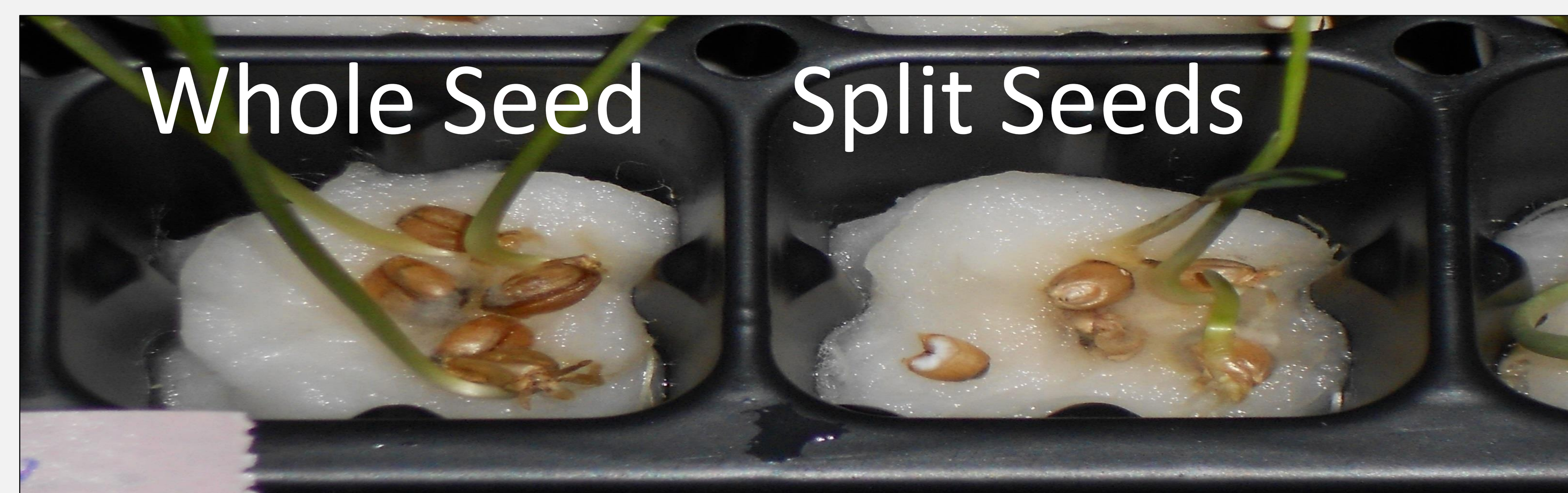

Figure 2) Examination of split seeds and half seeds to determine if split seeds germinate as well as whole seeds.

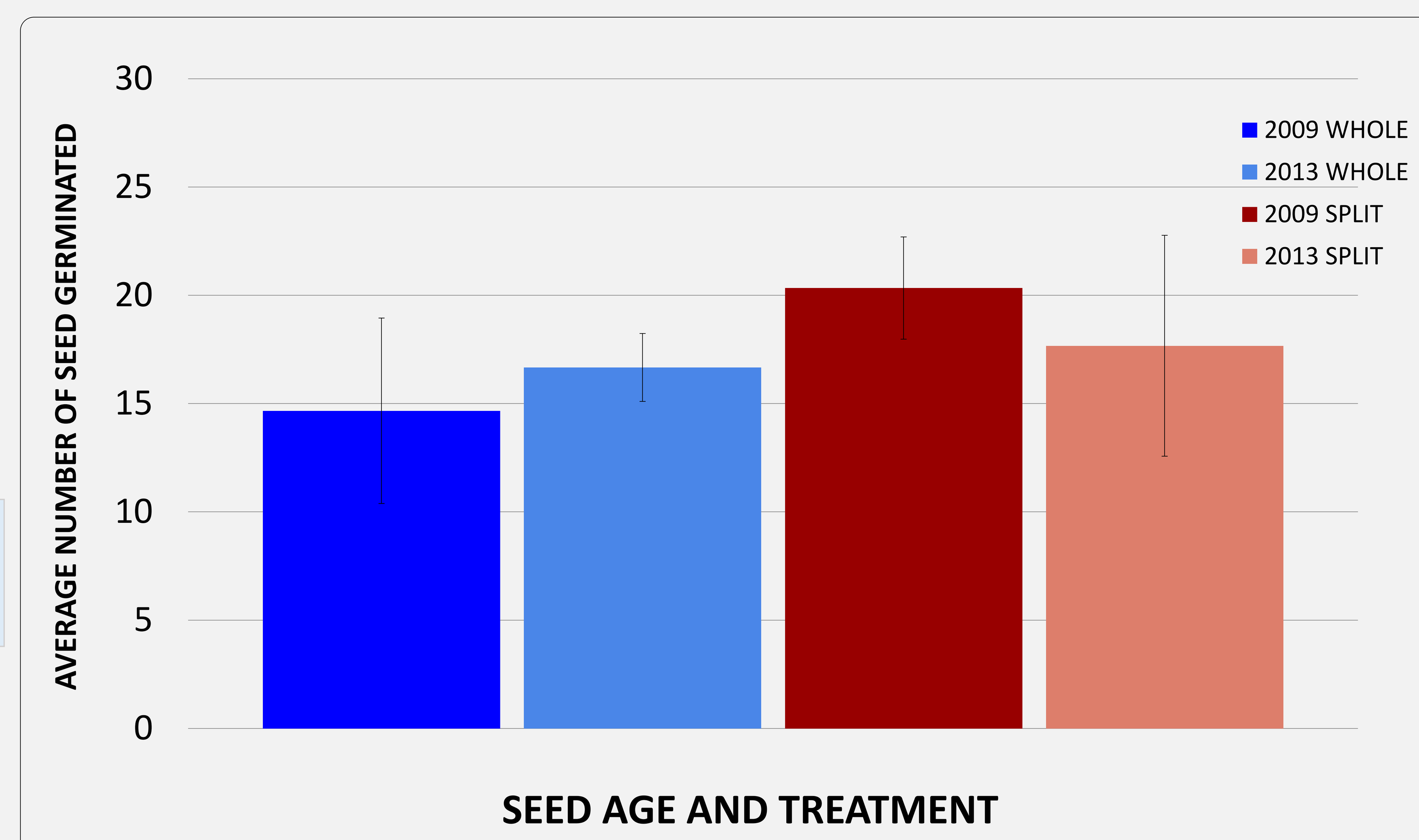

Figure 3) Germination comparison of whole and split seeds from different growing years

Discussion

BioSprint DNA amplified better than QuickExtract DNA (Figure 1), the Lr34 primer amplified the water controls as well (this means there is a problem with the primer). The lack of amplification in the control marker, along with the spectroscopy indicates that there is not enough DNA in the QuickExtract sample. Split seeds germinated more than the whole seeds but the difference was not significant.(Fig 1, Fig 2)

Further Research and Conclusion

We were able to prove that split seed germination is as effective as whole seed germination and so we can continue researching into this method.
However, there is clearly room for improvement with the QuickExtraction protocol in order to yield better results. Possible areas for improvement is to reduce contamination of seeds during the splitting process or using other primers.

References and Acknowledgements

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