Rapid isolation and characterization of soybean group IX ERF promoters using two different validation tools
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Abstract
Promoters are DNA sequences located upstream of gene coding regions that play an enormous role in controlling gene expression. Promoters are also important in transgenic crops as they contain the main elements that directly regulate the introduced transgene. Because of their importance, large efforts in our laboratory have recently focused on identification and characterization of plant promoters. Promoters are usually characterized in stably-transformed plants, which take some time to generate. The main goal of this research was to identify and characterize soybean ERF (GmERF) group IX promoters. The GmERF gene family encodes for a large group of transcription factors induced by both biotic and abiotic stress. Specifically, the ERF group IX genes are induced by wounding or pathogen attack.

Introduction
The use of transgenic technology is helpful not only for basic research but also for crop improvement. Transgenes are usually composed of an open reading frame (ORF) encoding for a protein of interest, a terminator sequence needed to signal the end of transcription, and a promoter which is a DNA sequence located just upstream of the ORF that drives gene expression. Transgenic soybean is the most widely grown transgenic crop in Ohio, the US and the world. For most transgenic soybeans, the promoters used to regulate the genes of interest are of viral origin. Since viral promoters may be undesirable for use in future transgenic crops, large efforts have been focused in our laboratory on identification and characterization of native soybean promoters.

The ability to identify and clone soybean promoters has been tremendously facilitated by the recent release of the soybean genome (www.phytozone.net/soybean.php). The soybean genome has been sufficiently annotated (using other plant genomes) so that most soybean genes can be predicted with good reliability. Promoters for annotated soybean genes lie 5' to the predicted translational start, which is relatively easy to identify using computational biology approaches with consensus sequences. Identification of the physical limits (within the DNA) of the promoter itself cannot currently be predicted. The only way to validate promoter is to test the contribution of the promoter using a marker gene or gene of interest following re-introduction.

Material and Methods
Promoter identification and construct design
Ten GmERF Group IX genes were identified in the soybean genome database based on similarities in the predicted amino acid sequence with previously reported tobacco ERF Group IX proteins (Ruash et al. 2008). The upstream regions of the ORF for these 10 GmERF genes were PCR-amplified and cloned in front of the gfp gene contained in pFLEV (Finer Laboratory Expression Vector), a vector designed for promoter and promoter element validation. The 10 promoters were subsequently subcloned into the binary vector pCambia1300 for introduction into A. rhizogenes (strain K599) using the freeze-thaw method (Cho et al. 2000). Cotyledons from 5-d old germinating seedlings were wounded with a scalpel dipped in bacterial suspension cultures harboring the GmERF promoters and co-cultivated for 3 d on wet filter paper. Inoculated cotyledons were transferred onto culture medium containing 400 mg l-1 Timentin in order to prevent the bacterial growth. Isolated hairy roots, obtained 14-20 d post-inoculation, were excised and subcultured for 3 d before image collection for expression in stably-transformed tissues.

Results
Numerous GFP-positive hairy roots were observed after 14-20 d after inoculation of soybean cotyledons.

Stably expression analysis in soybean hairy roots
Promoter constructs were introduced into A. rhizogenes strain K599 using the freeze-thaw method (Cho et al. 2000). Cotyledons from 5-d old germinating seedlings were wounded with a scalpel dipped in bacterial suspension cultures harboring the GmERF promoters and co-cultivated for 3 d on wet filter paper. Inoculated cotyledons were transferred onto culture medium containing 400 mg l-1 Timentin in order to prevent the bacterial growth. Isolated hairy roots, obtained 14-20 d post-inoculation, were excised and subcultured for 3 d before image collection for expression in stably-transformed tissues.

Phylogenetic tree for the 10 GmERF promoters.

Discussion
The GmERF promoters drive different GFP expression levels in both transient and stable expression analysis.

The GmERF promoters were isolated and partially characterized in both transiently expressing and stably transformed tissues.

References

Acknowledgments
We would like to thank Dr. Harold Trick, Kansas State University, for providing the A. rhizogenes strain K599. Funding from the United Soybean Board is appreciated.

Diagram showing the methodology for transient expression analysis of promoters in transformed lima bean cotyledons.

Table showing transient GFP expression driven by 8 different GmERF promoters and CaMV35S. 24 h after bombardment.