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 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 study was to isolate and characterize a soybean ERF group IX promoter family using two new gene expression validation tools. Ten GmERF (*Glycine max* Ethylene Response Factor) promoters were identified, cloned and initially characterized using quantitative analysis of transient GFP expression in bombarded lima bean cotyledons. Some of the GmERF promoters gave high levels of transient GFP expression, while profiles of transient GFP expression over varies among GmERF promoters. Generation of stably-transformed soybean hairy roots was also evaluated as an additional validation tool for analysis of the GmERF promoter family. Soybean cotyledon explants, inoculated with *Agrobacterium rhizogenes*, exhibited numerous GFP-positive hairy roots 14-20 days after inoculation. GFP detection in these roots was greatly facilitated by the lack of chlorophyll, which could otherwise interfere with GFP detection. GFP expression intensity was somewhat variable between different events but consistent within events. In summary, 10 different soybean GmERF promoters were isolated and partially characterized in both transiently expressing and stably-transformed tissues.

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 (*Glycine max* Merrill.) 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.phytozome.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.

The main goal of this research was to identify and characterize promoters from a new soybean promoter family; the soybean (*Glycine max)* ERF (GmERF) gene family group IX. 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.

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 projected amino acid sequence with previously reported tobacco ERF Group IX proteins (Rushton 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.

Transient expression analysis in lima bean cotyledons

All 10 GmERF promoters were evaluated through transient expression using lima bean cotyledonary tissues. Cotyledons from 3-4-days (d) old germinating seedlings were excised, bombarded, and placed adaxial side up on culture medium, for automated image collection. Images of cotyledons expressing GFP were collected every hour for 100 h. GFP intensity was scored through ImageJ, which is a publicly-available Java-based software.

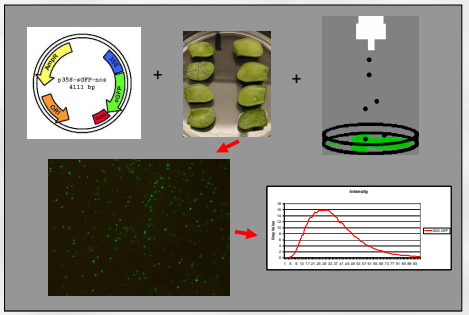
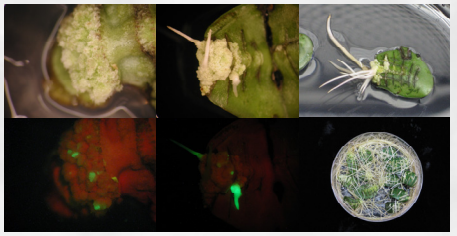


Diagram showing the methodology for transient expression analysis of promoters in transformed lima bean cotyledons.

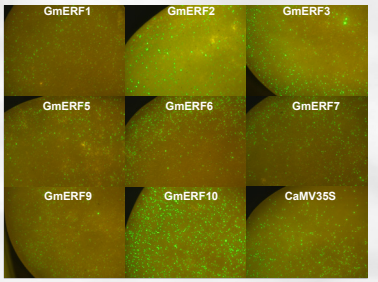
Stably expression analysis in soybean hairy roots

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 mgl^{-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.

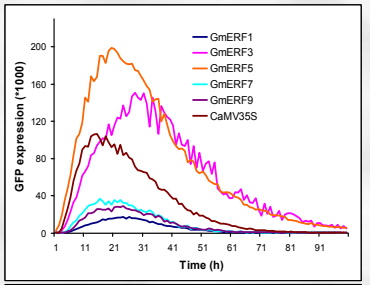


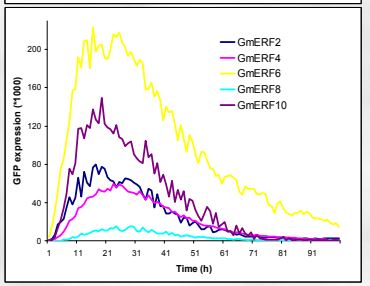
Induction of soybean hairy roots containing GmERF promoters fused to the *gfp* reporter gene.

Results



Transient GFP expression driven by 8 different GmERF promoters and CaMV35S 24 h after bombardment.





Time course of transient expression analysis of GmERF promoters in lima bean cotyledons.

- Some GmERF promoters showed higher GFP expression than the CaMV35S promoter.
- Expression profiles (curve shapes) varied among different promoters.
- Expression profiles could be associated with promoters features and promoter element composition.
- It does not appear to be a direct relationship between the transient expression analysis and the phylogenetic tree of promoter sequences.

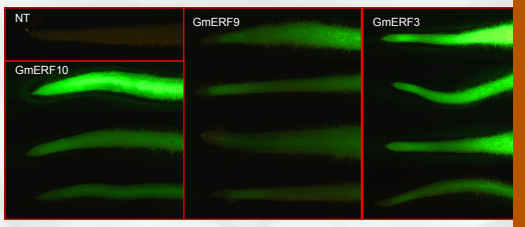
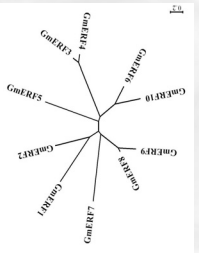


Image collection of hairy roots containing 3 GmERF promoters.

- Numerous GFP-positive hairy roots were observed after 14-20 d after inoculation of soybean cotyledons.
- Variation in GFP expression was observed among different events (roots).
- GFP expression was easily detected in hairy roots as they do not contain chlorophyll, which could interfere with GFP detection.
- Hairy roots seemed to be functional and showed rapid growth during *in vitro* subculture.



Phylogenetic tree for the 10 GmERF promoters.

Discussions and Conclusions

- The GmERF promoters drive different GF expression levels in both transient and sta expression analysis.
- The lima bean cotyledonary system leads rapid analysis of transient expression prof
- The soybean hairy root system provides a validation tool than any other method in s transformed soybean tissues.
- The intensity of GFP expression seems to correlated between the two validations to

References

Cho HJ, Farrand SK, Noel GR, Widholm JM (2000) efficiency induction of soybean hairy roots and propa of the soybean cyst nematode. Planta 210:195-204

Rushton PJ, Bokowiec MT, Han S, Zhang H, Branno Chen X, Laudeman TW, Timko MP (2008) Tobacco transcription factors: novel insights into transcription regulation in the solanaceae. Plant Physiol 147:280-

Acknowledgments

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