Identification and Analysis of Nematode-Induced Cis-Regulatory Regions in *Solanum sisymbriifolium*

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INTRODUCTION

- Pale cyst nematode (*Globodera pallida*) threatens the genus Solanum, which contains potatoes and tomatoes. Root-knot nematodes (*Meloidogyne* spp.) are the second greatest nematode threat to potatoes.
- Unmanaged *G. pallida* infestations can cause up to 70% yield losses[1]. Root-knot nematode infestations that damage >15% tubers can cause the entire lot to be culled[2].
- In *Solanum sisymbriifolium*, a distant South American relative of potato, the expression of 277 genes change during a *G. pallida* infestation. *S. sisymbriifolium* is a trap crop, releasing hatching factors but is resistant to *G. pallida* reproduction[3].
- Ascaroside #18 (Ascr#18) is a conserved signaling molecule in nematodes recognized by the plant receptor NILR1[4].

METHODS

BAC Library Screening: A *S. sysimbriifolium* BAC library was screened for 8 genes with PCR using a 7-pool matrix system. BAC DNA was sequenced using long read sequencing at the WSU Laboratory for Biotechnology and Bioanalysis.

Ligation: A > 1 kb region upstream of each gene's translational start site was ligated into β-glucuronidase-containing vector pCAMBIA 1391z. Transformation into potato c.v. Desiree is ongoing.

PlantPAN 4.0 Analysis: Shared regulatory elements within the 3 kb region upstream (cis-regulatory region) of six genes were identified. Root-and floral-associated elements had locations and observed minus expected binding sites recorded.

GUS Assay Analysis: Leaves and roots were imaged for each experiment, followed by GUS quantification with CompuEye Leaf & Symptom Area software.

Gene Name	Function
CytoP450	Cytochrome P450
Oxi-B1	Oxidative burst gene 1
Quail	Cell-wall associated kinase
TS9	Terpene synthase-like 9
VE1	Tomato verticillium wilt resistance gene homologue
Warbler	Leucine-rich repeat receptor-like kinase 3

Table 1. Names and functions for 6 genes identified by Wixom et al. (2020) as being differentially expressed in *S. sisymbriifolium* in the presence of *G. pallida*.

EXPERIMENTS

Stable Experiment 1: A GUS assay was run to compare gene expression between transformed potato lines containing a 691 bp cis-regulatory region (CRR) and pCAMBIA 1301 direct from tissue culture.
Stable Experiment 2: Stable Experiment 1 was repeated in 3:1 sand-loam, treated with 0 or 1000 northern root-knot nematode (*M. hapla*) J2s and harvested 3 days later.
Stable Experiment 3: Stable Experiment 1 was repeated, with a 0 or 10 nM Ascr#18 root dip, followed by 3 days in ½ MS liquid media.
Transient Experiment 1: Five >1 kb CRRs and the 691 bp CRR were transformed into GV2260 competent *Agrobacterium tumefaciens* cells, then infiltrated into *Nicotiana benthamiana* leaves. GUS assay was performed 24 hours later.
Transient Experiment 2: Transient Experiment 1 was repeated with co-infiltration of 0 or 2 µM Ascr#18, with harvest 24 hours later.

Stable Experiment 2

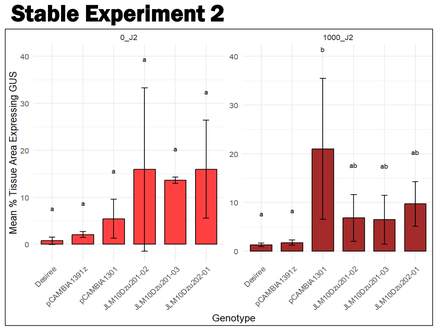

Fig. 1a. Mean percent area of roots expressing GUS 72 hours after inoculation of stable transformant lines with *M. hapla* J2s. Neither Ascr#18 concentration significantly altered GUS expression. pCAMBIA 1391z = negative control; pCAMBIA 1301 = positive control (constitutive promoter); 691 bp Quail = JLM10Dzu201-02, JLM10Dzu201-03, JLM10Dzu202-01.

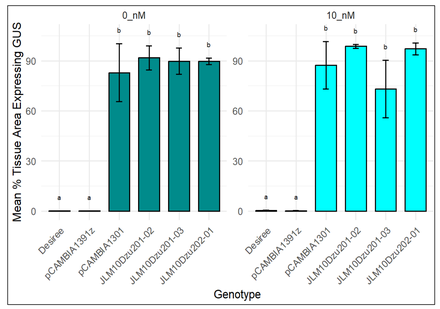

Fig. 1b. Mean percent area of leaves expressing GUS 72 hours after inoculation of stable transformant lines with *M. hapla* J2s. Neither Ascr#18 concentration significantly altered GUS expression. pCAMBIA 1391z = negative control; pCAMBIA 1301 = positive control (constitutive promoter); 691 bp Quail = JLM10Dzu201-02, JLM10Dzu201-03, JLM10Dzu202-01.

Stable Experiment 3

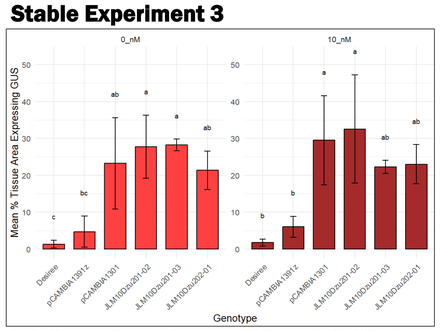

Fig. 2a. Mean percent area of roots expressing GUS 72 hours after Ascr#18 root dip in stable transformant lines. Neither Ascr#18 concentration significantly altered GUS expression. pCAMBIA 1391z = negative control; pCAMBIA 1301 = positive control (constitutive promoter); 691 bp Quail = JLM10Dzu201-02, JLM10Dzu201-03, JLM10Dzu202-01.

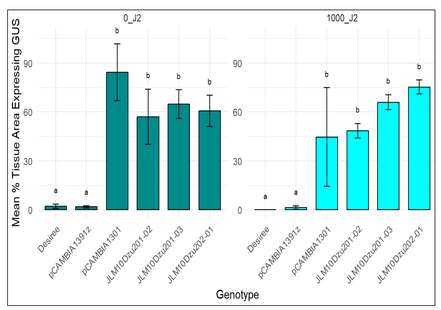

Fig. 2b. Mean percent area of leaves expressing GUS 72 hours after Ascr#18 root dip in stable transformant lines. Neither Ascr#18 concentration significantly altered GUS expression. pCAMBIA 1391z = negative control; pCAMBIA 1301 = positive control (constitutive promoter); 691 bp Quail = JLM10Dzu201-02, JLM10Dzu201-03, JLM10Dzu202-01.

Transient Experiment 2

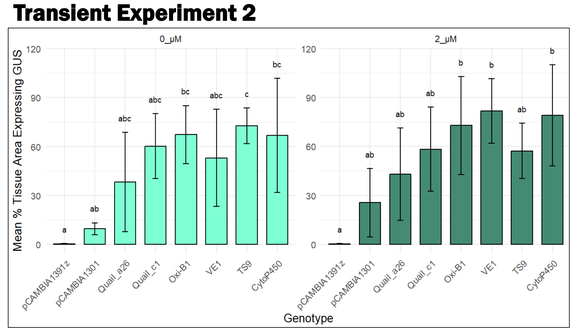

Fig. 3. Mean percent area expressing GUS 24 hours after infiltration of *N. benthamiana* with *A. tumefaciens*. Quail_a26 = 691 bp CRR; Quail_c1 = 1305 bp CRR. Quail_a26 is similar to the constitutive promoter (pCAMBIA 1301) at both Ascr#18 treatment levels. TS9 was significantly higher than pCAMBIA 1301 at 0 µM but had similar GUS expression at 2 µM Ascr#18.

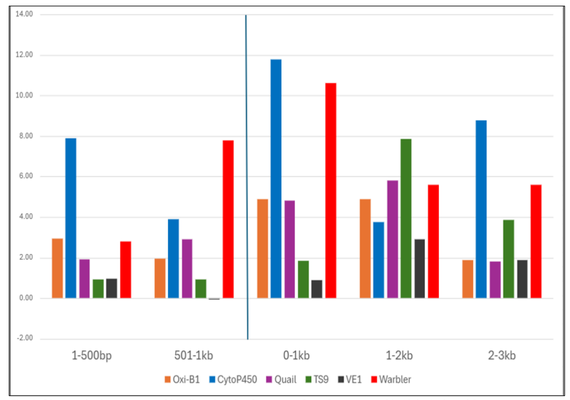

Fig. 4. Observed minus expected binding sites for Sequence Over-Represented in Light-Induced Promoters (SORLIP). The most enriched (more prevalent than expected) root-associated elements across the CRRs were 263 (Sequence Over-represented in Light-Induced Promoters), 407 (Sporamin binding factor binding site), 384 and 388 (Sporamin A TATA boxes). Frequency of these elements, including SORLIP, peaked within 1,000 bases of the TATA box.

DISCUSSION

- This study assessed if 6 cis-regulatory regions from a nematode-resistant wild relative of potato were induced by the presence of root-knot nematodes or ascaroside #18.
- The four most enriched root-associated elements were concentrated near the gene start, suggested the six CRRs have root-associated function.
- The 691 bp Quail CRR functioned similarly to a constitutive promoter, while some of the five long CRRs had significantly higher gene expression than a constitutive promoter.
- While computational analysis suggests root-related function, exposure of the 691 bp Quail CRR to Northern root-knot nematode or to ascaroside #18 (at the tested conditions) did not induce gene expression in roots. Transient expression of the six CRRs was not induced in leaves by ascaroside #18.

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Selected References:

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