Research Article

Microarray Detection Call Methodology as a Means to Identify and Compare Transcripts Expressed within Syncytial Cells from Soybean (Glycine max) Roots Undergoing Resistant and Susceptible Reactions to the Soybean Cyst Nematode (Heterodera glycines)

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Received 23 April 2009; Revised 23 September 2009; Accepted 14 February 2010

Academic Editor: Tanya Parish

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Background. A comparative microarray investigation was done using detection call methodology (DCM) and differential expression analyses. The goal was to identify genes found in specific cell populations that were eliminated by differential expression analysis due to the nature of differential expression methods. Laser capture microdissection (LCM) was used to isolate nearly homogeneous populations of plant root cells. Results. The analyses identified the presence of 13,291 transcripts between the 4 different sample types. The transcripts filtered down into a total of 6,267 that were detected as being present in one or more sample types. A comparative analysis of DCM and differential expression methods showed a group of genes that were not differentially expressed, but were expressed at detectable amounts within specific cell types. Conclusion. The DCM has identified patterns of gene expression not shown by differential expression analyses. DCM has identified genes that are possibly cell-type specific and/or involved in important aspects of plant nematode interactions during the resistance response, revealing the uniqueness of a particular cell population at a particular point during its differentiation process.

1. Introduction

Microarray analyses are a way to study the expression of thousands of genes simultaneously. Microarray analyses are important because they can provide information on genes that are expressed differentially between a control and an experimental sample [1]. However, part of the problem of differential expression methodology is that genes must be expressed in both sample types, the experimental and control samples, for statistical analyses to be possible. Without gene expression data available for a probe set in each sample, the probe set will be discarded by the analysis procedure. The drawback of the differential expression methodology, therefore, is obvious when specific cell types with vastly different identities are being compared [2].

Microarray analyses, however, do provide useful information on the transcripts that are present or absent within samples [3]. Detection call methodology (DCM) reveals the types of transcripts that are present or absent within samples. The DCM is typically used on a single array to
answer whether a transcript of a particular gene is present or absent in a sample. Several recent papers have used DCM successfully to understand transcription in various experimental systems [4–6]. The DCM is useful when cost is an issue because the method can be performed on a single array. More importantly, DCM can be used to compare transcripts between different cell types or of the same cell type at different points during a time course [2, 3, 7]. None of these examples compared detection calls during a pathological infection. Nonetheless, DCM can provide extremely useful information about the samples under investigation, especially in the analysis of plant pathological systems where a pathogen interacts intimately with a specific cell population within a complex tissue or organ (e.g., root).

The infection of plants by parasitic nematodes is a major agricultural problem that is currently poorly understood (reviewed in [8–12]). Infection results in damage totaling 157 billion U.S. dollars, annually [13]. Among the most costly is Heterodera glycines infection of Glycine max, accounting for an estimated $460 to $818 million in production losses annually in the U.S. [14]. The G. max-H. glycines system is a powerful research tool because both resistant and susceptible reactions can be studied in the same genotype (e.g., G. max[Peking]). Information learned through its genetic and genomic studies can be translated directly to improve resistance in one of the most important global agriculturally relevant plants.

The genetic basis of G. max to overcome H. glycines infection (an incompatible reaction resulting in resistance) is complex (reviewed in [15]). Several recessive resistance loci (rhg1, rhg2, and rhg3) [16] and dominant resistance loci (Rhg4) [17] and (Rhg5) [18] have been identified (reviewed in [15]). The understanding of resistance to H. glycines has also been aided by other genetic marker technology (e.g., quantitative trait loci (QTL) mapping). Those studies have identified QTLs that map to 17 linkage groups. G. max[Peking] has nine QTLs that map to different linkage groups (reviewed in [15]). One of those QTLs present in G. max[Peking] that maps to linkage group G explains more than 50% of resistance to H. glycines [19]. It also is responsible for resistance to several different populations of H. glycines. The major QTL that is located on linkage group G was identified using the RFLP marker C006V and is designated as rhg1 [20]. Further studies have shown that molecular marker Satt309 is only 0.4 centimorgans from rhg1 [21]. Importantly, much of the resistance that has been bred into elite G. max varieties originates from the G. max[Peking] genotype.

Genomic approaches have also identified transcriptional changes in whole roots during infection [22–26]. Importantly, a time course microarray analysis was used to investigate the G. max-H. glycines interaction [23]. The analysis demonstrated that differential expression of genes was occurring in G. max roots undergoing a compatible reaction, a reaction that results in susceptibility. The analysis used time points both prior to and after feeding site selection [23]. Importantly, the differential expression of genes was occurring in G. max roots even before the nematodes had selected their feeding sites [23]. Thus, the plant is reacting in important ways to the presence of the nematode before the nematodes have begun to initiate the formation of their feeding sites during a compatible reaction.

The G. max-H. glycines interaction is an exceptional model because it is possible to compare gene expression occurring during incompatible (resistant) and compatible reactions. The experiments are possible because even resistant genotypes like G. max[Peking] undergo infection [12, 25–33]. The comparisons can be made because well-defined incompatible and compatible H. glycines races (populations) are available [12, 25, 26, 31–35]. A time course microarray analysis has examined H. glycines infection during both an incompatible and a compatible reaction in whole roots at time points both prior to and after nematodes have established feeding sites [25]. Importantly, those microarray analyses were performed in the same G. max genotype (e.g. G. max[Peking]) by using incompatible and compatible populations of H. glycines [25, 26]. Thus, no possibility existed for G. max genotype differences complicating the identification of important gene expression events during those reactions. The analyses have shown that G. max behaves differently as it undergoes the incompatible or compatible reaction and these differences in gene expression are detectable as early as 12 hours post infection (hpi) [25]. The 12 hpi time point is a point before the nematode has selected its feeding site. The analyses also showed how expression of G. max[Peking] genes differs over time between roots undergoing an incompatible or compatible reactions.

The aforementioned investigations were not designed to study gene expression of the syncytium. However, several labs have made histological studies of the infection process. The studies showed that H. glycines infest the roots and migrate through the cortex during the early stages of the infestation process. After 24 hpi the nematodes reach the stele where they select and establish their feeding sites [27–30, 36]. Consequently, the feeding site initial (FSi), a cell that is usually a pericycle cell, fuses with neighboring cells. The process occurs when the cell walls dissolve and the cytoplasm of adjacent cells (e.g., cortex) merges with the feeding site initial. Cell fusion, thus, results in the formation of a syncytium. Syncytial cells continue to develop in compatible roots into sites from which H. glycines feed (Figure 1) [27–30]. Conversely, syncytial cells of incompatible roots collapse four to five days post infection (dpi) and the nematodes die [27, 28, 30].

Understanding the localized resistance reaction at the site of infection may also lead to better measures to control H. glycines parasitism. The problem, however, has been in isolating these cells to some amount of homogeneity for expression analysis. Hand dissections have been performed to obtain giant cells from galls induced by the root knot nematode (Meloidogyne incognita) during a compatible interaction in tomato (Lycopersicon esculentum) [37]. The experiments permitted the isolation of cDNA from those cells [37]. However, relatively few of them turned out to be gall specific [38]. The experiments, nonetheless, demonstrated the efficacy of the approach in isolating RNA from those cell types. Unfortunately, it is not possible to use this method to study syncytium formation during H. glycines infection.
Figure 1: Life cycle of *H. glycines*. Cysts, encasing the eggs, are able to remain dormant in the soil for years. At some point, the eggs hatch. The second-stage juveniles (J2s) migrate toward the root and burrow into it. The infective J2s (i-J2s) then migrate toward the root stele. A stylet emerges from the anterior end of the nematode. The nematode selects a pericycle cell or neighboring root cell, for its feeding site. The i-J2 then presumably releases substances that then cause major changes in the physiology of the root cell. Those root cells (yellow) subsequently fuse with neighboring cells (light blue), producing a common cytoplasm. The repeated cell fusion events produces a syncytium (orange) that contains approximately 200 merged root cells and serves as the *H. glycines* feeding site. After the establishment of the syncytium, male nematodes feed for several days. Feeding proceeds until the end of their J3 stage. Meanwhile, the males become sedentary. Subsequently, the males stop feeding, followed by a molt into vermiform J4 males. The males burrow out of the root in preparation for copulation. In contrast to the males, the females become and remain sedentary after the establishment of their feeding site. The female nematodes then increase in size while undergoing both J3 and J4 molts. The J4s then mature, becoming adult feeding females. Ultimately, the female develops into the cyst that encases the eggs. (a) Cysts (dark red) with eggs (white) hatch. (b) Second-stage juveniles (J2) (gray) hatch and migrate toward the root. (c) The J2 nematodes burrow into the root and migrate toward the root stele (dark gray). (d) Feeding site selection (yellow). (e) i-J2 nematodes molt into J3 and then J4. The female is shown here in red. During this time, the original feeding site (yellow) is incorporating adjacent cells (magenta) via cell wall degradation and fusion events. Meanwhile, the male discontinues feeding at the end of its J3 stage. (f) The male and female J4 nematodes mature into adults. By this time, the feeding site has matured into a syncytium (green) as shown here where the female is actively feeding. The vermiform male (blue) migrates out of the root and subsequently copulates with the female (red). (g) After ~30 days, the female is clearly visible externally because its body emerges from the root tissue. The figure is adapted from Klink et al. [11].

Laser capture microdissection (LCM) is an alternative means that affords a high degree of precision and accuracy to isolate homogeneous cell populations that are otherwise recalcitrant to their isolation [39–42]. The method has proven to be especially valuable to study the development of the syncytium during *G. max* infection by *H. glycines* during a compatible and incompatible reaction [26, 33, 43, 44] because *H. glycines* can be used as an in situ physical marker for the syncytium. Microarray analysis studying gene expression of the syncytium has allowed for the identification of genes that exhibit differential expression in these cell types [26, 33]. However, it was unclear whether the true diversity of gene expression was being revealed by the differential expression methodology.

In the analysis presented here, DCM was used to compare detection calls made between the different cell types involved in the formation of the syncytium using samples isolated by LCM. The DCM was used to compare how the cell types under investigation (e.g., the syncytium) differed from the cell type(s) from which they originated (e.g., pericycle). Using a comparative analysis aided by customized computer scripts, a broader understanding was obtained of the differences between (1) syncytia and pericycle cells, (2) syncytia undergoing incompatible and compatible reactions, and (3) syncytia at different points of their development during a compatible reaction as they mature into a functional feeding site.

2. Materials and Methods

2.1. Female Index. The *H. glycines* [NL1-RHg] population used in the analyses presented here has been used extensively as race 3 for analyses requiring susceptible reactions in *G. max* genotype Kent (G. max[Kent]) [22, 23, 31–33, 43, 45] and resistant reactions in *G. max* [PI 548402/Peking] [25, 26, 31, 33, 46]. For a description of the 16 nematode races and the HG-type test, please refer to Niblack et al. [35]. The HG-type test is derived from the original Index of
The determination of the HG-type test [47]. The determination of the HG-type test is based on the performance of the nematode race to infect indicator lines. The indicator lines are G. max genotypes (including G. max[PI 548402/Peking]) having varying ability to resist infection by the 16 known races of H. glycines. Based on the accepted variation of infection by H. glycines on the different indicator lines, an HG-type is given to an unknown sample. The numerous G. max genotypes are named by an accepted plant introduction (PI) classification scheme. The indicator lines now used in the HG-type test are G. max[PI 88788], G. max[PI 548402/Peking], G. max[PI 90763], G. max[Pickett], G. max[PI 457645] (G. max[Hartwig]), G. max[PI 89772], G. max[PI 548316] (G. max[Cloud]), G. max[PI 209332], and G. max[PI 438489B]. Of note, G. max[PI 438489B] was added to the HG-type test to allow a more accurate test because it was classified as being resistant to five H. glycines races (1, 2, 3, 5, and 14) in the greenhouse [48]. The HG-type test is based off of the presence of an expected number of females, given as a proportion, which will develop on each indicator line. The number is called the female index (FI). The FI is the number of mature females that develop on a test genotype divided by the number of females that develop on a known susceptible genotype (i.e., G. max[Lee] and/or G. max[Essex]) multiplied by 100. According to the original Index of Parasitism [47] any genotype with a female number less than 10% of the number determined on G. max[Lee] would be considered resistant (−) and any number above 10% would be susceptible (+). The HG-type test as determined by Niblack et al. [35] has changed the Index of Parasitism reaction [50]. The determination of the HG-type test [47] and improved race test [34] to include several other categories. Now, the FI categories for the HG-type test have been selected for a single-cyst descent on G. max[PI 90763] [50]. Originally, H. glycines[TN8/HG-type 1.3.6.7] was maintained on the G. max[PI 90763] genotype according to Niblack et al. [50]. The HG-type test allowed a more accurate test because it was based on the determination of infection by the HG-type test [47] and improved race test [34] to include several other categories. Now, the FI categories for the HG-type test are Highly Resistant, FI: 0%–9%; Resistant, FI: 10%–24%; Moderately Resistant, FI: 25%–39%; Low Resistance, FI: 40%–59%; and No Effective Resistance, FI: >60%. The HG-type test for H. glycines[NL1-RHg] was determined independently in the lab of Dr. Terry Niblack (Department of Crop Sciences, University of Illinois) [11, 25] during June-July, 2007 using the published methods of Niblack et al. [35]. The performance of H. glycines[NL1-RHg] on those indicator lines was compared to the susceptible genotypes G. max[Lee] and G. max[Essex]. An FI of 0 (0%) was found for H. glycines[NL1-RHg] on G. max[PI 548402/Peking]. The HG-type test determined that G. max[PI 548402/Peking] is considered highly resistant to H. glycines[NL1-RHg]. Based on the infectivity of H. glycines[NL1-RHg] on the indicator lines, the HG-type test also determined that H. glycines[NL1-RHg] is race 3, as previously published [22, 23, 31, 43, 45, 49]. H. glycines[NL1-RHg] (incompatible) is HG-type 7 (H. glycines[NL1-RHg/HG-type 7]). The reaction of H. glycines[TN8] (HG-type 1.3.6.7 [race 14]) (H. glycines[TN8/HG-type 1.3.6.7]) (compatible) on G. max[PI 548402/Peking] is a susceptible reaction [50].

2.1.1. Plant Nematode Procurement. The methods have been published previously [26, 33]. Briefly, plant and nematode materials were grown at the United States Department of Agriculture, Soybean Genomics and Improvement Laboratory (SGIL). A single G. max genotype (G. max[PI 548402/Peking]) was used in the experiments to obtain both incompatible and compatible reactions by the use of two different populations of H. glycines, H. glycines[NL1-RHg/HG-type 7] and H. glycines[TN8/HG-type 1.3.6.7]. The H. glycines populations were maintained in the greenhouse using the moisture replacement system (MRS) [51]. The origin of H. glycines[TN8/HG-type 1.3.6.7] was by selection of a single-cyst descent on G. max[PI 90763] [50]. Originally, H. glycines[TN8/HG-type 1.3.6.7] was maintained on the G. max[PI 90763] genotype according to Niblack et al. [50]. The HG-type test allowed a more accurate test because it was based on the determination of infection by the HG-type test [47] and improved race test [34] to include several other categories. H. glycines[TN8/HG-type 1.3.6.7] and H. glycines[NL1-RHg/HG-type 7] have always been exposed to the same G. max genotypes. The method virtually eliminated variations among the different G. max genotypes in influencing the experiments.

Seedlings were grown in sterilized sand in 20 × 20 × 10 cm flats for a period of one week. The plants were gently removed from the sand and rinsed with sterile water. Seedlings were placed on moistened germination paper (Anchor Paper; St. Paul, MN) inside the flats. Mature female nematodes were harvested by massaging the roots in water. Mature nematodes were collected by filtering the solution through nested 850 and 150 μm sieves. Females were further purified by sucrose flotation [45]. The females were crushed gently with a rubber stopper within a 7.5 cm diameter apparatus containing 250 μm sieves. The process released the eggs. The eggs passed through the sieve into a small plastic tray. Debris smaller than the eggs was removed. Debris removal was done by washing the debris in a 25 μm mesh sieve. The eggs were placed in a small plastic tray containing 1 cm of water. The tray was covered with plastic wrap and subsequently placed on a rotary shaker at 25 rpm. After 3 days, the second-stage juvenile nematodes (J2s) were separated from the unhatched eggs. Separation was done by passing them through a 41 μm mesh cloth. The J2s were concentrated by centrifugation in an IEC clinical centrifuge for 30 seconds at 1720 rpm to 5,000 J2/mL. The nematodes were used to infest the roots. There were 2 mL of nematode-containing solution added directly on the roots for a final concentration of 2,000 J2/root. The control mock-infested replicates received the same amount of water. The roots were covered with a moistened sheet of germination paper. The plants were placed in a 45 × 50 × 20 cm plastic tray.
with a one cm of water in the bottom to add humidity. A
semitransparent bag was then wrapped around the tray. The
trays were then placed under fluorescent lights of 16/8 hour
light/dark photoperiod. Light intensities were identical for
all experiments. Infested roots were grown for three or 8 dpi.
The mock-infested control samples and susceptible and
resistant reactions were washed. The process removed the
extraneous nematodes that had not yet penetrated the root,
preventing additional nematodes from entering the root.
The process ensured that tissue that was the most highly
infested with nematodes was obtained. The process was then
repeated, providing two independent sets of samples. Seven
independent replicates were pooled to obtain each replicate
for each sample type in the analysis. Thus, there are a total of
14 replicates used in the analysis. At least 50 serially sectioned
infested with nematodes was obtained. The process was then
repeated after the drying step was done. LCM was performed
on a Leica ASLMD microscope (Leica). Microdissection
cutting parameters were determined empirically for each
session by examining how amenable the tissue was to LCM.
However, cutting parameters for dissections performed on
the 40× objective were approximately power, 55–85; speed,
2–4; specimen balance, 1–3; and offset, 40. Similar quantities
of cells were obtained for each sample type for the analyses.

Tissue was collected in OptiCaps (Leica Cat. number
11505169) and subsequently washed to the bottom of the
OptiCap PCR tube. The process was done by micropipetting
20 μL of XB buffer (Arcturus) onto the microdissected tissue.
The cap was spun for five minutes at 500 rpm to pellet the
tissue into the bottom of the Opticap. LCM tissue was ground
with a micropestle. The process was done in 40 μL RNA
extraction buffer (Arcturus). The RNA was extracted and
subsequently processed using the PicoPure RNA Isolation Kit
(Arcturus). The process was done according to the manu-
facturer’s instructions. A DNase treatment was added, just
before the second column wash, using DNAfree (Ambion;
Austin, TX). RNA quality and yield were determined.
The processing was done using the RNA 6000 Pico Assay
(Agilent Technologies; Palo Alto, CA) using the Agilent 2100
Bioanalyzer according to the manufacturer’s instructions.
RNA amplification of LCM samples was performed with the
GeneChip Two-Cycle cDNA Synthesis Kit (Affymetrix;
Santa Clara, CA: Cat. number 900432). Probe preparation
and hybridizations were performed according to Affymetrix
guidelines at the Laboratory of Molecular Technology, SAIC-
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2.3. Microarray Analyses and G. max Probe Set Annotations.
The GeneChip Soybean Genome Array (Affymetrix; Cat.
number 900526) containing 37,744 G. max transcripts
(35,611 transcripts) was used for the microarray analyses.
Details of the GeneChip soybean genome array can be obtained
(http://www.affymetrix.com/index.affx). Annotations
were made by comparison to the Arabidopsis thaliana
gene ontology (GO) database [54] based on their best
match obtained by BLAST searches [55]. They were updated
(2009).

2.2. LCM. Slides were prepared according to Klink et al.
[26]. MembraneSlides (Leica, Germany; Cat# 11505158)
were placed on a slide warmer set at 40°C. DEPC-treated
RNase-free water (∼0.5–1 mL) was placed onto the slide and
allowed to warm. The tissues used for these analyses were
obtained from the same tissue used in whole-root microarray
experiments [26]. Serial sections (10 μm) from control
mock-inoculated roots and roots undergoing incompatible
(3 dpi) and compatible (3 and 8 dpi) reactions were prepared
according to Klink et al. [26, 33, 43]. Serial sections for
the independent sample types were placed directly onto the
pool of DEPC-treated water. DEPC-treated water was blotted
off with a sterile KimWipe after the serial sections were
adequately spread. Tissue was allowed to warm on the slide
warmer for an additional hour to promote tissue binding to
the slide surface. Slides were deparaffinized for five minutes
in xylene. The processing was followed by a two-minute
incubation in 1:1 (v/v) xylene: ETOH. That was followed
subsequently with two one-minute incubations in ETOH.
Slides were then dried on the lab bench on filter paper
covered with KimWipes. The slides were used immediately
for LCM after the drying step was done. LCM was performed
on a Leica ASLMD microscope (Leica). Microdissection
cutting parameters were determined empirically for each
session by examining how amenable the tissue was to LCM.
However, cutting parameters for dissections performed on
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Figure 2: *G. max*[PI 548402/Peking] seedlings were inoculated with incompatible or compatible *H. glycines* J2 nematodes. Roots were harvested and prepared for histological observation to confirm the establishment of feeding sites at three and 8 dpi. (a) 3 dpi *G. max*[PI 548402/Peking] infected with a compatible nematode, black arrowhead; area encircled in red, syncytial cell. (b) 8 dpi *G. max*[PI 548402/Peking] infected with a compatible nematode, black arrowhead; area encircled in red, syncytial cell. (c) 3 dpi *G. max*[PI 548402/Peking] infected with an incompatible nematode, black arrowhead; area encircled in red, syncytial cell. (d) 8 dpi *G. max*[PI 548402/Peking] infected with an incompatible nematode, black arrowhead; area encircled in red, syncytial cell. Bar = 50 μm.

All microarray hybridizations were performed at the Laboratory of Molecular Technology, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, MD 21701, USA. Local normalization was used. The presence or absence of a particular probe set’s (gene’s) transcript on a single array was determined using the Bioconductor implementation of the standard Affymetrix DCM. In summary, the DCM consists of four steps: (1) removal of saturated probes, (2) calculation of discrimination scores, (3) *P*-value calculation using the Wilcoxon’s rank test, and (4) making the detection (present/marginal/absent). Ultimately, the algorithm determines if the presence of a probe set’s transcript is provably different from zero (present (P)), uncertain (marginal (M)), or not provably different from zero (absent (A)). Details of the standard Affymetrix DCM can be found in their Statistical Algorithms Description Document (http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf.). For a particular condition (e.g., 3 dpi syncytia during the incompatible response), a probe set was considered present only if it was present on both replicate microarrays corresponding to that condition. Otherwise, it was considered to be absent. All original data sets, the normalized data sets, statistics, and data supplemental to each table and figure are available at the MAIME compliant [http://bioinformatics.towson.edu/SGMD3] [56].

3. Results

3.1. Histological Analysis of Incompatible and Compatible Responses in the Whole Root. Morphological and anatomical details of compatible and incompatible disease responses by *G. max* to *H. glycines* infection have been published previously [27–29, 36, 57–61]. Infection during the first 8 dpi (Figure 2) was focused on for this analysis because syncytial cells complete the incompatible reaction by 8 dpi under the experimental conditions in *G. max*[PI 548402/Peking]. During a compatible reaction, nematodes have selected and are establishing feeding sites by 3 dpi (Figure 2(a)) that are continuing to develop by 8 dpi (Figure 2(b)). During an incompatible reaction, nematodes have also selected and are establishing feeding sites at 3 dpi (Figure 2(c)). However, during an incompatible reaction, syncytial cells collapse by 8 dpi (Figure 2(d)). Syncytial cells (Figure 3(a)) for the various analyses were collected by LCM (Figure 3(b)).

3.2. The Use of Detection Calls to Identify Genes Present in Syncytium Samples. The DCM was used to make a comparative analysis of the probe sets measuring the presence of a transcript (present transcript) within LCM-derived cell samples. The analyses would allow (1) the determination of the total number of present transcripts, (2) the determination of the
numbers of present transcripts within a sample, and (3) a comparison of the present transcripts between the different sample types while estimating the differences between those samples (4) the identification of whether transcripts that are common between the two sample types under comparison had been identified in a prior differential expression analysis [26]. Only probe sets that measured detection on both arrays for a particular sample type (Figure 4) were evaluated further (see below).

While detection calls are generally used for single array analyses, the DCM presented here used two arrays for each sample type in a comparison. Thus, for a particular comparison between cell types, four arrays were taken into consideration. Detection calls were analyzed for each of the two arrays for each sample type (e.g., pericycle). Detection calls were made for each of the two arrays independently to determine if the probe sets were consistently measuring present or absent for a particular sample type. For example, the probe set had to obtain a like measurement (e.g., present/present; absent/absent) for each of the two arrays for each sample type to be considered for subsequent analyses (Figure 4). The arrays that measured present on both arrays within a sample type are considered present. The arrays that measured absent on both arrays within a sample type are considered absent (Figure 4). The probe sets that failed to produce like measurements (e.g., any combination of present/absent; present/marginal for the two arrays) and those that measured marginal amounts of a
of a transcript on the four arrays under comparison were considered common and present between two sample types (e.g., pericycle and 3 dpi incompatible syncytia-common). The probe sets measuring absent on the four arrays (common and absent), although potentially interesting, were not taken into consideration in this analysis. The samples whose probe sets measured present for both arrays but only in one of the two sample types would be considered present and unique for a particular sample type (e.g., pericycle-unique or 3 dpi incompatible syncytia-unique) (Figure 5). Probe sets that measure detectable amounts of gene activity in both sample types can either be differentially expressed or not differentially expressed (Figure 6). The differential expression calls used in some of the comparative analyses had been presented previously [26]. The DCM analysis presented here is employed as a different way of examining the data with the goal of identifying genes at low thresholds of expression that are missed in differential expression analyses. More importantly, DCM is also a way of identifying genes that may be expressed at high thresholds in one sample type and are undetectable in a second sample type used for comparative purposes in a differential expression analysis. In cases like these, statistical analyses cannot be done because no expression data is available for the second sample type and thus the probe set is excluded from the differential expression analysis. Therefore, probe sets that measured detectable amounts of a transcript uniquely in one sample type (e.g., unique-present) (Figure 5) cannot measure differential expression (Figure 6). An example of genes identified in a comparative analysis of two hypothetical gene pools (Figure 7) illustrates the different gene categories investigated in the analysis (Figure 8). As illustrated, all genes that are identified as differentially expressed had to be present in each gene pool (Figure 7). It became clear from the analysis that many genes that were unique to a specific sample type (e.g., A or B) were being excluded from the differential expression analysis because the probe sets measured detectable levels of gene activity only in one of the two sample types (Figure 7). The probe sets that match this criterion, A or B and not A U B, became the focus of the analysis (Figure 8).

3.3. The DCM Identifies Many Genes Expressed in the Experimental Cell Types. The DCM identified a total of 13,291 transcripts as being present between the pericycle, 3 dpi incompatible syncytium, 3 dpi compatible syncytium, and the 8 dpi compatible syncytium samples. Direct comparisons were made between each of the sample types. The analyses focused on two types of transcripts that were determined to be present. The transcript types are (1) unique and (2) common. Unique transcripts were defined as those that were present and found in only one of the two sample types being compared. Common transcripts were defined as those that were present and overlap between the two sample types being compared. Data from five of the comparisons (Figures 8(a), 8(c), 8(e), 8(g), 8(i)) are presented as Venn diagrams. The annotated probe sets were divided into seven subcategories (histograms) per functional category (Figures 8(b), 8(d), 8(f), 8(h), 8(j) (A–R—see figure legends)), based on the particular comparison being made (see below). The comparison in Figure 8(g), presented as a Venn diagram, was divided into eight subcategories (histograms) per functional category (see below).

3.4. 3 dpi Syncytia Undergoing an Incompatible Reaction. The DCM was used to compare present transcripts (genes) within the 3 dpi microdissected syncytia undergoing an incompatible reaction to the pericycle sample (Figures 8(a) and 8(b)). A total of 3,908 genes were present in these two samples. The DCM identified 1,966 genes that were present and unique to the pericycle sample (Figures 8(a) (see Table 1 in Supplementary Material available online at 10.1155/2010/491217)). Further analysis identified 1,002 genes that were present
Figure 8: Continued.
Selected gene lists comprising the (1) Disease and Defense, (2) Signaling, and (3) Transcription categories are provided (Table 1 (supplementary Table 3)).

3.5. 3 dpi Syncytia Undergoing a Compatible Reaction. The DCM was used to compare genes within the 3 dpi microdissected syncytia undergoing a compatible reaction to the pericycle sample (Figures 8(c) and 8(d)). A total of 4,925 genes were present in these two samples. From these analyses, 711 genes were identified that were present and unique to the pericycle sample (Figure 8(c) (supplementary Table 4)). Further analysis identified 2,257 genes that were present and common between the pericycle and 3 dpi compatible syncytium sample (Figure 8(c) (supplementary Table 5)). Only these genes could be used for differential expression analyses because expression data was available for each

and common between the pericycle and 3 dpi incompatible syncytium sample (Figure 8(a) (supplementary Table 2)). Only the genes that are present and common can be used for differential expression analyses because expression data was available for each sample type. An analysis identified 940 genes present and unique to the 3 dpi incompatible syncytium sample (Figure 8(c) (supplementary Table 5)). Therefore, in the analysis presented here, a total of 1,942 genes were present in these two samples. From these analyses, 711 genes were identified that were present and unique to the pericycle sample (Figure 8(c) (supplementary Table 4)). Further analysis identified 2,257 genes that were present and common between the pericycle and 3 dpi compatible syncytium sample (Figure 8(c) (supplementary Table 5)). Only these genes could be used for differential expression analyses because expression data was available for each
Table 1: Select genes that were unique to the 3 dpi syncytia undergoing an incompatible reaction but that were not differentially expressed as compared to a pericycle control sample (Figure 8(b)) comprising the Disease and Defense, Signaling, and Transcription categories.

| I-3 dpi | Probe set ID | Public ID | Avg P-value | Gene |
|---------|--------------|-----------|-------------|------|
| Disease & defense | Gma.4886.2.S1.at | AW234624 | 0.005201937 | haem peroxidase |
| | GmaAffx.69994.1.S1.at | CD417025 | 0.017952293 | phosphate-responsive protein (phi-1) |
| | Gma.8449.1.S1.s.at | AF002258.1 | 0.019563038 | CoA ligase 4 |
| | GmaAffx.14986.1.S1.at | BE657889 | 0.020669698 | phosphate-responsive protein (phi-1) |
| | GmaAffx.93611.1.S1.s.at | CF809336 | 0.034312943 | disease resistance response protein (DRRG49-C) |
| Signal transduction | Gma.1965.1.S1.x.at | L01432.1 | 0.005201937 | calmodulin (SCaM-3) |
| | GmaAffx.50980.1.S1.x.at | BE823095 | 0.006003594 | protein phosphatase 2C (PP2C) |
| | Gma.11041.1.S1.at | BI970555 | 0.006660588 | Pti1-like kinase-like |
| | GmaAffx.33721.1.S1.at | BI967195 | 0.006660588 | protein kinase |
| | Gma.6290.1.S1.at | AW311265 | 0.007290178 | BOTRYTIS-INDUCED KINASE1 (BKI) |
| | Gma.13604.1.S1.at | CD401537 | 0.011411572 | protein kinase-like |
| | Gma.9902.1.A1.at | AW395328 | 0.011756578 | FUSCA 5 MAP kinase kinase (FUS5) |
| | GmaAffx.56323.1.S1.at | BU764214 | 0.01212639 | protein kinase |
| | Gma.5162.1.A1.at | BI971156 | 0.013412317 | Curculin-like (mannose-binding) protein kinase |
| | Gma.4455.3.S1.at | CB063632 | 0.014076915 | PROTEIN KINASE 2B (APK2B) |
| | GmaAffx.21787.1.A1.at | AW348555 | 0.015096504 | leucine-rich repeat transmembrane protein kinase (CLAVATA1) |
| | GmaAffx.66511.1.S1.at | AW350917 | 0.019563038 | calcium and calmodulin-dependent protein kinase (ATCDPK1) |
| | GmaAffx.34312.1.S1.at | AJ965735 | 0.023684433 | protein phosphatase 2C (PP2C) |
| | GmaAffx.64402.1.S1.at | AW317282 | 0.025399823 | leucine-rich repeat |
| | Gma.4801.1.S1.s.at | BU548608 | 0.028086024 | protein phosphatase 1 (PP1) |
| | Gma.11291.1.S1.at | AW351207 | 0.028086024 | inositol 1,3,4-trisphosphate 5/6-kinase |
| | GmaAffx.73451.1.S1.at | BG046889 | 0.034312943 | CALMODULIN-RELATED PROTEIN 2 |
| Transcription | Gma.7212.1.S1.at | BE658102 | 0.005553292 | SUPPRESSOR OF FRIGIDA4 (SUF4) |
| | GmaAffx.67609.1.S1.at | BG551013 | 0.009290923 | SAR DNA-binding protein-1 |
| | Gma.4165.1.S1.at | BI969143 | 0.015813164 | homeodomain-related |
| | Gma.4164.1.S1.at | BI968666 | 0.016772715 | MYB transcription factor (MYB112) |
| | GmaAffx.1165.1.S1.at | BI425542 | 0.01738237 | jasmonate-responsive promoter element |
| | Gma.15724.2.S1.at | AW350291 | 0.018668953 | CCR4 associated factor 1-related protein |
| | Gma.1772.1.S1.at | CD406036 | 0.020753499 | transcription factor IIA (TFIIA) |
| | GmaAffx.52970.1.S1.at | BU548330 | 0.021323422 | zinc finger (DHHHC type) family protein |
sample type. The detection call analysis identified 1,957 genes present and unique to the 3 dpi compatible syncytium sample (Figure 8) (supplementary Table 6)). Therefore, in the analysis presented here, a total of 4,214 genes were present within 3 dpi compatible syncytial cell samples. A histogram of the functional categorizations of the 1957 genes present and unique to the 3 dpi compatible syncytium sample described in this section is presented (Figure 8(d)). Selected gene lists comprising the (1) Disease and Defense, (2) Signaling, and (3) Transcription categories are provided (Table 2 (supplementary Table 6)).

3.6. 8 dpi Syncytia Undergoing a Compatible Reaction. The DCM was used to compare genes within the 8 dpi microdissected syncytia undergoing a compatible reaction to the pericycle sample (Figures 8(e) and 8(f)). A total of 4,823 genes were present in these two samples. From these analyses, 656 genes that were present and unique to the pericycle sample were identified (Figure 8(e) (supplementary Table 7)). Further analysis identified 2,312 genes that were present and common between the pericycle and 8 dpi compatible syncytium sample (Figure 8(e) (supplementary Table 8)). Only these genes could be used for differential expression analyses because expression data was available for each sample type. The detection call analysis identified 1,855 genes present and unique to the 8 dpi compatible syncytium sample (Figure 8(e) (supplementary Table 9)). Therefore, in the analysis presented here, a total of 4,167 genes within 8 dpi compatible syncytial cell samples were present. A histogram of the functional categorizations of the 4,167 genes present and unique to the 8 dpi compatible syncytium sample described in this section is presented (Figure 8(f)). Selected gene lists comprising the (1) Disease and Defense, (2) Signaling, and (3) Transcription categories are provided (Table 3 (supplementary Table 9)).

3.7. Direct Comparison: 3 dpi Incompatible versus 3 dpi Compatible Syncytia. The DCM was used to compare genes within the 3 dpi microdissected syncytia undergoing an incompatible reaction directly to the 3 dpi syncytia undergoing a compatible reaction (Figures 8(g) and 8(h)). A total of 4,793 genes were present in these two samples. From these analyses, 2,851 genes were identified that were present and unique to the 3 dpi compatible syncytium sample (Figure 8(g) (supplementary Table 10)). Further analysis identified 1,363 genes that were present and common between the 3 dpi syncytia undergoing compatible and incompatible reactions (Figure 8(g) (supplementary Table 11)). Only these genes could be used for differential expression analyses because expression data was available for each sample type. The detection call analysis identified 579 genes present and unique to the 3 dpi incompatible syncytium sample (Figure 8(g) (supplementary Table 12)). A histogram of the functional categorizations of the 579 genes present and unique to the 3 dpi incompatible syncytium (as directly compared to the present and unique to the 3 dpi compatible syncytium sample genes) described in this section is presented (Figure 8(h)). Selected gene lists for the incompatible syncytium (Table 4) and compatible syncytium (Table 5) comprising the (1) Disease and Defense, (2) Signaling, and (3) Transcription categories are provided.

3.8. Direct Comparison: 8 dpi Compatible versus 3 dpi Compatible Syncytia. The DCM was used to compare genes within the 8 dpi microdissected syncytia undergoing a compatible reaction to the 3 dpi syncytia undergoing a compatible reaction (Figures 8(i) and 8(j)). A total of 5,475 genes were present in these two samples. From these analyses, 1,308 genes were identified that were present and unique to the 3 dpi compatible syncytium sample (Figure 8(i) (supplementary Table 13)). The detection call analysis identified 2,906 genes that were present and common between the three and 8 dpi syncytia undergoing compatible reactions (Figure 8(i) (supplementary Table 14)). Only these genes could be used for differential expression analyses because expression data was available for each sample type. Further analysis identified 1,261 genes present and unique to the 8 dpi compatible syncytium sample (Figure 8(i) (supplementary Table 15)). A histogram of the functional categorizations of the 1,261 genes present and unique to the 8 dpi compatible syncytium sample described in this section is presented (Figure 8(j)). Selected gene lists comprising the (1) Disease and Defense, (2) Signaling, and (3) Transcription categories are provided (Table 6 (supplementary Table 15)).

4. Discussion

Microarray experiments typically rely on differential expression analysis methods to identify differences in relative levels
## Table 2: Select genes that were unique to the 3 dpi syncytia undergoing a compatible reaction but that were not differentially expressed as compared to a pericycle control sample (Figure 8(d)) comprising the Disease and Defense, Signaling, and Transcription categories.

| C-3 dpi                  | Public ID  | Avg P-value | Gene                                                                 |
|--------------------------|------------|-------------|----------------------------------------------------------------------|
| **Disease & defense**    |            |             |                                                                      |
| Gma.3749.1.S1_at         | CD392491   | 0.002923594 | heat shock protein 70 precursor protein                              |
| GmaAffx.90134.1.S1_s_at  | CF805859   | 0.008396837 | purple acid phosphatase-like protein                                 |
| GmaAffx.78614.1.S1_at    | BQ611991   | 0.008396837 | Suppressor-of-White-APricot/surp domain-containing protein           |
| Gma.13217.1.S1_at        | CD391191   | 0.01175678  | wound-responsive protein-related protein                              |
| GmaAffx.11893.1.A1_at    | CD414188   | 0.01212639  | regulator of chromatin condensation-1 (RCC1)                        |
| GmaAffx.91519.1.S1_s_at  | CF807244   | 0.013412317 | double-stranded DNA-binding family protein                           |
| GmaAffx.29692.1.S1_at    | AW348396   | 0.016772715 | chitinase (class II)                                                 |
| GmaAffx.71331.1.S1_at    | AW597101   | 0.018428453 | galactosyltransferase family protein                                 |
| Gma.6640.1.S1_at         | BQ628278   | 0.022040082 | haem peroxidase                                                       |
| Gma.8022.1.S1_at         | BQ628998   | 0.024040827 | epoxide hydrolase                                                     |
| GmaAffx.24201.1.S1_at    | BQ740972   | 0.025685834 | Avr9 elicitor response like protein                                  |
| Gma.9638.1.A1_at         | CA936403   | 0.029056963 | ADR6                                                                  |
| **Signal transduction**  |            |             |                                                                      |
| Gma.3893.1.S1_at         | U44850.1   | 0.002298236 | Guanine nucleotide-binding protein subunit beta-like protein (ArcA) |
| Gma.3286.1.S1_at         | BQ298747   | 0.003021268 | putative presenilin                                                   |
| GmaAffx.33721.1.S1_at    | B167195    | 0.003317825 | calcium/calmodulin-dependent protein kinase (CDPK)                   |
| Gma.17655.1.S1_at        | BE057259   | 0.003317825 | protein phosphatase 2A (PP2A)                                        |
| Gma.10697.2.S1_at        | AW424151   | 0.003591192 | BRII-associated receptor kinase 1                                     |
| Gma.10649.1.S1_at        | BE659256   | 0.004034485 | BRII-associated receptor kinase 1                                     |
| Gma.1965.1.S1_x_at       | L01432.1   | 0.00603523  | calmodulin (SCaM-3)                                                  |
| GmaAffx.52826.3.S1_at    | BE596503   | 0.00846925  | protein phosphatase 2C (PP2C)                                        |
| Gma.7517.1.S1_at         | BU548272   | 0.009290923 | U box-containing protein kinase                                       |
| GmaAffx.50980.1.S1_s_at  | BE823095   | 0.010694912 | protein phosphatase 2C (PP2C)                                        |
| GmaAffx.57055.1.S1_at    | AW203411   | 0.015413718 | mitogen-activated protein kinase                                      |
| Gma.16954.2.A1_at        | BE822903   | 0.017813909 | phospholipase C                                                      |
| GmaAffx.85565.1.S1_at    | BE611082   | 0.018428453 | calcium-dependent protein kinase 29 (CDPK)                           |
| Gma.7177.2.A1_a_at       | BI425372   | 0.018428453 | MHK kinase                                                           |
| GmaAffx.35805.2.S1_at    | BF324178   | 0.019563038 | MAP3K delta-1 protein kinase-like                                     |
| Gma.9902.1.A1_at         | AW395328   | 0.019678474 | FUSCA 5 MAP kinase kinase (FUS5)                                     |
| Gma.2314.1.S1_at         | AW310385   | 0.019678474 | FUS3-COMPLEMENTING GENE 1                                            |
| GmaAffx.21787.1.A1_at    | AW348555   | 0.020429855 | CLAVATA1 receptor kinase (CLV1)                                      |
| GmaAffx.69025.1.S1_at    | BM271195   | 0.020753499 | RIO kinase                                                           |
## Table 2: Continued.

| C-3 dpi | Probe set ID | Public ID | Avg P-value | Gene |
|---------|--------------|-----------|-------------|------|
| **Signal transduction** | | | | |
| Gma Affx.62926.1.S1_at | BE804949 | 0.020828644 | G-protein alpha subunit |
| Gma.11015.1.S1_at | CD398110 | 0.023684433 | leucine-rich repeat transmembrane protein |
| Gma.3185.2.S1_at | BM890715 | 0.023684433 | leucine-rich repeat transmembrane protein |
| Gma Affx.15664.1.S1_at | BE607642 | 0.024040827 | LRR receptor-like protein kinase |
| Gma Affx.77602.1.S1_at | BQ627622 | 0.025399823 | protein phosphatase-2c (PP2C) |
| Gma.5722.1.S1_at | BU546228 | 0.029056963 | Ste20-related protein kinase |
| Gma.8364.1.S1_at | BE659226 | 0.034312943 | putative protein kinase (PK12) |
| **Transcription** | | | | |
| Gma.4165.1.S1_at | BI969143 | 0.005201937 | homeodomain-related |
| Gma.4205.1.S1_at | AF464906.1 | 0.005679433 | repressor protein |
| Gma Affx.50673.1.S1_at | BF425742 | 0.005679433 | No apical meristem (NAM) protein |
| Gma Affx.67609.1.S1_at | BG551013 | 0.007110254 | SAR DNA-binding protein-1 |
| Gma.16172.1.S1_at | CD411627 | 0.009049965 | CONSTANS-like B-box zinc finger |
| Gma Affx.52855.1.S1_at | AW308923 | 0.010397582 | transcription initiation factor IIE (TFIIE) |
| Gma.3176.1.S1_at | BU549115 | 0.010649919 | transcription factor IIA (TFIIB) |
| Gma.2702.2.S1_at | AI855587 | 0.010649919 | no apical meristem (NAM) |
| Gma.8298.1.S1_at | CD392694 | 0.012696314 | trithorax 4-nuclear SET-domain containing protein |
| Gma.4116.2.S1_at | BM177935 | 0.014076915 | transcription initiation factor IIE, beta subunit (TFIIE beta) |
| Gma Affx.85579.1.S1_at | BQ273352 | 0.014307059 | lipoamide dehydrogenase |
| Gma Affx.81234.1.A1_at | BE823765 | 0.014307059 | REF-LIKE 8 (TRFL8) |
| Gma Affx.66085.1.S1_at | BQ630399 | 0.015096504 | Basic Helix-Loop-Helix (bHLH) |
| Gma.13174.1.S1_at | CD414686 | 0.016772715 | indoleacetic acid-induced protein 1 (IAA13) |
| Gma Affx.71523.1.S1_at | BU544012 | 0.01738237 | lipoamide dehydrogenase |
| Gma Affx.92212.1.A1_s_at | CF807937 | 0.018428453 | MYB transcription factor (MYB92) |
| Gma.7891.1.S1_at | AW310625 | 0.020753499 | Basic Helix-Loop-Helix (bHLH) |
| Gma.3632.1.A1_at | BJ316950 | 0.020828644 | zinc finger protein |
| Gma.17664.1.S1_at | AW348917 | 0.020828644 | zinc finger (DHHHC type) family protein |
| Gma.2243.2.S1_s_at | BE807162 | 0.02208694 | transcription initiation factor IIF beta (TFIIF beta) |
| Gma.1270.1.S1_at | CD405147 | 0.022830045 | LIM domain-containing, zinc-binding protein |
| Gma.752.1.A1_at | AW432463 | 0.022830045 | Helix-loop-helix DNA-binding |
| Gma.5274.1.S1_at | BM178426 | 0.025399823 | transcription factor EIL2 |
| Gma.7776.1.A1_at | CD399260 | 0.025868534 | ATBRM/CHR2 |
| Gma Affx.52970.1.S1_at | BU548330 | 0.027055662 | zinc finger (DHHHC type) family protein |
| Gma.7212.1.S1_at | BE658102 | 0.027055662 | Zinc finger, BED-type predicted |
| Gma Affx.91768.1.S1_s_at | CF807493 | 0.031457154 | WRKY27 |
of gene expression. However, it is possible that very large differences in gene activity are present when the analysis involves comparing gene expression within homogeneous populations of cells, especially cells that are at different stages of differentiation or become genomically reprogrammed as a consequence of a pathological infection. In the analysis presented here, the DCM was used as an alternative method to identify genes that are expressed in a particular cell type but not active in another cell type. Importantly, the resulting DCM analysis identified a group of genes that were present uniquely within a sample type. However, those same genes were eliminated by differential expression analyses methods because differential expression analyses require expression data from each sample in the comparison.

The DCM is a statistically sound method based on a four-step procedure. The procedure incorporates (1) removal of saturated probes, (2) calculation of discrimination scores, (3) P-value calculation using the Wilcoxon’s rank test, and (4) making the detection call. The DCM has been used in a variety of analyses to understand gene expression in various experimental systems [4–6]. The DCM analyses have demonstrated the utility of the method. The DCM takes into consideration only the presence of the transcript as measured by the probe set on the microarray. Thus, DCM can be used as a measurement of the diversity of transcripts within those samples. In the analysis presented here, DCM identified thousands of genes in the 3 dpi incompatible and compatible syncytium samples, the 8 dpi compatible syncytium samples, and the pericycle samples that were isolated by LCM. The DCM, along with customized computer scripts, was then used to compare the transcripts present in those samples. The method allowed for the identification and comparison of transcripts that were found in those samples. The DCM analyses presented here identified transcripts that are found in the incompatible or compatible syncytium samples that did not meet the criteria in a differential expression analysis [26]. Thus, DCM provided a broader (or different) estimate of the similarities and differences in those samples. In all of the comparisons made, the samples exhibited substantial differences in transcript composition. The DCM demonstrated vast differences in transcripts when directly comparing 3 dpi incompatible to compatible syncytia, even though the anatomy of these cells at that time appears similar [26–28, 33].

Comparisons of detection calls between the pericycle control and syncytia undergoing an incompatible reaction resulted in the identification of a disproportionate number of transcripts in the syncytia undergoing an incompatible reaction belonging to the “No Homology to Known Proteins” category. Conversely, the pericycle control had a disproportionate number of transcripts in the “Metabolism” and “Hypothetical Protein Supported by cDNA” categories (Figure 8(b)). Detection calls can also be used to determine other features of the cells under study. For example, detection calls can be used to arrive at an estimate of how different (or similar) two cell samples or sample types are from each other. Thus, when examining the development of specialized cell types like the syncytium, certain types of gene activity can be identified and used for comparative purposes by using DCM. For example, rapid elicitation of gene expression can be followed by a return to preinfestation levels during a reaction to a pathogen [62, 63]. The DCM may allow for the identification of genes expressed at lower levels that are not identified in a differential expression analysis. The DCM will also identify gene expression that is at a high threshold in one sample and absent in the other. This category of genes would be excluded in a differential expression analysis because no statistics can be performed on probe sets lacking expression data.

In this study, DCM aided in identifying additional genes expressed during nematode infection. In the analyses many genes from (1) Disease and Defense, (2) Signaling, and (3) Transcription categories that were unique to one sample type and thus excluded from a differential expression analysis were focused on because of their obvious importance in a variety of plant defense pathways. The analyses here focus on the incompatible analyses. Recent proteomic work by Afzal et al. [64] provides an additional bank of genes to obtain a broader understanding of H. glycines infection of G. max. The genes identified in the analysis make reasonable candidates for further functional tests [32].

### 4.1. DCM Is Used to Compare the 3dpi Incompatible Syncytium to Pericycle Cells

The DCM analyses identified genes that were present only in the incompatible syncytial cell sample as compared to the pericycle sample. The genes included various defense response genes. Some of these were DRRG49-C (CF809336), Pto-interacting-kinase (Pti) (B1970555), BOTRTIS-INDUCED KINASE 1 (BIK1) (AW311265), and two leucine-rich repeat genes (LRRs) (AW348555, AW317282). LRRs near rhgl exist [65]. The DRRG49-C gene is induced during pathogen attack [66]. The Pti-kinase [67] and BIK1 [68] are examples of genes that are induced during a response to pathogenic attack and are
Table 3: Select genes that were unique to the 8 dpi syncytia undergoing a compatible reaction but that were not differentially expressed as compared to a pericycle control sample (Figure 8(f)) comprising the Disease and Defense, Signaling, and Transcription categories.

| C-8 dpi                  | Public ID | Avg P-value | Gene                                      |
|--------------------------|-----------|-------------|-------------------------------------------|
| **Disease & defense**    |           |             |                                           |
| GmaAffx.91273.1.S1_at    | CF805964  | 0.002747901 | wound-induced protein                      |
| Gma.4305.1.S1_at         | AW350687  | 0.00337326  | haem peroxidase                           |
| GmaAffx.90134.1.S1_at    | CF805859  | 0.004659843 | purple acid phosphatase-like protein       |
| Gma.8512.1.S1_at         | AF236108.1| 0.014307059 | purple acid phosphatase-like protein       |
| Gma.7301.1.S1_at         | BM528250  | 0.019563038 | gamma-glutamyl transferase                |
| GmaAffx.78614.1.S1_at    | BQ611991  | 0.019678474 | Suppressor-of-White-APricot splicing regulator |
| GmaAffx.59573.1.S1_at    | AW350986  | 0.019951841 | purple acid phosphatase-like protein       |
| GmaAffx.91519.1.S1_at    | CF807244  | 0.027055562 | double-stranded DNA-binding protein        |
| Gma.11154.1.S1_at        | AW309927  | 0.031457154 | putative elicitor-responsive gene-3        |
| GmaAffx.83232.1.S1_at    | BE023128  | 0.037684072 | MILDEW RESISTANCE LOCUS O 10 (MLO10)       |
| **Signal transduction**  |           |             |                                           |
| Gma.1965.1.S1_at         | L01432.1  | 0.001946244 | calmodulin (SCaM-3)                       |
| GmaAffx.90377.1.S1_at    | CF806102  | 0.001946244 | PP2A regulatory subunit                   |
| Gma.9902.1.A1_at         | AW395328  | 0.002571602 | FUSCA 5 MAP kinase kinase (FUS5)           |
| Gma.7517.1.S1_at         | BU548272  | 0.002923594 | U box-containing protein kinase calcium/calmodulin-dependent protein kinase |
| Gma.17655.1.S1_at        | BE057259  | 0.00492857 | protein phosphatase 2C (PP2C)              |
| GmaAffx.50980.1.S1_at    | BE823095  | 0.007394226 | PROTEIN KINASE 2B (APK2B)                 |
| Gma.455.3.S1_at          | CB606362  | 0.009290923 | protein phosphatase type-2C               |
| Gma.5188.1.S1_at         | AW349454  | 0.010397582 | LysM domain-containing receptor-like kinase 7 |
| Gma.9853.1.A1_at         | AW350335  | 0.010397582 | calmodulin-binding receptor-like cytoplasmic kinase 3 (CRCK3) |
| Gma.10515.1.S1_at        | BM528701  | 0.011324733 | membrane-associated progesterone-binding protein 2 (ATMAPR2) |
| Gma.5304.2.S1_at         | CD410657  | 0.01212639 | protein kinase 2                           |
| Gma.1068.1.S1_at         | L19360.1  | 0.013412317 | MITOGEN-ACTIVATED PROTEIN KINASE 1 (ATMPK1) |
| Gma.4631.1.S1_at         | BE824210  | 0.013412974 | Ste20-related protein kinase               |
| Gma.5722.1.S1_at         | BUS46228  | 0.013451556 | 14-3-3-like protein                       |
| Gma.596.1.S1_at          | AF228501.1| 0.015413718 | Inositol monophosphatase                    |
| Gma.2222.1.S1_at         | CD416168  | 0.015413718 | protein phosphatase 2C (PP2C)              |
| GmaAffx.82748.1.S1_at    | BM085604  | 0.016427052 | RhoGAP small G protein family protein      |
| GmaAffx.67957.1.S1_at    | BG157622  | 0.01677215  | calmodulin                                |
| Gma.15250.1.S1_at        | AJ856228  | 0.01677215  | BR11-associated receptor kinase 1          |
| Gma.10649.1.S1_at        | BE659256  | 0.016932704 |                                           |
### Table 3: Continued.

| C-8 dpi | Probe set ID | Public ID | Avg P-value | Gene |
|---------|--------------|-----------|-------------|------|
| **Signal transduction** | | | | |
| Gma.8364.1.S1_at | BE659226 | 0.016932704 | ARABIDOPSIS | FUS3-COMPLEMENTING GENE (AFC1)  |
| Gma.10697.2.S1_at | AW424151 | 0.01738237 | protein phosphatase 2A (PP2A) catalytic subunit |
| Gma.9706.1.S1_at | BE657400 | 0.018668953 | protein phosphatase 1 (PP1) transmembrane protein |
| GmaAffx.65281.1.S1_at | CA819808 | 0.019563038 | ARABIDOPSIS | FUS3-COMPLEMENTING GENE (AFC2)  |
| Gma.2314.1.S1_at | AW310385 | 0.022008082 | root hair defective 3 (RHD3) |
| Gma.2471.1.S1_at | AI938029 | 0.025399823 | FUSCA PROTEIN (FUS6)  |
| Gma.4629.1.A1 | CA820195 | 0.028086024 | SGT1 |
| GmaAffx.78968.2.S1_at | BM188587 | 0.030941813 | cysteine protease |
| Gma.1518.2.S1_a_at | BM524684 | 0.030941813 | cysteine protease |
| GmaAffx.19821.1.S1_at | CA782536 | 0.030941813 | protein kinase |
| Gma.4536.1.A1_at | BJ945486 | 0.034312943 | receptor-like protein kinase |
| **Transcription** | | | | |
| GmaAffx.92861.1.S1_s_at | CF808586 | 0.002923594 | HMG-protein  |
| Gma.1748.2.S1_a_at | CA820372 | 0.003591192 | no apical meristem (NAM) protein (NAC1) |
| Gma.17736.1.S1_at | AW598570 | 0.00603523 | zinc finger, C2H2-type |
| Gma.4165.1.S1_at | BJ96143 | 0.007394226 | Homeodomain-related |
| Gma.6739.1.S1_s_at | AI856042 | 0.007667593 | RNA polymerase II (RPB15.9) |
| Gma.2844.1.S1_at | BJ972378 | 0.008019584 | auxin-induced protein 2 |
| Gma.2258.2.S1_a_at | BG237280 | 0.008396837 | pre-mRNA processing ribonucleoprotein (NOP5) |
| GmaAffx.54382.1.A1_at | BE807592 | 0.00846925 | calmodulin-binding transcription activator 4 |
| GmaAffx.50673.1.S1_at | BF425742 | 0.009049965 | no apical meristem (NAM) protein |
| GmaAffx.41946.1.S1_at | BM528357 | 0.009290923 | helix-loop-helix DNA-binding |
| GmaAffx.78992.1.S1_at | BU760819 | 0.010397582 | HMG-I and HMG-Y DNA-binding protein |
| Gma.2465.1.S1_at | UD390577 | 0.010694912 | ARABIDOPSIS THALIANA HOMEBOX PROTEIN 54 (ATHB54) |
| GmaAffx.42667.1.S1_at | BU761164 | 0.011051366 | SCARECROW-LIKE 1 (SCL1) |
| GmaAffx.66085.1.S1_at | BQ630399 | 0.011411572 | basic helix-loop-helix (bHLH) zinc finger, CCCH-type; Zinc finger, RING-type |
| Gma.4975.1.S1_at | BJ970178 | 0.011756758 | NIM1-like protein 1 (NPR-1) |
| Gma.6838.1.S1_at | AW349633 | 0.011756758 | WRKY27 |
| GmaAffx.91768.1.S1_s_at | CF807493 | 0.013412317 | bZIP transcription factor bZIP123 |
| GmaAffx.81622.1.S1_at | BM093159 | 0.013412317 | helix-loop-helix DNA-binding lipoamide dehydrogenase-UNE12 (unfertilized embryo sac 12) |
| Gma.3609.1.S1_at | CD392010 | 0.013412317 | BEL1-like homeodomain transcription factor |
involved in important defense responses. The identification of a Pti-like kinase was particularly interesting. In *L. esculentum*, *Pti4* and *Pti5* are induced by the virulent *Pseudomonas syringae* pv. *tomato*, the nonhost pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae* (strain PXO61 avrXa10), and the nonpathogenic bacterium *Pseudomonas fluorescens* (strain 2–79) [69]. Interestingly, Pti kinases are observed at 3 dpi in both compatible and incompatible reactions. The Pti-kinase identified in the 3 dpi incompatible reaction was most closely related to *Pti1* isolated from tomato [70]. In that analysis, *Pti1* was shown to be phosphorylated by *Pto* and to be involved in the hypersensitive response [70]. The LRR genes have a long history as being important for plant defense [71, 72]. The genes also have been shown to confer resistance to parasitic nematodes [73–78]. Thus, due to the transient nature of expression of some of these genes in other systems, it is not surprising that they were not identified as being differentially expressed in syncytium samples [26].

4.2. DCM Is Used to Compare the 3dpi Incompatible Syncytium Directly to the 3dpi Compatible Syncytium. The DCM analyses identified genes that were present only in the 3dpi incompatible syncytium as compared directly to the 3dpi compatible syncytium sample (supplementary Table 12). The probe sets included genes like
Table 4: An analysis compared the 3 dpi syncytia undergoing an incompatible reaction directly to the 3 dpi compatible syncytium samples. Selected genes that were unique to the 3 dpi syncytia undergoing an incompatible reaction but that were not differentially expressed as compared directly to the 3 dpi compatible syncytium samples (Figure 8(h)) comprising the Disease and Defense, Signaling, and Transcription categories are provided.

| Probe set ID       | Public ID | Avg P-value | Gene                                           |
|--------------------|-----------|-------------|------------------------------------------------|
| **Disease & defense** |           |             |                                                |
| Gma.4886.2.S1_at   | AW234624  | 0.005201937 | haem peroxidase                                |
| Gma.405.1.A1_at    | AI443411  | 0.008396837 | leucine-rich repeat family protein (DRT100)    |
| Gma.2044.2.S1_at   | BE821230  | 0.011411572 | abscisic stress ripening-like protein          |
| GmaAffx.92230.1.A1_s_at | CF807955  | 0.012305657 | thaumatin-like protein PR-5b                   |
| Gma.7542.2.S1_at   | CA936764  | 0.016772715 | defender against cell death 1 (DAD-1)          |
| Gma.8449.1.S1_s_at | AF002258.1| 0.019563038 | CoA ligase 4                                   |
| GmaAffx.14986.1.S1_at | BE657889 | 0.020669698 | PHOSPHATE-INDUCED 1 (phi-1)                    |
| GmaAffx.2203.1.S1_at | CD415745  | 0.020669698 | cadmium-induced protein                        |
| GmaAffx.91141.1.S1_at | CF806866  | 0.020828644 | peroxidase 1 precursor                         |
| GmaAffx.46214.1.S1_at | BE659266  | 0.022934167 | polyphenol oxidase                             |
| **Signal transduction** |           |             |                                                |
| Gma.13604.1.S1_at  | CD401537  | 0.011411572 | protein kinase                                 |
| GmaAffx.56323.1.S1_at | BU764214 | 0.01212639 | protein kinase                                 |
| Gma.6092.1.S1_at   | BI968757  | 0.012305657 | COP9 signalosome subunit 3                    |
| Gma.5162.1.A1_at   | BI971156  | 0.013412317 | Curculin-like (mannose-binding) lectin protein kinase |
| Gma.4455.3.S1_at   | CB063632  | 0.014076915 | PROTEIN KINASE 2B (APK2B)                      |
| GmaAffx.66511.1.S1_at | AW350917  | 0.019563038 | calcium and calmodulin-dependent protein kinase (ATCDPK1) |
| GmaAffx.34312.1.S1_at | AI965735  | 0.023684433 | protein phosphatase 2C (PP2C)                 |
| GmaAffx.64402.1.S1_at | AW317282  | 0.025399823 | leucine-rich repeat                            |
| Gma.4801.1.S1_s_at | BU548608  | 0.028086024 | protein phosphatase 1 (PP1)                   |
| Gma.11291.1.S1_at  | AW351207  | 0.028086024 | inositol 1,3,4-trisphosphate 5/6-kinase       |
| GmaAffx.73451.1.S1_at | BG046889  | 0.034312943 | CALMODULIN-RELATED PROTEIN 2                  |
| **Transcription**  |           |             |                                                |
| GmaAffx.89077.1.A1_s_at | CK605647  | 0.009323331 | CONSTANS interacting protein 2a               |
| Gma.3996.1.S1_at   | AW394946  | 0.014307059 | WRKY52                                         |
| Gma.9678.1.S1_at   | CD404894  | 0.016707249 | RNA polymerase II pathogenesis-related         |
| Gma.12330.2.S1_s_at | BI972758  | 0.027055562 | transcriptional factor                         |
| Gma.6838.1.S1_at   | AW349633  | 0.027055562 | NIM1-like protein 1 (NPR-1)                   |
| Gma.16645.1.S1_at  | BM143429  | 0.028086024 | Arabidopsis NAC domain-containing protein 1 (ATAF1) |
Table 5: An analysis compared the 3 dpi syncytia undergoing an incompatible reaction directly to the 3 dpi compatible syncytium samples. Selected genes that were unique to the 3 dpi syncytia undergoing a compatible reaction but that were not differentially expressed as compared directly to the 3 dpi incompatible syncytium samples (Figure 8(h)) comprising the Disease and Defense, Signaling, and Transcription categories.

| C-3 dpi genes in the comparison of I-3 dpi to C-3 dpi |  |
|-----------------------------------------------|---|
| Probe set ID | Public ID | Avg P-value | Gene |
| **Disease & defense** | | | |
| GmaAffx.36484.1.S1.at | BI425441 | 0.001672877 | PR1a |
| Gma.6091.1.S1.at | AW510762 | 0.00221961 | haem peroxidase |
| Gma.2523.1.S1.at | CA852440 | 0.002747901 | R 14 protein |
| GmaAffx.85114.1.S1.at | AW760844 | 0.003021268 | Malus major allergen (Mal d 1.07) |
| Gma.4312.3.S1.at | BF424240 | 0.003822926 | glutathione peroxidase (GSH-PX3) |
| Gma.257.2.S1_at | CD400364 | 0.005553929 | cysLteine proteinase inhibitor |
| GmaAffx.36514.1.S1.at | BE658341 | 0.005553929 | cationic peroxidase |
| Gma.6299.3.S1.at | BU547701 | 0.00603523 | selenium binding protein |
| GmaAffx.92699.1.S1.at | CF808424 | 0.00603523 | PR-5 protein |
| Gma.5141.1.S1.at | BI971102 | 0.007667593 | laccase 3 (LAC3) |
| Gma.9504.1.S1.at | CA803130 | 0.007680178 | plant disease resistance response protein |
| Gma.18084.1.S1.at | BI317557 | 0.008396837 | RESPIRATORY BURST OXIDASE HOMOLOG (ATRBOHB) |
| Gma.8144.1.A1.at | BU548599 | 0.009323331 | cationic peroxidase |
| GmaAffx.11893.1.A1.at | CD414188 | 0.01212639 | regulator of chromatin condensation-1 (RCC1) |
| Gma.4077.1.S1.at | CD414118 | 0.012696314 | ASR protein |
| Gma.7257.2.S1.at | BG155489 | 0.016427052 | soluble epoxide hydrolase |
| GmaAffx.71331.1.S1.at | AW597101 | 0.018428453 | galactosyltransferase family protein |
| GmaAffx.52146.1.S1.at | CA934966 | 0.02806024 | PATHOGENESIS-RELATED 4 (PR4) |
| Gma.9638.1.A1.at | CA936403 | 0.029056963 | ADR6 |
| **Signal transduction** | | | |
| GmaAffx.92136.1.S1.at | CF807451 | 0.001672877 | Curculin-like (mannose-binding) lectin protein kinase |
| Gma.6338.1.S1.at | AI442775 | 0.001946244 | protein phosphatase 2C (PP2C) |
| Gma.3893.1.S1.at | U44850.1 | 0.002298236 | Arabidopsis thaliana Homolog of the Tobacco ArcA (ATARCA) |
| Gma.4228.1.S1.at | AI856764 | 0.002298236 | RelA-SpoT like protein (RSH3) |
| Gma.13033.1.A1.at | CD392795 | 0.002298236 | calcium-dependent calmodulin-independent protein kinase (CDPK) |
| Gma.6709.1.S1.at | BE823291 | 0.002747901 | CBL-interacting protein kinase 22 |
| Gma.10697.2.S1.at | AW424151 | 0.003591192 | protein phosphatase 2A catalytic subunit |
| Gma.11006.1.S1.at | AW706204 | 0.004928567 | CBL-interacting protein kinase caltractin-like |
| Gma.6359.1.S1.at | CD398481 | 0.005201937 | 14-3-3 protein |
| GmaAffx.92229.1.S1.at | CF806381 | 0.005553929 | leucine-rich repeat protein |
| Gma.4507.1.S1.at | BG653255 | 0.006308596 | LRR receptor-like protein kinase |
Table 5: Continued.

| Probe set ID          | Public ID | Avg P-value | Gene                                                                 |
|-----------------------|-----------|-------------|----------------------------------------------------------------------|
| Signal transduction   |           |             |                                                                     |
| GmaAffx.90655.1.S1_at | CF806380  | 0.007680178 | 14-3-3-like protein C (SGF14C)                                        |
| GmaAffx.91570.1.A1_at | CF807732  | 0.008019584 | JUN-activation-domain-binding protein                                 |
| Gma.4049.1.S1_at     | BQ786519  | 0.008184263 | wall-associated kinase (WAK-like kinase)                             |
| Gma.3083.1.S1_at     | BE474466  | 0.008396837 | protein kinase                                                        |
| Gma.7517.1.S1_at     | BU548272  | 0.012056573 | WD-40 repeat protein                                                 |
| GmaAffx.25928.1.S1_at| BE4714013 | 0.014307059 | leucine-rich repeat protein                                           |
| Gma.15907.1.A1_at    | CD407154  | 0.014771313 | serine/threonine-protein phosphatase PP1                              |
| Gma.3852.1.S1_at     | CD399104  | 0.015413718 | Arabidopsis thaliana MAP kinase (ATMPK20)                            |
| GmaAffx.57055.1.S1_at| AW203411  | 0.015413718 | Pti1-like kinase                                                      |
| Gma.10215.1.S1_at    | AY263347.1| 0.016307345 | calcium-dependent protein kinase 29 (CPK29)                          |
| GmaAffx.85565.1.S1_at| BE611082  | 0.018428453 | Cdc2-related protein kinase                                           |
| Gma.7177.2.A1_at     | BI425372  | 0.018428453 | MAP3K delta-1 protein kinase-like                                     |
| GmaAffx.35805.2.S1_at| BF324178  | 0.019563038 | protein phosphatase 2A (PP2A)                                        |
| Gma.10798.1.S1_at    | CD401168  | 0.019563038 | PSEUDOLYPHAGOID SYNOPTIC 1-LIKE (PSE)                                 |
| Gma.2314.1.S1_at     | AW310385  | 0.019678474 | calcium dependent calmodulin independent protein kinase (CDPK)       |
| Gma.1517.2.A1_at     | BE078459  | 0.020696938 | VERNALIZATION INDEPENDENCE 3 (VIP3)                                  |
| GmaAffx.69025.1.S1_at| BM271195  | 0.020753499 | RIO kinase                                                           |
| GmaAffx.62926.1.S1_at| BE804949  | 0.02082644  | extra-large GTP-binding protein 3 (XLG3)                             |
| GmaAffx.40750.1.S1_at| BG352469  | 0.022028694 | protein phosphatase 2A regulatory subunit (PP2A)                     |
| GmaAffx.44305.1.S1_at| BU551393  | 0.022830045 | leucine-rich repeat transmembrane protein                            |
| Gma.3185.2.S1_at     | BM890715  | 0.023684433 | leucine-rich repeat transmembrane protein                            |
| Gma.11015.1.S1_at    | CD398110  | 0.023684433 | leucine-rich repeat transmembrane protein                            |
| GmaAffx.15664.1.S1_at| BE607642  | 0.024040827 | leucine-rich repeat transmembrane protein                            |
| Gma.1423.1.S1_at     | AJ960045  | 0.024040827 | BRASSINAZOLE-RESISTANT 1 (BZR1)                                      |
| Gma.4044.1.S1_at     | BE821233  | 0.025399823 | Pescadillo-like                                                      |
| GmaAffx.89525.1.S1_at| CK606517  | 0.027055562 | protein phosphatase 1 (PP1)                                          |
| Gma.7387.1.A1_at     | CD396910  | 0.027055562 | pseudo-response regulator                                            |
| Gma.5722.1.S1_at     | BU546228  | 0.029056963 | Ste20-related protein kinase                                         |
Table 5: Continued.

C-3 dpi genes in the comparison of I-3 dpi to C-3 dpi

| Probe set ID     | Public ID  | Avg P-value | Gene                                |
|------------------|------------|-------------|-------------------------------------|
| **Transcription**|            |             |                                     |
| Gma.1538.1.S1_a_at| AW351115   | 0.002298236 | salt tolerance protein 6            |
| Gma.12279.1.A1_at| CD397826   | 0.00337326  | basic helix-loop-helix (bHLH)       |
| Gma.5331.1.S1_at | BI892702   | 0.004307852 | no apical meristem (NAM) protein NAC4 |
| Gma.593.2.S1_x_at| CA800286   | 0.00492857  | MYB transcription factor (MYB173)   |
| GmaAffx.5069.2.A1_at | BM121565 | 0.005201937 | basic-leucine zipper (bZIP111)      |
| GmaAffx.50673.1.S1_at | BF425742 | 0.005679433 | no apical meristem (NAM) protein (NAC) |
| GmaAffx.93436.1.A1_s_at | CF809161 | 0.007680178 | AP2/EREBP transcription factor ERF-2 |
| GmaAffx.38951.1.S1_at | BI322098 | 0.008396837 | basic-leucine zipper (bZIP)         |
| Gma.16172.1.S1_at | CD411627   | 0.009049965 | CONSTANS-LIKE 13                   |
| Gma.3730.2.S1_a_at | BI320846   | 0.009290923 | WRKY27                             |
| Gma.15862.1.S1_at | BI970593   | 0.009323331 | pathogenesis-related transcriptional factor and ERF |
| Gma.2702.2.S1_at | AL855587   | 0.010649919 | no apical meristem (NAM) protein    |
| Gma.163.1.S1_at  | AB029269.1 | 0.014076915 | MYB transcription factor (MYB12)    |
| Gma.16613.1.S1_s_at | BU760651 | 0.014307059 | zinc finger                         |
| GmaAffx.81234.1.A1_at | BE823765 | 0.014307059 | MYB-TRFL8 (TRF-LIKE 8)              |
| GmaAffx.15471.1.S1_at | BQ611747 | 0.014526581 | MYB transcription factor (MYB139)   |
| Gma.17432.1.S1_s_at | AW277783 | 0.015813164 | RNA polymerase subunit (RPB5)       |
| Gma.13174.1.S1_s_at | CD414686 | 0.016772175 | aux/IAA protein (IAA13)             |
| GmaAffx.71523.1.S1_at | BU544012 | 0.01738237  | Polycomb group-NAP1-RELATED PROTEIN 1 (NRP1) |
| Gma.15460.1.S1_at | CD403496   | 0.018032044 | ethylene-induced calmodulin binding protein (EICBP) |
| GmaAffx.92212.1.A1_s_at | CF807937 | 0.018428453 | MYB transcription factor (MYB92)    |
| Gma.5483.1.S1_s_at | CD414581   | 0.019563038 | basic-leucine zipper (bZIP105)      |
| Gma.7891.1.S1_at  | AW310625   | 0.020753499 | basic helix-loop-helix              |
| Gma.3632.1.A1_at  | BI316950   | 0.020828644 | zinc finger                         |
| Gma.17664.1.S1_at | AW348917   | 0.020828644 | zinc finger (DHHC type) family protein |
| Gma.752.1.A1_at   | AW432463   | 0.022830045 | helix-loop-helix DNA-binding        |
| Gma.7341.1.A1_s_at | CA953350  | 0.022830045 | aux/IAA protein (IAA3)              |
| GmaAffx.44143.1.S1_at | BU547730 | 0.023648433 | CCR4-Not complex component (Not1)   |
| GmaAffx.50295.1.S1_at | BI424123 | 0.024040827 | zinc finger (C2H2 type, AN1-like)   |
| GmaAffx.76537.1.S1_at | CD416147 | 0.025399823 | MYC1                               |
| Gma.5274.1.S1_at  | BM178426   | 0.025399823 | transcription factor EIL2           |
| GmaAffx.91768.1.S1_s_at | CF807493 | 0.031457154 | WRKY27                            |
| Gma.6571.2.S1_a_at | BE191621   | 0.031457154 | transcription initiation factor IIA (TFIIA) |
| Gma.4281.1.S1_at  | AW156348   | 0.037684072 | WRKY                              |
ing the overexpression of a potato (*Solanum tuberosum* L.) PPO in tomato (*Lycopersicon esculentum* Mill. cv. Money Maker), resulted in transgenic plants expressing 30-fold more PPO transcripts [107]. Quantification of PPO protein functionality showed a 5- to 10-fold increase in PPO activity in the transgenic plants [107]. Consequently, the overexpressing PPO transgenic lines produce 15-fold fewer lesions as well as strong inhibition of bacterial growth [107]. Bacterial population growth counts demonstrate at least a 100-fold reduction of bacterial populations in the infected leaves [107]. Thus, PPO could provide a terminal step in plant defense and may provide a localized resistance reaction to *H. glycines* infection.

WRKY transcription factor homologs, involved directly in plant defense, are also identified in syncytia undergoing a resistant reaction. WRKY transcription factors are important in defense [93–96, 98, 108]. Shen et al. [108] demonstrated that WRKY genes are important to the resistance response in the specific cells that contain the signaling proteins that are secreted by the pathogen. Shen et al. [108] demonstrated that this is accomplished through leucine rich repeat receptor-like kinase genes (LRRs) involved in resistance. Many LRRs are essential in gene-for-gene resistant (R) interactions [72]. Shen et al. [108] demonstrated that the signals were transduced through R-genes to WRKY transcription factors, resulting in resistance to the pathogen. Importantly, R genes have been shown to confer resistance to parasitic nematodes [73, 75]. WRKY gene expression in the syncytial cells during the resistance response is consistent with their suggested roles in plant defense.

The nonexpressor of PR genes (NPR1) (also known as *nim1* for noninducible immunity 1) and *sail* [109, 110] is the regulator of salicylic acid-mediated defense. Mutants of NPR1 block SA signaling in *A. thaliana* [100, 110–112]. In the uninduced state, NPR1 exists in the cytoplasm as an oligomer. The oligomer is formed through intermolecular disulfide bonds [113]. Oligomerization is mediated by S-nitrosylation of NPR1 by S-nitrosoglutathione which occurs at cysteine-156 [114]. During systemic acquired resistance (SAR), NPR1 experiences a thioredoxin-mediated reaction that results in its monomerization [114]. This monomerization is induced by mutations at residues Cys82 and Cys216 that facilitated NPR1 monomer accumulation. It also resulted in constitutive nuclear localization. Importantly, the monomerization promoted NPR1-mediated gene expression in the absence of the pathogen [114]. Experiments in *A. thaliana* using mutants in NPR1 (*npr1-2* and *npr1-3*), impaired in SA signaling, demonstrate an increased susceptibility to the beet cyst nematode *H. schachtii*.
Table 6: Select genes that were unique to the 8 dpi syncytia undergoing a compatible reaction but that were not differentially expressed as compared directly to the 3 dpi compatible syncytium samples (Figure 8(j)) comprising the Disease and Defense, Signaling, and Transcription categories.

| C-8 dpi as compared to C-3 dpi | Probe set ID | Public ID | Avg P-value | Gene |
|-------------------------------|--------------|-----------|-------------|------|
| **Disease & defense** | GmaAffx.8704.2.S1_at | BG042982 | 0.003021268 | Peroxidase |
| Gma.8512.1.S1_at | AF236108.1 | 0.014307059 | purple acid phosphatase |
| GmaAffx.93342.1.S1_at | CF809067 | 0.014526581 | glutathione peroxidase 1 |
| Gma.7301.1.S1_at | BM528250 | 0.019563038 | GAMMA-GLUTAMYL TRANSPEPTIDASE 3 (GGT3) |
| GmaAffx.59573.1.S1_at | AW350986 | 0.019951841 | purple acid phosphatase |
| Gma.13182.1.S1_at | CD392298 | 0.020669698 | copper-binding protein (CUTA) |
| Gma.320.1.S1_at | AF019116.1 | 0.024040827 | Peroxidase |
| Gma.11154.1.S1_at | AW309927 | 0.031457154 | elicitor-responsive gene |
| **Signal transduction** | GmaAffx.21217.3.S1_at | AW569872 | 0.004392849 | protein phosphatase 2C (PP2C) |
| Gma.4455.3.S1_at | CB063632 | 0.009290923 | PROTEIN KINASE 2B (APK2B) |
| Gma.2407.1.S1_at | BI970419 | 0.009755834 | putative protein kinase membrane-associated progesterone-binding protein 2 (ATMAPR2) |
| Gma.5304.2.S1_at | CD410657 | 0.01212639 | |
| Gma.1007.1.S1_at | CD402215 | 0.015096504 | calmodulin-related protein |
| Gma.2222.1.S1_at | CD416168 | 0.015413718 | inositol monophosphatase |
| Gma.596.1.S1_at | AF228501.1 | 0.015413718 | 14-3-3-like protein |
| GmaAffx.67957.1.S1_at | BG157622 | 0.016772715 | RhoGAP small G protein family protein |
| GmaAffx.73932.1.S1_at | BU550426 | 0.017952293 | CTR1-like protein kinase |
| Gma.4487.2.S1_at | AW508329 | 0.020753499 | calcium ion binding |
| GmaAffx.91867.1.S1_at | CF807592 | 0.028086024 | 14-3-3 protein |
| Gma.4629.1.A1_at | CA820195 | 0.028086024 | SGT1 |
| Gma.1518.2.S1_at | BM524684 | 0.030941813 | cysteine protease |
| GmaAffx.19821.1.S1_at | CA782536 | 0.030941813 | serine/threonine protein kinase |
| **Transcription** | GmaAffx.92861.1.S1_at | CF808586 | 0.002923594 | HIGH MOBILITY GROUP B 1 (HMGB1) |
| Gma.3419.2.S1_at | BE658641 | 0.005201937 | zinc finger, C2H2-type |
| Gma.6739.1.S1_at | AI856042 | 0.007667593 | RNA polymerase II 15.9 (RPB15.9) |
| GmaAffx.41946.1.S1_at | BM528357 | 0.009290923 | helix-loop-helix DNA-binding |
| GmaAffx.42667.1.S1_at | BU761164 | 0.011051366 | SCARECROW-LIKE 1 (SCL1) |
| Gma.6476.2.S1_at | BQ453135 | 0.011324733 | polynucleotidyl transferase |
| GmaAffx.30434.1.S1_at | BQ081227 | 0.011676724 | helix-loop-helix DNA-binding |
| Gma.4975.1.S1_at | BI970178 | 0.011756578 | zinc finger, CCCH-type-RING-type |
| Gma.6838.1.S1_at | AW349633 | 0.011756578 | NIM1-like protein 1 (NPR-1) |
| GmaAffx.58899.1.S1_at | BI317760 | 0.016307345 | C2-H2 zinc finger protein |
| Gma.16645.1.S1_at | BM143429 | 0.017952293 | no apical meristem (NAM) protein (NAC2) |
| GmaAffx.65829.1.A1_at | CD392418 | 0.020669698 | pathogenesis-related transcriptional factor and ERF |
Conversely, the npr1-suppressor mutation snl shows decreased susceptibility to the nematode [115]. Thus, the highly induced expression of thioredoxin during the resistance responses of G. max[PI 548402/Peking] is consistent with functional tests involving npr1-2 and npr1-3 in A. thaliana. Induced levels of NPR1 are not observed in syncytium samples of G. max[PI 548402/Peking]. Thioredoxin has been shown to be involved in this process [114]. Therefore, it is possible that thioredoxin transcription accompanies infection. Thus, thioredoxin could be recruited during the defense response to monomerize NPR1 already present in root tissues to accomplish the resistant reaction.

Calmodulin dependent protein kinases (CDPKs) such as calmodulin kinase II (CaMKII) are proteins reliant on calcium for their proper function. The identification of CaMKII indicates that calcium may be playing important roles in resistance. Calcium performs many interesting cellular roles. Calcium, as a second messenger, encodes information through Ca\(^{2+}\) gradients, amplitude, and oscillation frequency [116]. Thus, proteins relying on Ca\(^{2+}\) gradients and calmodulin may be important during the establishment of the resistant reaction. CaMKII functions by decoding Ca\(^{2+}\) oscillation frequencies [117]. At the cellular level, calmodulin is implicated in successful plant-pathogen interactions by its interaction with CDPKs. For example, the arbuscular mycorrhizal interaction in Medicago truncatula requires the CDPK, DMI3 [118]. Other symbioses as well are dependent on CDPKs [118, 119]. The expression analyses show that calmodulin may be performing some function analogous to those observed for the arbuscular mycorrhizal interaction in M. truncatula.

### 4.4. Orthogonality of the DCM.

The DCM has resulted in the identification of probe sets that measure detectable amounts of gene activity in one cell type (present) while absent in the other cell type (Figures 7 and 8). The DCM analysis has also identified genes that were common to the two cell types under investigation. As would be expected, there is orthogonality of the DCM probe set lists as compared to probe set lists obtained by the differential expression analysis method. However, since statistical analyses for differential expression analyses can only happen if statistically significant (e.g., measuring present) amounts of gene activity are present in the two cell types under study (e.g., A \(\cup\) B), many genes are eliminated from differential expression analyses. The elimination of the genes occurs because measurable amounts of gene activity as measured by a particular probe set are not present in one of the two samples under study. The exclusion of genes from differential expression analyses is probably less common and less of a problem when the RNA under study is obtained from a whole organism or whole organs (i.e., roots). The problem would be minimized in analyses of whole organisms or organs because they are composed of heterogeneous cell populations, each having unique gene expression programs. The RNA pools of those individual cell types become homogenized during the RNA extraction procedures. In contrast, LCM purifies cells to near homogeneity. Thus, gene expression of homogeneous samples of one cell type may be very different from gene expression found in their neighboring cells or a cell at an earlier point during its developmental process. As shown here, many genes are excluded from a differential expression analysis of nearly homogeneous populations of pericycle cells as compared to syncytia at various stages of their resistant or susceptible reactions. The genes identified in the DCM analyses that are present, but not differentially expressed, became the focus of the analysis presented here. As shown in the multiple analyses, genes that pertain to important classes of genes involved in various plant defense responses to pathogens have been identified by DCM.
5. Summary

The DCM was used to compare syncytium and pericycle samples isolated by LCM. The comparisons presented here are an alternative method of examining microarray gene expression data and are different from those presented in a differential expression analysis of the syncytium [26]. The DCM comparisons are powerful when considering that the cells under investigation are homogeneous (e.g., syncytia). The power of DCM is that it reveals that nearly homogeneous populations of cells have gene activity that is unique to each type. Importantly, differential expression analyses would miss the uniqueness of gene activity of the various cell types because gene expression data is required from each cell type for the analysis to be performed. Therefore, differential expression analyses actually may be underestimating the uniqueness of gene activity profiles for the different cell types under study. The genes identified here represent an additional and significant pool to take into consideration and explore with regard to the interaction between G. max and H. glycines. The genes can be investigated in functional analyses to study the interaction between G. max and H. glycines [31, 120]. In the broader sense, DCM should be seriously considered as an analysis tool when comparing homogeneous populations of cells.

Abbreviations

EST: Expressed sequence tag
hpi: Hours post inoculation
dpi: Days post inoculation
J2: Second stage juvenile
FS: Farmer’s solution
PFA: Paraformaldehyde
DEPC: Diethylpyrocarbonate
LCM: Laser capture microdissection
MRS: Moisture replacement system
DCM: Detection call methodology.

Acknowledgments

The authors greatly appreciate the continued support provided by the United Soybean Board under Grant 5214. The authors thank Dr. David Munroe and Nicole Lum at the Laboratory of Molecular Technology, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, MD 21701, USA for the Affymetrix array hybridizations and data acquisition. Brandon Le and Anhthu Bui of Dr. Robert Goldberg’s lab (University of California-Los Angeles) very kindly provided the G. max annotations. The authors thank Veronica Martins for careful editing of the manuscript. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

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