Transgenic expression of *Medicago truncatula* PR10 and PR5 promoters in alfalfa shows pathogen induced up-regulation of transgene expression

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Abstract

Genetic modification of alfalfa to introduce novel traits requires promoters for controlling gene expression. Promoters that are induced upon pathogen invasion are needed for engineering plants with disease resistance. *Medicago truncatula* promoter regions of pathogenesis-related (PR) genes, PR5 and PR10, were identified as being highly up-regulated during the initial stages of infection by root and foliar pathogens (1). These promoters were PCR amplified and cloned into plant transformation vectors ahead of the β-glucuronidase (*gus*) gene. *Agrobacterium*-mediated transformation was used to create transgenic lines of alfalfa (cultivar Regen SY27x). The transgenic plants were stained for GUS activity. Quantitative PCR assays were done to quantify pathogen-induced GUS expression, as well as expression of PR5 and PR10 in infected leaves. In plants with the PR10:GUS or PR5:GUS constructs, GUS transcripts accumulated 41- to 378-fold over the mock-inoculated plants at 7 days after inoculation with *Phoma medicaginis*, depending on the plant line. GUS transcripts were also strongly up-regulated in response to *Colletotrichum trifolii* and *Pseudomonas syringae* pv. *syringae*. In response to *P. medicaginis*, transcripts of the PR10 gene were up-regulated 31- to 221-fold at 7 days after inoculation and transcripts of the PR5 gene were up-regulated 44- to 60-fold. These experiments show that the *M. truncatula* PR10 promoter is functional in alfalfa for expression of transgenes and up-regulates genes after infection by a range of alfalfa pathogens.

Objectives

- Evaluate PR5 and PR10 promoter functionality in alfalfa for expression of transgenes.
- Determine if the promoters are induced by a range of alfalfa pathogens.
- Reveal the promoter with greater activity.

Materials and Methods

Generation of Transgenic Plants

A pBI101.2 transformation vector was used with the promoter (PR5 or PR10) ahead of the *gus* gene. Alfalfa (cultivar Regen SY27x) was transformed by co-cultivating leaf explants with *A. tumefaciens* LBA4404 containing the transformation vectors as described previously (2). The transgenic plants were identified by PCR amplification of the *nptII* gene, primers targeting the PR5 or PR10 promoter, and the *gus* gene from genomic DNA.

GUS Staining

Samples of stems, leaves, and roots with nodules were placed in X-gluc staining solution at 37°C for 24 h. This process was repeated with detached leaves that had been inoculated with *P. medicaginis* or *C. trifolii*.

Quantitative real-time PCR

For the detached leaf assay, fully expanded trifoliates from the top 2 to 3 nodes of transgenic lines were removed and placed in moist chambers. Drops of a conidial or bacterial suspension were placed on leaflets with mock-inoculated leaves receiving drops of sterile water. For the whole plant assay, conidial suspensions with 50 ppm Tween 20 were sprayed until runoff, then placed in a mist chamber for 48 h. RNA was extracted from symptomatic infected leaves after inoculation and converted to cDNA. Using specific primers, transcript accumulation was compared between cDNA from mock-inoculated and inoculated plant tissue. Relative quantification was based on the comparative Ct method.

References

1. Samac, D.A., Peñuela, S., Schnurr, J.A., Hunt, E.N., Foster-Hartnett, D. Vandenbosch, K.A., Gantt, J.S. 2011. Expression of coordinately regulated defence response genes and analysis of their role in disease resistance in *Medicago truncatula*. Molecular Plant Pathology. 12(8):786-798

2. Samac, D.A., Austin-Phillips, S. 2006. Alfalfa (*Medicago sativa* L.). Methods in Molecular Biology 343: 301-311.

Results

In uninoculated plants, GUS activity was primarily seen in the root vascular tissues in both PR5 and PR10 lines. Staining appeared to be intensified near regions of developing nodules. No activity was observed in uninoculated leaves. With fungal pathogen (*P. medicaginis* or *C. trifolii*) infection, staining was greatly enhanced and allowed for stain visualized near centers of infection and vascular tissue in the leaves. This indicates that the transgenic promoters are functioning in alfalfa and pathogen induced.

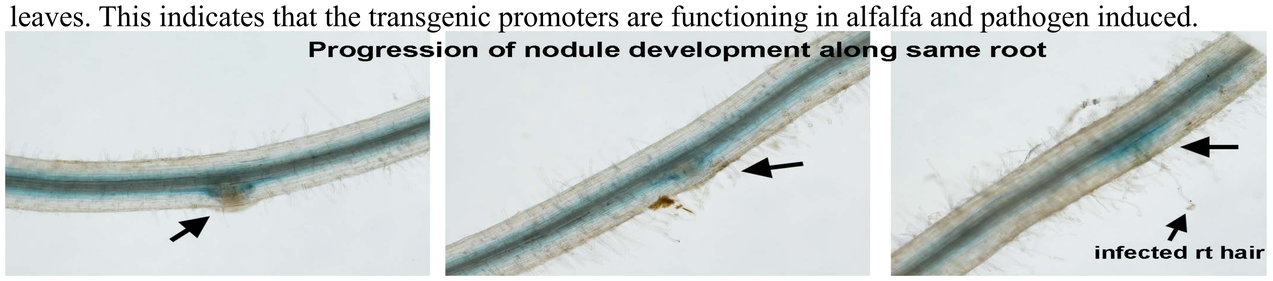

Figure 1. GUS staining in uninoculated PR10-12 alfalfa root showing activity in developing nodule.

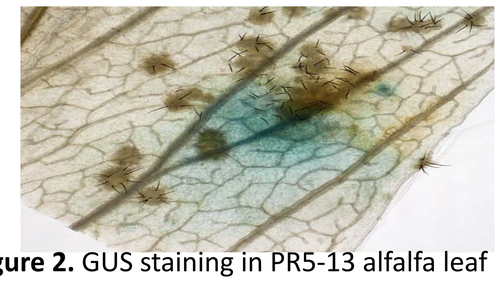

Figure 2. GUS staining in PR5-13 alfalfa leaf inoculated with *Colletotrichum trifolii*.

Figure 3. GUS staining in PR10-25 alfalfa leaf inoculated with *Phoma medicaginis*.

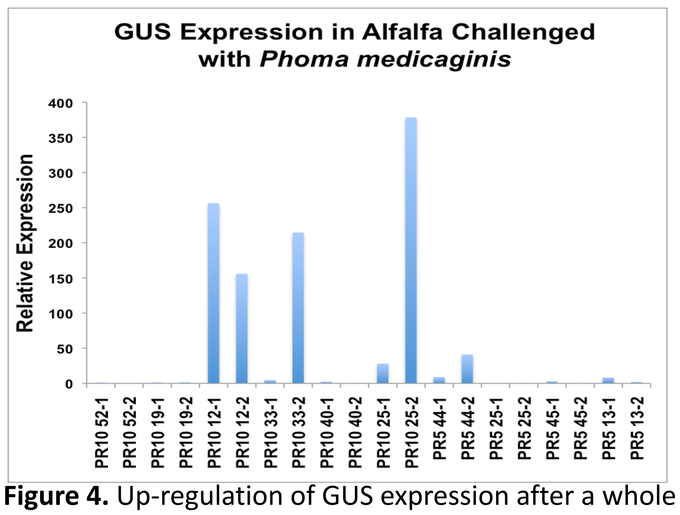

Figure 4. Up-regulation of GUS expression after a whole plan inoculation in PR5 and PR10 lines.

In response to pathogen infection, the PR10 promoter resulted in greater up-regulation than PR5 (Fig. 4). In the whole plant assay, transcripts of the PR10 gene were up-regulated 31- to 221-fold (Fig. 5) compared to transcripts of the PR5 gene that were up-regulated 44- to 60-fold (Fig. 6).

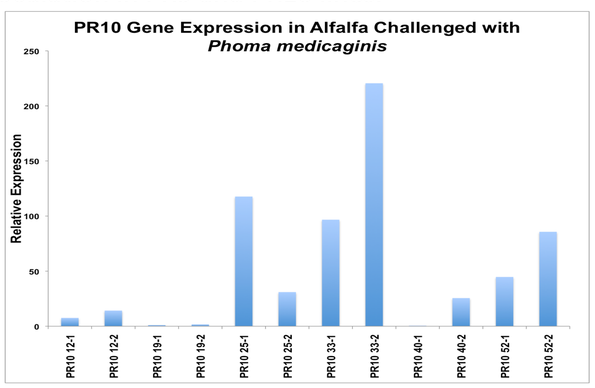

Figure 5. Up-regulation of PR10 expression after inoculation.

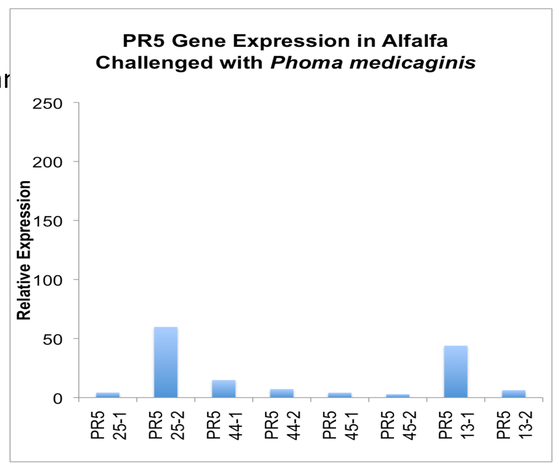

Figure 6. Limited up-regulation of PR5 expression after inoculation.

Conclusions

- The PR5 and PR10 promoters were demonstrated to be pathogen-induced.
- The PR10 promoter had greater activity than the PR5 promoter.
- The *M. truncatula* PR10 promoter is functional in alfalfa for expression of transgenes and up-regulates genes after infection by a range of alfalfa pathogens. This promoter potentially could be used for the transgenic expression of antimicrobial peptides or other avirulence factors.