An expanded role of the SNARE-containing regulon as it relates to the defense process that *Glycine max* has to *Heterodera glycines*  

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Abstract  

The defense regulon has been defined genetically in *Arabidopsis thaliana* to involve the syntaxin PENETRATION1 (PEN1), the secreted glucosidase (PEN2) and an ATP-binding cassette (ABC) transporter (PEN3). Experiments in *Glycine max* (soybean) have identified homologous genes being expressed in root cells undergoing defense processes to *Heterodera glycines* parasitism. These experiments have not examined proteins involved in cargo delivery to the infection site. A good candidate fulfilling this role would be myosin XI. In related studies, prior microscopic analyses have shown the accumulation of callose at these defense sites. Experiments presented here show that callose synthase expression impairs *H. glycines* parasitism. The experiments presented here have expanded on prior results demonstrating the central defense role of the plant vesicular trafficking apparatus and callose synthase to the defense process that *G. max* has toward *H. glycines* parasitism.

Introduction  

Plant defense is a process functioning on a number of levels (Jones and Dangl 2006). The process installs barriers that impede pathogen success and ensures host recovery. Consequently, the process would be expected to require many products necessary to accomplish the task faithfully and it would not be surprising if these components exhibit cross-talk at some level. Therefore, impairing the expression or function of any of these genes could have drastic, negative impacts on plant defense, host recovery and even growth itself. Revealing whether coordinated crosstalk of member components occurs would have great value in understanding the defense apparatus as a whole and, perhaps, lead to the identification of cellular surveillance systems previously not well characterized or known.

The plant secretion apparatus is a basic fundamental platform employed to accomplish a number of tasks, including defense, with part of this process involving vesicle transport and membrane fusion. A substantial part of this secretion apparatus is composed of the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) and associated proteins (Nielsen and Thordal-Christensen 2013; Klink et al. 2017). While the processes involving membrane fusion and vesicle transport are extensive, efforts have been focused in on the co-regulated expression of components that compose the regulon, a structure defined in *A. thaliana* through analyses of the coordinated roles of the PENETRATION1, PEN2 and PEN3 proteins occurring during defense (Collins et al. 2003; Lipka et al. 2005; Stein et al. 2006; Humphry et al. 2010). However, more recent work focusing in on the analysis of defense in an understudied organ, the root, has led to an expansion of the number of these proteins functioning in defense with some of them previously not shown to have defense functions. To demonstrate this concept, developmental genomics analyses have been performed on the allotetraploid agricultural crop *G. max* as it interacts with the plant parasitic nematode *H. glycines*. In comparisons with original research performed in *S. cerevisiae*, work performed in *A. thaliana* has allowed for an understanding of the relationship between these membrane fusion genes and an expansive defense platform (the regulon) (Humphry et al. 2010; Johansson et al. 2014; Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). In contrast to earlier studies, the analyses have led to the identification that the component parts of the regulon exhibit various levels of co-regulation where the expression of one component influences the expression of others (Matsye et al. 2012; Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). This feature of the regulon had not previously been reported and may explain how the defense regulon sustains its expression and functionality as it is recruited...
to perform different, but related roles during the defense process.

Experiments have shown rapid cellular rearrangements involving actin, as the cell is under pathogen attack, resemble those produced by mechanical stimulation that would be expected to happen by nematode parasitism (Hardham et al. 2008). A number of experiments that have been performed in A. thaliana have demonstrated the importance of molecular motor components such as myosin (myosin XI) functioning in the delivery of vesicles to the defense site (Yang et al. 2014). These defense vesicles travel along actin filaments to deliver defense cargo while also functioning to include the deposition of important structural defense barriers. One of these barriers is callose, a β-1,3- and β-1,6-branched glucose-derived polysaccharide (Ostergaard et al. 2002; Meyer et al. 2009; Nielsen and Thordal-Christensen 2013; Ellinger et al. 2013; Yang et al. 2014). Callose relates to papillae, structures long known to perform important defense functions (Aist 1976; Assaad et al. 2004). A question that has remained is whether myosin and callose synthesis function during defense in the G. max–H. glycines pathosystem which is likely due to the observation of callose at defense sites (Sharma et al. 2016).

The work presented here experimentally tests components that would be expected to function within the regulon, but lie outside of the genetically defined apparatus (Humphry et al. 2010). The selected genes encoding myosin XI and callose synthase (CS) relate to their previously reported defense roles and how the roles relate to vesicle trafficking (Ellinger et al. 2013; Ellinger, Glöckner, et al. 2014; Ellinger, Sode, et al. 2014; Ellinger and Voigt 2014; Yang et al. 2014; Peremyslov et al. 2015; Leslie et al. 2016; De Benedictis et al. 2018; Sassmann et al. 2018; Wang et al. 2018). Furthermore, an examination of CS has been performed due to our previous observations that have shown an apparent increase in callose labeling at sites of engineered resistance in transgenic lines overexpressing GmSYP121-1 (GmSYP121-1-OE), leading to a successful defense response while in contrast GmSYP121-1-RNAi transgenic lines exhibit an impaired defense response and an apparent decrease in callose at sites of successful parasitism (Sharma et al. 2016). The results presented here unify earlier genomics analyses that have examined gene expression occurring within these sites of infection undergoing various types of defense processes and the identification that GmSec17α-SNAP functions in defense (Klink et al. 2007; Klink, Hosseini et al. 2009, 2010, 2011; Klink, Overall, et al. 2010; Matsye et al. 2011, 2012; Pant et al. 2014; Sharma et al. 2016). These observations relate well to the identification of nematode effectors that bind to GmSec17α-SNAP, presumably to impair the secretion process, unifying many observations made regarding defense in this pathosystem (Bekal et al. 2015; McNeese et al. 2017, 2019). Lastly, these results are the first to demonstrate how the defense vesicles through myosin could be delivered to the subcellular site of defense in this pathosystem.

Results

Myosin XI functions in G. max defense to H. glycines

Myosin XI has been shown to function in vesicle transport by acting as part of an apparatus that captures vesicles and serving as an ATPase to facilitate transport along the actin cytoskeleton. These observations have prompted this analysis of the G. max genome, clearly identifying genes relating to myosin XI that are expressed in syncytia undergoing the process of defense (Table 1; Supplemental Table 1). To determine if these myosins perform a defense function, five myosins have been genetically engineered as overexpression (OE) and RNAi cassettes using previously published analysis procedures (Sharma et al. 2016). In our hands, the genetic engineering of the GmMyosin-OE cassettes never led to an ability to obtain transgenic roots over many attempts while at the same time the pRAP15-OE control experiments did lead to the generation of plants having transgenic roots. Furthermore, these experiments have been performed side by side with the GmMyosin-RNAi and pRAP17 control experiments and the GmCS-OE, pRAP15-OE-control, GmCS-RNAi and pRAP17-RNAi control experiments which led to successful generation of transgenic plants. These results indicate that the GmMyosin-OE cassettes may lead to a lethal condition in the cells expressing this cassette. These experiments have not been pursued further.

In contrast, the expression of GmMyosin-RNAi cassettes leads to the generation of roots, observed both by expression of the eGFP reporter and the decrease of the respective GmMyosin transcript levels in root tissue (Supplemental Figure 1). Since transgenic GmMyosin-RNAi-expressing roots had been obtained, it became possible to examine the effect that the expression of the RNAi cassettes had on H. glycines parasitism (Figure 1). The experiments reveal that H. glycines parasitism is increased in the GmMyosin-RNAi lines (Figure 2). No statistically significant effect has been observed on root growth for the GmMyosin-RNAi lines (data not presented). Consequently, the level of parasitism is similar when comparing the FI of the whole root (wr) mass and per gram (pg) of root tissue (Figure 2).

Callose synthase functions in G. max defense to H. glycines

Prior experiments have revealed that sites surrounding syncytia cells undergoing the process of defense contain callose

| Gene          | Accession | control-0 | 3 | 6 |
|---------------|-----------|-----------|---|---|
| Myosin-1      | Glyma03g32660 | n/a       | n/a | n/a |
| Myosin-2      | Glyma03g40950 | n/a       | n/a | n/a |
| Myosin-3      | Glyma04g05920 | n/a       | n/a | n/a |
| Myosin-4      | Glyma06g05910 | N/M       | N/M | M |
| Myosin-5      | Glyma08g17170 | n/a       | n/a | n/a |
| Myosin-6      | Glyma09g41921 | n/a       | n/a | n/a |
| Myosin-7      | Glyma09g42180 | N/M       | N/M | N/M |
| Myosin-8      | Glyma10g03963 | n/a       | n/a | n/a |
| Myosin-9      | Glyma10g04750 | n/a       | n/a | n/a |
| Myosin-10     | Glyma10g03670 | n/a       | n/a | n/a |
| Myosin-11     | Glyma10g43230 | n/a       | n/a | n/a |
| Myosin-12     | Glyma12g34780 | N/M       | N/M | N/M |
| Myosin-13     | Glyma13g16710 | n/a       | n/a | n/a |
| Myosin-14     | Glyma13g18140 | n/a       | n/a | n/a |
| Myosin-15     | Glyma13g19080 | N/M       | N/M | N/M |
| Myosin-16     | Glyma13g35790 | N/M       | M   | M |
| Myosin-17     | Glyma14g11170 | N/M       | N/M | N/M |
| Myosin-18     | Glyma15g42030 | M          | M  | M |
| Myosin-19     | Glyma17g05970 | M          | M  | M |
| Myosin-20     | Glyma19g35410 | M          | M  | M |
| Myosin-21     | Glyma20g00320 | M          | M  | M |
| Myosin-22     | Glyma20g00510 | n/a       | n/a | n/a |
| Myosin-23     | Glyma20g23660 | M          | N/M | N/M |
| Myosin-24     | Glyma20g36970 | n/a       | n/a | n/a |

Footnote: Yellow, genes under study.
Sharma et al. (2016). These results are consistent with observations showing callose is present at defense sites in a number of different plant pathosystems. How callose arrives at the defense site has been revealed in genetic experiments showing A. thaliana PEN2 and PEN3 mediate callose deposition (Clay et al. 2009). However, the process is complex, possibly involving additional syntaxins and vesicle associated membrane proteins (VAMPs) (Kwon et al. 2008; Meyer et al. 2009; Nielsen and Thordal-Christensen 2013; Caillaud et al. 2014).

Since components of the G. max SNARE particle and myosin function in H. glycines defense, experiments are presented here to determine if enzymes functioning in callose synthesis (callose synthase) also have a role.

The G. max genome is shown here to have at least 21 genes that are related to callose synthase (Table 2; Supplemental Table 1). Table 2. DCM reveals CS is expressed within G. max syncytia undergoing the process of defense while combating H. glycines parasitism. Analysis details are provided (Supplemental Table 1).

| Gene    | Accession | Time point (dpi) |
|---------|-----------|------------------|
|         |           | control-0 3 6    |
| CS-1    | Glyma04g36725 | n/a n/a n/a |
| CS-2    | Glyma04g39120 | n/a n/a n/a |
| CS-3    | Glyma05g32500 | n/a n/a n/a |
| CS-4    | Glyma06g15860 | n/a n/a n/a |
| CS-5    | Glyma06g18216 | n/a n/a n/a |
| CS-6    | Glyma06g44770 | n/a n/a n/a |
| CS-7    | Glyma08g16660 | n/a n/a n/a |
| CS-8    | Glyma08g16730 | n/a n/a n/a |
| CS-9    | Glyma08g42110 | N/M N/M N/M |
| CS-10   | Glyma08g42150 | N/M M M |
| CS-11   | Glyma08g47670 | N/M N/M N/M |
| CS-12   | Glyma10g44150 | N/M M M |
| CS-13   | Glyma13g31310 | N/M N/M M |
| CS-14   | Glyma13g3560  | n/a n/a n/a |
| CS-15   | Glyma13g37290 | n/a n/a n/a |
| CS-16   | Glyma15g08020 | n/a n/a n/a |
| CS-17   | Glyma15g39420 | n/a n/a n/a |
| CS-18   | Glyma15g42330 | M M M |
| CS-19   | Glyma18g12870 | n/a n/a n/a |
| CS-20   | Glyma18g13105 | n/a n/a n/a |
| CS-21   | Glyma18g53823 | n/a n/a n/a |

Footnote: Yellow, genes under study.
to GmCS-OE lines having increased CS transcript levels while the GmCS-RNAi lines have decreased transcript levels in root tissue, revealing that the cassettes are affecting relative levels of transcript abundance as expected (Supplemental Figure 3). The GmCS-OE roots exhibit a decrease in *H. glycines* parasitism in the wr and pg experiments, but there have been no apparent differences observed in root mass. These results indicate there is no effect occurring on root growth as a consequence of the genetic engineering of these genes (data not presented). Analyses of parasitism has resulted in FI of 29–33 in the wr analyses with similar results observed in the pg analyses (Figure 3). The similarity of the FI that has been observed between the wr and pg analyses demonstrate that there is little observed effect on root growth.

In contrast to the observations made for myosin RNAi lines, wr analysis of *H. glycines* parasitism is shown to exhibit no statistically significant change in parasitism as compared to the control (Figure 3). However, pg analyses of the amount of *H. glycines* parasitism has identified a significant increase in parasitism in the GmCS-10, GmCS-12, GmCS-13 and GmCS-18 RNAi lines (Figure 4). These observations indicate that the genetic engineering of GmCS as an RNAi cassette has a negative effect on root growth. To examine this hypothesis, analyses of the root masses of GmCS-10, GmCS-12, GmCS-13 and GmCS-18 demonstrate they have less mass than controls (Supplemental Figure 4). These results are not surprising when considering that in *A. thaliana*, the SNARE protein VAMP721 co-immunoprecipitates with the CS gene PLASMODESMATA-LOCATED PROTEIN 1 (PDLP1) leading to the regulation of callose deposition at developing encastrments at infection sites as the plant overcomes *Hyaloperonospora arabidopsis* infection (Gaillaud et al. 2014).

### Discussion

**The breadth of the defense regulon is broad**

The work presented here has described the defense regulon functioning in the root of *G. max* as it defends itself from parasitism by *H. glycines*. The regulon has been shown to be a structure that circumvents pathogen entry into the plant body by coordinately delivering antimicrobial compounds across the cell membrane (Collins et al. 2003; Lipka et al. 2005; Stein et al. 2006; Humphry et al. 2010; Johansson et al. 2014). Developmental genomics analyses presented here have allowed for an expansion of the regulon. While not directly described as part of the regulon, myosin and callose would be expected to be parts of the apparatus (Meyer et al. 2009; Humphry et al. 2010; Nielsen and Thordal-Christensen 2013; Johansson et al. 2014; Yang et al. 2014).

#### Myosin may function to deliver vesicles to the site of defense

The results presented here are the first to demonstrate how the defense vesicles, possibly through myosin, could be delivered to the subcellular site of defense in this pathosystem. The results presented here regarding the involvement of myosin XI genes are consistent with the role that the regulon plays in defense. While at first it may appear surprising that transgenic myosin XI-RNAi roots do not exhibit significant developmental defects, the lack of developmental defects observed for myosin-XI motor genes is not unprecedented. For example, RNAi for two different combinations of two myosin XI genes, xi-k xi-1 and xi-k xi-2, show no statistically significant developmental defects for mesophyll cell diameter, plant height, rosette span or flowering time, but are reduced in epidermal cell length only for *A. thaliana* plants engineered for RNAi of xi-k xi-2 (Peremysoy et al. 2015). The syncytium is not an epidermal cell, but a product of the pericycle and some surrounding cells. In the experiments presented by Peremysoy et al. (2015) it had been shown that *A. thaliana* undergoing RNAi did not experience significant developmental defects until five myosin paralogs had been suppressed in their expression. Our results show that the very specific process of defense may be restricted in some ways to the cell in which the defense process is occurring (Peremysoy et al. 2015; Kurth et al. 2017). This observation may explain why the RNAi of a single myosin as observed in the experiments presented here, while not experiencing significant observable developmental defects, are impaired in their defense response. In contrast, the lack of roots obtained in the myo-OE experiments could be consistent with the induced expression of these myosin genes in syncytia undergoing a defense response, leading to the lethality of the plant cell and ultimately nematode death. Future experiments aimed to further clarify the role of myosin XI in defense in the *G. max*–*H. glycines* pathosystem certainly will reveal the intricacies and specificity of this defense process (Figure 5).

#### Materials and methods

**The studied myosin and callose synthase genes**

The myosin and CS genes have been selected from microarray data generated from prior experiments (Klink, Hosseini et al. 2009, 2010; Matsye et al. 2011). A cutoff has been set whereby a gene is considered expressed in a given sample if probe signal is measurable (M) above threshold (p < 0.05) on all 6 arrays (combining the 3 arrays from *G. max* [PI 548402] and *G. max* [PI 88788]) according to the described methods (Klink, Hosseini, et al. 2010; Matsye et al. 2011). The expression of a gene has been considered not measured (N/M) if probe signal is not detected at a statistically significant

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**Figure 3.** CS functions in *G. max* defense to *H. glycines*. The calculated FI for the OE lines as compared to the controls: * statistically significant, p < 0.05 calculated by the Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test. The experimental error representing standard deviation is presented. The results are the average of three independently run biological replicates.

**Figure 4.** The effect that the expression of the CS-OE cassette has on *H. glycines* parasitism in *G. max*.
level ($p \geq 0.05$) on any one of the 6 total arrays. Due to how the Affymetrix® microarray has been constructed, some genes have had no probe set fabricated onto the array. In these cases, gene expression could not be quantified according to the analysis procedures so the gene expression analysis is not applicable (n/a) to those genes.

**Gene cloning**

The pRAP15 (OE) pRAP17 (RNAi) destination vectors are based off of the published Gateway® cloning vector platform (Curtis and Grossniklaus 2003; Klink, Kim et al. 2009; Matsye et al. 2012). The PCR-generated amplicons used in the experiments have been ligated into the pENTR/D-TOPO® entry vector (Invitrogen®) according to the manufacturer’s instructions (Curtis and Grossniklaus 2003; Klink, Kim et al. 2009; Matsye et al. 2012) (Supplemental Table 3). LR Clonase® (Invitrogen®) has been used to facilitate the transfer the gene amplicon to the pRAP15 overexpression and pRAP17 RNAi destination vectors according to the manufacturer’s instructions. The unengineered pRAP15 or pRAP17 control has the ccdB gene located in the position where, otherwise, the myosin or CS amplicon is inserted during the LR clonase reaction. This feature makes the pRAP15-ccdB and pRAP17-ccdB (RNAi control) unengineered vectors suitable controls for non-specific effects caused by gene overexpression and RNAi (Klink, Kim et al. 2009; Matsye et al. 2012). The pRAP15-ccdB, pRAP15-gene-OE, pRAP17-ccdB and pRAP17-gene-RNAi vectors have been used in freeze–thaw incubation experiments to transform chemically competent Agrobacterium rhizogenes K599 (K599) with the designated gene cassette (Hofgen and Willmitzer 1988; Haas et al. 1995; Collier et al. 2005; Klink, Kim et al. 2009).

**Production of genetically mosaic transgenic G. max plants**

The enhanced green fluorescent reporter (eGFP) has been used for visual selection of transgenic G. max roots (Supplemental Figure 1) (Haseloff et al. 1997; Klink, Kim et al. 2009; Matsye et al. 2012). The resultant plant is a genetic mosaic called a composite plant with a non-transgenic shoot having a transgenic root system (Tepfer 1984; Haas et al. 1995; Collier et al. 2005; Klink, Kim et al. 2009; Matsye et al. 2012). The transgenic mosaic plants have been grown as described previously (McNeece et al. 2019).

**Figure 4.** CS functions in G. max defense to H. glycines. The calculated FI for the RNAi lines as compared to the controls; *statistically significant, $p < 0.05$ calculated by the Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test. The experimental error representing standard deviation is presented. The results are the average of three independently run biological replicates.

**Figure 5.** Components of the G. max-H. glycines defense regulon. The model presents a number of tested genes functioning in defense in the G. max-H. glycines pathosystem under the described procedures. Defense signals that lead to the propagation of defense include harpin (Aljaafri et al. 2017). Harpin treatment leads to increased transcript levels of a number of genes that have been proven to function in defense. These genes include those signaling both effector triggered immunity (ETI) and pathogen activated molecular pattern (PAMP) triggered immunity (PTI). Harpin increases transcript levels of the coiled-coil nucleotide binding leucine rich repeat (CC-NB-LRR) NON-RACE SPECIFIC DISEASE RESISTANCE 1 (NDR1)/HARPIN INDUCED1 (HIN1) and the cytoplasmic receptor-like kinase BOTRYTIS INDUCED KINASE1 (BIK1). Components of salicylic acid signaling are also increased in their transcript abundance, including the PTI genes ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1), NONEXPRESSOR of PR1 (NPR1), TGA2 and LESION SIMULATING DISEASE1 (LSD1). The induced transcription of several secreted proteins that function in defense, including and xyloglucan endotransglycosylase (XTH) and $\alpha$-hydroxynitrile glycosidase ($\beta$g). The secreted proteins would enter the vesicle transport system experience requisite modifications and become secreted into the apoplast to perform their defense role. S, SNARE, including synaptotagmin and associated proteins; G, conjugated glycoside; ABC-G, ABC-G-type transporter.
Assessment of gene expression by qPCR

*G. max* root mRNA has been isolated according to our published procedures using the UltraClean® Plant RNA Isolation Kit (Mo Bio Laboratories®, Inc.) according to the manufacturer’s instructions (Matsye et al. 2012). Assessment of engineered myosin or CS expression in *G. max* and an examination of proven defense gene expression has been accomplished by qPCR using Taqman® 6-carboxyfluorescein (6-FAM) probes and Black Hole Quencher (BHQ1) (MWG Operon; Birmingham, AL) (Supplemental Table 3) (Sharma et al. 2016). The qPCR control that has been used in the *G. max* experiments has been designed from an expressed sequence tag of ribosomal S21 protein coding gene which functions like other proven control genes (Klink et al. 2005; Sharma et al. 2016). The fold change in gene expression caused by the genetic engineering event has been calculated using $2^{\Delta \Delta \text{CT}}$ according to our prior published methods (Livak and Schmittgen 2001; McNeece et al. 2019). Statistical analyses have been performed according to Yuan et al. (2006). The qPCR experiments have been run using cDNA generated from transgenic experiments run in biological triplicates. Each of those individual qPCR experiments from each experimental replicate has been run with triplicate replicates. The average of the experimental triplicates from the biological triplicates have been used to generate the gene expression data according to prior procedures (McNeece et al. 2019).

Analysis of the effect the genetic constructs have on *H. glycines* parasitism in *G. max*

An analysis of the effect that the myosin or CS genetic constructs have on *H. glycines* parasitism in *G. max* has been calculated and presented as the female index (FI), which is the community-accepted standard representation of the obtained data (Golden et al. 1970). The FI = (Ns/Nx) X 100, where Nx is the average number of females on the test cultivar and Ns is the average number of females on the standard susceptible cultivar (Golden et al. 1970). Using the formula of Golden et al. (1970), Ns is the pRAP17-α control line which has been calculated historically (Golden et al. 1970). The cyst per gram mass of the whole root (wr) and also cysts per gram (pg) of root according to our published methods (McNeece et al. 2019). The whole root analysis is how the data has been presented historically (Golden et al. 1970). The cyst per gram analysis accounts for possible altered root growth caused by the influence of the overexpression or RNAi of the myosin or CS genes. Three biological replicates have been made for each construct with 10–20 individual transgenic plants each. A statistical analysis has been performed using the Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test, p < 0.05 cutoff (Mann and Whitney 1947; McNeece et al. 2019). The MWW Rank-Sum Test, as stated, is a nonparametric test of the null hypothesis not requiring the assumption of normal distributions (Mann and Whitney 1947).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Aist JR. 1976. Papillae and related wound plugs of plant cells. Annu Rev Phytopathol. 14:145–163.

Aljaafri WAR, McNeece BT, Lawaju BR, Sharma K, Niraula PM, Pant SR, Long DH, Lawrence KS, Lawrence GW, Klink VP. 2017. A harpin elicitor induces the expression of a coiled-coil nucleotide binding leucine rich repeat (CC-NB-LRR) defense signaling gene and others functioning during defense to parasitic nematodes. Plant Physiol Biochem. 121:161–175.

Assaad FF, Qiu JL, Youngs H, Ehrhardt D, Zimmerli L, Kalde M, Wanner G, Peck SC, Edwards H, Ramonell K, et al. 2004. The PEN1 syntaxin defines a novel cellular compartment upon fungal attack and is required for the timely assembly of papillae. Mol Biol Cell. 15:5118–5129.

Bekal S, Domier LL, Gonfa B, Lakshnasi N, Meksem K, Lambert KN, Castagnone-Sereno P. 2015. A SNARE-like protein and bixin are implicated in soybean cyst nematode virulence. PLoS One. 10: e0154601. doi:10.1371/journal.pone.0154601

Caillaud MC, Wirthmueller L, Sklenar J, Findlay K, Piquerez SJ, Jones AM, Robatsek S, Jones JD, Faulkner C, Birch P. 2014. The plasmodesmal protein PDLP1 localises to haustoria-associated membranes during downy mildew infection and regulates callose deposition. PLoS Pathog 10:e1004496.

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM. 2009. Glucosinolate metabolites required for an Arabidopsis innate immune response. Science. 323:95–101.

Collier R, Fuchs B, Walter N, Lutke KW, Taylor CG. 2005. Ex vitro composite plants: an inexpensive, rapid method for root biology. Plant J 43:449–457.

Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu JL, Hückelhoven R, Stein M, Freialdenhoven A, Somerville SC, Schulze-Lefert P. 2003. SNARE-protein mediated disease resistance at the plant cell wall. Nature. 425:973–977.
Curtis MD, Grossniklaus U. 2003. A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol 133:462–469.

De Benedictis M, Brunetti C, Brauer EK, Andreucci A, Popescu SC, Comisso M, Guazzo F, Sofo A, Ruffini Castiglione M, Vatamanuik OK, Santità di Toppi L. 2018. The Arabidopsis thaliana knockout Mutant for Phytochelatin Synthase 1 (cad-1-3) is defective in callus deposition, bacterial pathogen defense and auxin content, but shows an increased stem lignification. Front Plant Sci. 9:19. doi:10.3389/fpls.2018.00301.

Ellinger D, Glöckner A, Koch J, Naumann M, Stürtz V, Schütt K, Manisseri C, Somerville SC, Voigt CA. 2014. Interaction of the Arabidopsis GT-Pase RabA4c with its effector PMR4 results in complete penetration resistance to powdery mildew. Plant Cell. 26:3185–3200.

Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrov T, Manisseri C, Somerville SC, Voigt CA. 2013. Elevated early callus deposition results in complete penetration resistance to powdery mildew in Arabidopsis. Plant Physiol 161:1433–1444.

Ellinger D, Sode B, Falter C, Voigt CA. 2014. Resistance of callus synthase activity to free fatty acid inhibition as an indicator of Fusarium head blight resistance in wheat. Plant Signal Behav. 9: e29892.

Ellinger D, Voigt CA. 2014. The use of nanoscale fluorescence microscopic to decipher cell wall modifications during fungal penetration. Front Environ Microbiol. 61:2879–2884.

Hardham AR, Takemoto D, White RG. 2008. Rapid and dynamic subcellular reorganization following mechanical stimulation of Arabidopsis epidermal cells mimics responses to fungal and oomycete attack. BMC Plant Biol 8:63.

Haseloff J, Steimer KR, Prasher DC, Hodge S. 1997. Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic Arabidopsis plants brightly. Proc Natl Acad Sci USA. 94:2122–2127.

Hoügen R, Willmitzer L. 1988. Storage of competent cells for Agrobacterium transformation. Nucleic Acids Res 16:9877.

Humphry M, Bednarek P, Kemmerling B, Koh S, Stein M, Göbel U, Stüber K, Pislew ska-Bednarek M, Loraine A, Schulze-Lefert P, et al. 2010. A regulon conserved in monocot and dicot plants defines a functional module in antifungal plant immunity. Proc Natl Acad Sci USA. 107:21986–21991.

Johansson O, Strömquist E, Falberg P, Nilsson AK, Buholt N, Tör M, Andersson MX. 2014. Role of the penetration-resistance genes PEN1, PEN2 and PEN3 in the hypersensitive response and race-specific resistance in Arabidopsis thaliana. Plant J 79:466–476.

Jones JD, Dangl JL. 2006. The plant immune system. Nature. 444:323–329.

Klink VP, Alkahhar N, MacDonald M, Matthews BF. 2005. Laser capture microdissection (LCM) and analysis of Glycine max (soybean) syncytial cells formed by the soybean cyst nematode Heterodera glycines. Plant Mol Biol. 59:965–979.

Klink VP, Hosseini P, Matsu SE, Alkahhar NW, Matthews BF. 2009. A gene expression analysis of syncytia laser microdissected from the roots of the Glycine max (soybean) genotype PI 548402 (Peking) undergoing a resistant reaction after infection by Heterodera glycines (soybean cyst nematode). Plant Mol Biol. 71:525–567.

Klink VP, Hosseini P, Matsu SE, Alkahhar NW, Matthews BF. 2010. Syncytium gene expression in Glycine max (soybean) root undergoing a resistant reaction to the parasitic nematode Heterodera glycines. Plant Physiol Biochem. 48:176–193.

Klink VP, Hosseini P, Matsu SE, Alkahhar NW, Matthews BF. 2011. Differences in gene expression amplitude overlies a conserved transcriptomic program occurring between the rapid and potent localized resistant reaction at the syncytium of the Glycine max genotype Peking (PI 548402) as compared to the prolonged and potent resistant reaction of PI 86788. Plant Mol Biol. 75:141–165.

Klink VP, Kim K-H, Martins VE, MacDonald MH, Beard HS, Alkahhar NW, Lee S-K, Park S-C, Matthews BF. 2009. A correlation between host-mediated expression of parasite genes as tandem inverted repeats and abrogation of the formation of female Heterodera glycines cysts during infection of Glycine max. Planta. 230:53–71.

Klink VP, McNeece BT, Pant SR, Sharma K, Nirula PM, Lawrence GW. 2017. Components of the SNARE-containing region are co-regulated in root cells undergoing defense. Plant Signal Behav. 12:1274881.

Klink VP, Overall CC, Alkahhar NW, MacDonald MH, Matthews BF. 2007. Laser capture microdissection (LCM) and comparative microarray expression analysis of syncytial cells isolated from incompatible and compatible soybean roots infected by soybean cyst nematode (Heterodera glycines). Planta. 226:1389–1409.

Klink VP, Overall CC, Alkahhar NW, MacDonald MH, Matthews BF. 2010. Microarray detection calls as a means to compare transcripts expressed within compatible soybean cyst nematodes isolated from incompatible and compatible soybean (Glycine max) roots infected by the soybean cyst nematode (Heterodera glycines). J Biomed Biotechnol. 1–30.

Kurth EG, Peremyslov VV, Turner HL, Makorova KS, Ianzo J, Mechkedov SL, Koonin EV, Dolja VV. 2017. Myosin-driven transport network in plants. Proc Natl Acad Sci USA. 114:E1385–E1394.

Kwon C, Neu C, Pajonk S, Yun HS, Lipka U, Humphry M, Bau S, Straus M, Kwaaitaal M, Rempelt A, et al. 2008. Co-option of a default secretory pathway for plant immune responses. Nature. 451:835–840.

Leslie ME, Rogers SW, Heese A. 2016. Increased callose deposition in plants lacking DYNAMIN-RELATED PROTEIN 2B is dependent upon POWDERY MILDEW RESISTANT 4. Plant Signal Behav. 11: e1244594.

Lipka L, Dittgen J, Bednarek P, Bhat R, Wiermer M, Stein M, Landtag J, Brandt W, Rosahl S, Scheel D, et al. 2005. Pre- and postinfection defenses both contribute to nonhost resistance in Arabidopsis. Science. 310:1180–1183.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 25:402–408.

Mann HB, Whitney DR. 1947. On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat. 18: 50–60.

Matsye PD, Kumar R, Hosseini P, Jones CM, Tremblay A, Alkahhar NW, Matthews BF, Klink VP. 2011. Mapping cell fate decisions that occur during soybean defense responses. Plant Mol Bio. 77:513–528.

Matsye PD, Lawrence GW, Youssef RM, Kims K-H, Matthews BF, Lawrence KS, Klink VP. 2012. The expression of a naturally occurring, truncated allele of α- (SNAP) gene suppresses plant parasitic nematode infection. Plant Mol Bio. 80:131–155.

McNeece BT, Pant SR, Sharma K, Nirula PM, Lawrence GW, Klink VP. 2017. A Glycine max homolog of NON-RACE SPECIFIC DISEASE RESISTANCE 1 (NDR1) alters defense gene expression while functioning during a resistance response to different root pathogens in different genetic backgrounds. Plant Physiol Biochem. 114:60–71.

McNeece BT, Sharma K, Lawrence GW, Lawrence KS, Klink VP. 2018. The mitogen activated protein kinase (MAPK) gene family functions as a cohort during the Glycine max defense response to Heterodera glycines. Plant Physiol Biochem. 137:25–41.

Meyer D, Pajonk S, Micali C, O’Connell R, Schulze-Lefert P. 2009. Extracellular transport and integration of plant secretory proteins into pathogen-induced cell wall compartments. Plant J. 57:986–999.

Nielsen ME, Thordal-Christensen H. 2013. Transcription shuts the door for an unwanted guest. Trends Plant Sci. 18:611–616.

Ostergaard I, Petersen M, Mattsson O, Mundy J. 2008. Arabidopsis collese synthase. Plant Mol Bio. 49:559–566.

Pant SR, Matsye PD, McNeece BT, Sharma K, Krishnavahajala A, Lawrence GW, Klink VP. 2014. Syntaxin 31 functions in Glycine max resistance to the parasitic plant nematode Heterodera glycines. Plant Mol Bio. 85:107–121.

Peremyslov VV, Cole RA, Fowler JE, Dolja VV. 2015. Myosin-powered membrane compartment drives cytoplasmic streaming, cell expansion and plant development. PLoS One 10:e0139331.

Sassmann S, Rodrigues C, Milne SW, Nenninger A, Allwood E, Littlejohn GR, Talbot NJ, Soeller C, Davies B, Hussey PJ, Deeks MJ. 2018. An immune-responsive cytoskeletal-plasma membrane feedback loop in plants. Curr Biol. 28:2136–2144.e7.

Sharma K, Pant SR, McNeece BT, Nirula PM, Buson HE, Lawrence GW, Klink VP. 2016. Co-regulation of the Glycine max soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor
(SNARE)-containing regulon occurs during defense to a root pathogen. J Plant Interact. 11:74–93.
Stein M, Dittgen J, Sánchez-Rodríguez C, Hou B-H, Molina A, Schulze-Lefert P, Lipka V, Somerville S. 2006. Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell. 18:731–746.
Tepfer D. 1984. Transformation of several species of higher plants by Agrobacterium rhizogenes: sexual transmission of the transformed genotype and phenotype. Cell. 37:959–967.

Wang P, Sun Y, Pei Y, Li X, Zhang X, Li F, Hou Y. 2018. GhSNAP33, a t-SNARE protein from Gossypium hirsutum, mediates resistance to Verticillium dahliae infection and tolerance to drought stress. Front Plant Sci. 9:896.
Yang L, Qin L, Liu G, Peremyslov VV, Dolja VV, Wei Y. 2014. Myosins XI modulate host cellular responses and penetration resistance to fungal pathogens. Proc Natl Acad Sci USA. 111: 13996–14001.
Yuan JS, Reed A, Chen F, Stewart CN. 2006. Statistical analysis of real-time PCR data. BMC Bioinformatics. 7:85.