Technical advance

NEMATIC: a simple and versatile tool for the *in silico* analysis of plant–nematode interactionsJAVIER CABRERA¹, REGLA BUSTOS², BRUNO FAVERY³, CARMEN FENOLL¹ AND CAROLINA ESCOBAR^{1,*}¹Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-La Mancha, Avenida de Carlos III s/n, 45071 Toledo, Spain²Centro de Biotecnología y Genómica de Plantas U.P.M. I.N.I.A., Campus de Montegancedo, 28223, Pozuelo de Alarcón (Madrid), Spain³Institut Sophia Agrobiotech, Université de Nice Sophia Antipolis, INRA UMR 1355 CNRS UMR 7254, 400 route des chappes, BP 167, 0690 Sophia Antipolis Cedex, France**SUMMARY**

Novel approaches for the control of agriculturally damaging nematodes are sorely needed. Endoparasitic nematodes complete their life cycle within the root vascular cylinder, inducing specialized feeding cells: giant cells for root-knot nematodes and syncytia for cyst nematodes. Both nematodes hijack parts of the transduction cascades involved in developmental processes, or partially mimic the plant responses to other interactions with microorganisms, but molecular evidence of their differences and commonalities is still under investigation. Transcriptomics has been used to describe global expression profiles of their interaction with *Arabidopsis*, generating vast lists of differentially expressed genes. Although these results are available in public databases and publications, the information is scattered and difficult to handle. Here, we present a rapid, visual, user-friendly and easy to handle spreadsheet tool, called NEMATIC (NEMatode–*Arabidopsis* Transcriptomic Interaction Compendium; <http://www.uclm.es/grupo/gbbmp/english/nematic.asp>). It combines existing transcriptomic data for the interaction between *Arabidopsis* and plant-endoparasitic nematodes with data from different transcriptomic analyses regarding hormone and cell cycle regulation, development, different plant tissues, cell types and various biotic stresses. NEMATIC facilitates efficient *in silico* studies on plant–nematode biology, allowing rapid cross-comparisons with complex datasets and obtaining customized gene selections through sequential comparative and filtering steps. It includes gene functional classification and links to utilities from several databases. This data-mining spreadsheet will be valuable for the understanding of the molecular bases subjacent to feeding site formation by comparison with other plant systems, and for the selection of genes as potential tools for biotechnological control of nematodes, as demonstrated in the experimentally confirmed examples provided.

Keywords: functional classification, galls, giant cells, *in silico* analysis, plant–nematode interactions, syncytia, transcriptome comparison.

INTRODUCTION

Root-knot and cyst nematodes (RKNs and CNs) are obligate sedentary plant endoparasites that produce serious economic losses all over the world (Nicol *et al.*, 2011). RKNs and CNs induce their specialized feeding cells, termed giant cells (GCs) and syncytia, respectively, which they use for nourishment until reproduction within the root vascular cylinder (reviewed in Jones and Goto, 2011; Sobczak and Golinowski, 2011). Both nematode feeding cell (NFC) types are multinucleated, have dense cytosol and suffer endoreduplication (de Almeida Engler *et al.*, 2012). GCs induced by RKNs are immersed in galls, ‘pseudo-organs’ caused by root swelling and tissue hyperplasia (Bird, 1996). GCs develop from vascular cell precursors suffering repeated mitosis with aborted cytokinesis (Caillaud *et al.*, 2008). In contrast, syncytia develop from vascular cells, mainly procambial cells, which fuse and are incorporated into the syncytium (Hussey and Grundler, 1998). Although cytological and histological studies have described clear structural and morphological differences between both NFCs (Jones, 1981), molecular evidence of their differences and commonalities is still under investigation. Most of this evidence comes from the study of *Arabidopsis* promoter trap lines infected with either *Heterodera schachtii* (CN) or *Meloidogyne incognita* (RKN), some showing common or contrasting activation patterns in both NFCs (Barthels *et al.*, 1997; Goddijn *et al.*, 1993). Other comparisons have also been established over the years based mainly on the differential expression patterns of individual genes (reviewed in de Almeida Engler and Favery, 2011; Gheysen and Fenoll, 2002; Gheysen and Mitchum, 2009).

Over the last decade, microarray technology has been useful to elucidate the global expression profiles of *Arabidopsis*–RKN and *Arabidopsis*–CN interactions (Barcala *et al.*, 2010; Escobar *et al.*,

*Correspondence: Email: carolina.escobar@uclm.es

2011; Fuller *et al.*, 2007; Jammes *et al.*, 2005; Puthoff *et al.*, 2003; Szakasits *et al.*, 2009). Vast lists of Arabidopsis differentially expressed genes (DEGs) identified from comparisons between infected and uninfected roots are scattered across public databases and publications (reviewed in Escobar *et al.*, 2011). Initially, transcriptomic analyses were performed for whole roots or for nematode-induced organs, such as galls or roots containing syncytia (Fuller *et al.*, 2007; Jammes *et al.*, 2005; Puthoff *et al.*, 2003). The combination of transcriptomic analysis with newly developed cell biology techniques, such as laser capture microdissection and cytoplasm microaspiration, represented a step forward in the understanding of the specifically differentiated NFCs (Barcala *et al.*, 2010; Szakasits *et al.*, 2009). These approaches included not only Arabidopsis, but other plant species, such as *Glycine max* (Alkharouf *et al.*, 2006; Ithal *et al.*, 2007; Khan *et al.*, 2004; Klink *et al.*, 2007, 2009), tomato (Portillo *et al.*, 2013) and rice (Kyndt *et al.*, 2012). In parallel, microarray technology has been used in different fields of plant sciences to generate wide-ranging transcriptomic information on the genes involved in key processes, such as hormone responses (Nemhauser *et al.*, 2006), cell cycle regulation (Menges *et al.*, 2003) and root development (Brady *et al.*, 2007), generating complex datasets included in large databases.

It is believed that endoparasitic nematodes capture part of the transduction cascades involved in developmental processes, such as lateral root formation, or partially mimic the plant responses to other interactions with microorganisms, i.e. symbiosis or *Agrobacterium tumefaciens* (Barcala *et al.*, 2010; J. Cabrera *et al.*, unpublished data; Damiani *et al.*, 2012; Grunewald *et al.*, 2009; Mathesius, 2003). Commonalities and differences among transcriptomes of the plant–nematode interactions included in different databases could be very useful for understanding the molecular bases of plant–nematode interactions. Yet, many of the data are scattered, heterogeneous and difficult to handle in order to compare global transcriptional signatures of distinct plant–nematode interactions. The creation of visual and user-friendly Excel-based tools that facilitate the handling of the extensive amounts of genomic data based on the available transcriptomes would constitute a great advance to obtain conclusions from these comparative holistic approaches for different biological processes (Ogata *et al.*, 2009; Parizot *et al.*, 2010).

Thus, we developed a rapid, visual, user-friendly and easy to handle spreadsheet tool compiling existing transcriptomic data for the interaction between Arabidopsis and plant-endoparasitic nematodes. These range from gall transcriptomes at different developmental stages (Barcala *et al.*, 2010; Jammes *et al.*, 2005) and root segments infected with CNs (Puthoff *et al.*, 2003) to the transcriptomes of isolated NFCs (GCs, Barcala *et al.*, 2010; syncytia, Szakasits *et al.*, 2009). We also integrated data from different transcriptomic analyses regarding hormone and cell cycle regulation, development, different plant tissues and cells, and different

biotic stresses, as well as the functional classification of genes and links to different gene utilities from several databases, in a spreadsheet tool called NEMATIC (NEMatode–Arabidopsis Transcriptomic Interaction Compendium; <http://www.uclm.es/grupo/gbbmp/english/nematic.asp>). We provide three different examples for the use of this tool for a better understanding of its utility. We expect that NEMATIC, which will be periodically updated, will be useful to address questions and hypotheses regarding plant–nematode interactions in a simple and rapid manner, and therefore will facilitate knowledge advancement in this field.

RESULTS

NEMATIC, a simple and interactive tool for high-throughput biological data analysis centred on plant–nematode interactions

A spreadsheet tool (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>) was generated using Excel 2007 software for rapid, high-throughput analysis of biological data centred on plant–nematode interactions. It is composed of seven different sheets grouped into three different clusters according to their utilities.

1. 'Plant–Nematode' and 'Custom Selection' sheets: searching, selecting and filtering the genes of interest

In the 'Plant–Nematode' sheet, DEGs from Arabidopsis–nematode interactions and from transcriptomes considered to be of special interest were collected and fused in a single table. A dropdown menu was implemented to allow selection among the gene sets compiled in the tool. (i) A total of 9688 Arabidopsis loci found to be differentially expressed in any of the Arabidopsis–nematode transcriptomes compiled (Table 1; Fig. 1). These are laser micro-dissected GCs at 3 days post-inoculation (dpi) (Barcala *et al.*, 2010), hand-sectioned galls at 3, 7, 14 and 21 dpi (Barcala *et al.*, 2010; Jammes *et al.*, 2005), microaspirated syncytia at 5 and 15 dpi (treated together as originally described, because very few transcripts were different between the two time points; Szakasits *et al.*, 2009) and roots containing syncytia at 3 dpi (Puthoff *et al.*, 2003). The log₂ values shown were calculated by comparing the infected material with the corresponding uninfected control roots, all described in the references (Table S1, see Supporting Information). Only experiments with more than two independent biological replicates were considered, i.e. more than two independent chips hybridized. (ii) All genes classified by MapMan (Thimm *et al.*, 2004) into 34 functional categories. (iii) A total of 7002 up- and down-regulated genes after exogenous treatment with abscisic acid (ABA), gibberellins (GL), auxin (indole-3-acetic acid, IAA), ethylene (acetyl-CoA carboxylase, ACC), cytokinins (CK), brassinosteroids (BR) and jasmonate (JA) (Nemhauser *et al.*, 2006). (iv) A compiled list of 2620 transcriptional regulators classified into 30 families (Mitsuda and Ohme-Takagi, 2009)

Table 1 Common differentially expressed (DE) genes between pairs of plant–nematode transcriptomes compiled in the spreadsheet tool NEMATIC.

Number of common DE genes	3-dpi GCs	3-dpi galls	7-dpi galls	14-dpi galls	21-dpi galls	5 + 15-dpi syncytia	Roots containing 3-dpi syncytia
3-dpi GCs (Barcala <i>et al.</i> , 2010)	–	120	127	211	223	529	6
3-dpi galls (Barcala <i>et al.</i> , 2010)	–	–	91	131	147	248	15
7-dpi galls (Jammes <i>et al.</i> , 2005)	–	–	–	642	741	738	17
14-dpi galls (Jammes <i>et al.</i> , 2005)	–	–	–	–	1398	921	20
21-dpi galls (Jammes <i>et al.</i> , 2005)	–	–	–	–	–	1102	25
5 + 15-dpi syncytia (Szakasits <i>et al.</i> , 2009)	–	–	–	–	–	–	57
Roots containing 3-dpi syncytia (Puthoff <i>et al.</i> , 2003)	–	–	–	–	–	–	–

dpi, days post-inoculation; GC, giant cell.

originating from four representative databases of Arabidopsis: RARTF (rarge.gsc.riken.jp/rartf/) (Iida *et al.*, 2005), AGRIS (arabidopsis.med.ohio-state.edu/AtTFDB/) (Davuluri *et al.*, 2003), DATF (datf.cbi.pku.edu.cn/) (Guo *et al.*, 2005) and PlnTFDB (plntfdb.bio.uni-potsdam.de/v2.0/index.php?sp_id=ATH) (Riano-Pachon *et al.*, 2007). (v) A total of 1033 up-regulated genes during G1, G2, M and S cell cycle phases (Menges *et al.*, 2003; Parizot *et al.*, 2010). (vi) A total of 4790 genes enriched in 31 specific root cell types: lateral root cap, columella, non-hair cells, hair cells, cortex, endodermis, quiescent centre, stele, mature pericycle, xylem pole pericycle, phloem pole pericycle, lateral root primordia initials, maturing xylem cells, protoxylem and two-thirds of metaxylem, pericycle, protoxylem, metaxylem, phloem, developing proto-phloem and metaphloem, and companion cells (Brady *et al.*, 2007).

Another option, named 'Custom Selection' was included in the dropdown menu, by which it was possible to select a customized list of genes (Fig. 1) previously introduced into the 'Custom Selection' sheet.

The output generated after selection in the dropdown menu showed information on all the DEGs in any plant–nematode transcriptome compiled. The output was organized into 25 columns grouped into seven clusters: (i) seven columns with the log₂ values obtained from the reference publications for each gene selected (Barcala *et al.*, 2010; Jammes *et al.*, 2005; Puthoff *et al.*, 2003; Szakasits *et al.*, 2009); (ii) one column with the Arabidopsis Genome Initiative (AGI) code description from TAIR10; (iii) one column with the corresponding MapMan category (Thimm *et al.*, 2004); (iv) 14 columns with hormone regulation (Nemhauser *et al.*, 2006); (v) four columns with the cell cycle phase in which a gene was expressed (Menges *et al.*, 2003; Parizot *et al.*, 2010); (vi) one column with the transcription factor (TF) family to which the gene belonged (Mitsuda and Ohme-Takagi, 2009); and (vii) enrichment in a certain root cell type (Brady *et al.*, 2007; Fig. 1). Each row corresponded to a specific AGI code. The information was only shown if the gene was differentially expressed in any plant–nematode transcriptome compiled. The table cells were conditionally coloured to facilitate visualization according to the fold change, with red indicating induced, green repressed and grey not differentially expressed or not represented.

A filter button was added to the header of each transcriptome and information columns, facilitating the filtering of genes depending on the objectives of each user through the colour code or text filters. Filters were additive, i.e. it was possible to select sets of genes fulfilling more than one condition among the transcriptomes, e.g. up-regulated in a specific transcriptome, but down-regulated by a certain hormone.

In addition, a summary table showing the number of genes from the query found in each transcriptome was retrieved, as well as the percentages of these genes relative to the total number of genes in the plant–nematode set.

2. 'Anatomy', 'Cell Types', 'Development' and 'Biotic Stress' sheets: selecting and filtering genes by Genevestigator expression values

Data from experiments included in the Genevestigator database, performed on ATH1 Affymetrix 22K arrays (Hruz *et al.*, 2008), for each of the DEGs in any plant–nematode transcriptome (9688 genes), were collected. Thus, the corresponding Affymetrix probes for each AGI code and the expression values of different experiments divided in terms of anatomy, biotic stress, cell types and development could be easily accessed for the previously filtered genes on plant–nematode interactions by pasting them into the first column of these sheets (Fig. 1; Hruz *et al.*, 2008).

The 'Anatomy' sheet included experiments from 59 plant tissues/organs of seedlings, inflorescences, leaves and roots. The 'Cell Types' sheet included experiments from 20 different cell types. The 'Development' sheet included experiments from germinated seeds to mature siliques (nine stages). The 'Biotic Stress' sheet included experiments involving fungi infection/exposure (*Alternaria brassicicola*, *Botrytis cinerea*, *Blumeria graminis*, *Erysiphe* or *Golovinomyces cichoracearum*, *E. orontii*, *Gigaspora rosea*), insects (*Bemisia tabaci*, *Myzus persicae*), oomycetes (*Phytophthora infestans*, *P. parasitica*), bacteria (*Pseudomonas syringae*, *Escherichia coli*), viruses (*Cabbage leaf curl virus*, *Turnip mosaic virus*) and nematodes (*M. incognita* and *H. schachtii*) distributed in 118 columns. A custom selection of different categories from each group was possible. The corresponding Affymetrix probes for each AGI code in the input list were also provided.

Fig. 1 Flow chart representing an overview of the information captured and the putative different input data and the outputs obtained in the spreadsheet.

For the categories of Anatomy, Cell Types and Development, the table obtained displayed the average expression value of all samples calculated from those annotated as belonging to this category in Genevestigator. For the Biotic Stress category, the gene expression responses were calculated as $\log_2$ ratios between the experimental and control samples. The resulting values thus reflect the up- or down-regulation of genes.

A colour code was facilitated according to the intensity of the expression of a certain gene in a category (see Experimental Procedures S1, Supporting Information, for a detailed description of the colour code). The filter buttons implemented in each column allowed the filtering of genes by their expression values.

3. 'Gene Info' sheet: compiled information about filtered genes

A 'Gene Info' sheet was also provided to show information about any group of selected DEGs in the plant–nematode transcriptomes compiled and previously filtered, by pasting them into the first column of the sheet. For each AGI code, the TAIR description, MapMan category, subcellular localization, microRNA regulation, hormone regulation, KEGG pathways, InterPro domains and Salk mutant lines (Fig. 1) were provided. A system of filters could also be used in this sheet to select genes fulfilling certain restrictions. Putative functions, interaction networks, cross-species homology, co-expression analysis, mutant and transgenic lines available, etc., could also be easily obtained by clicking in the link provided for rapid access to 19 databases: TAIR, KEGG, GRAMENE, AGRIS, PLAZA, NCBI, EnsemblPlants, COP, PPDB, Genevestigator, e-NORTHERN, ATTED-II, eFPBrowser, AtGenExpress, PUBMED, GeneMania, AHD2.0, Salk and MapMan.

Gene information concerning descriptions, InterPro domains, KEGG pathways, subcellular localizations or microRNA regulation was downloaded from the TAIR FTP download section (<http://www.arabidopsis.org/download/index.jsp>). Salk mutant lines were obtained from the Salk homozygote T-DNA collection (<http://signal.salk.edu/cgi-bin/homozygotes.cgi>)

Example of utility 1: TFs induced by auxins in galls formed by *Meloidogyne* spp.

In this example, one of the most interesting advantages offered by this tool is shown. The user can score for genes fulfilling previously designed restrictions or limits through the subsequent use of filters in the data compiled in the spreadsheet tool.

Transcriptional regulators are expected to play a significant role in the cell differentiation processes that occur during the establishment of RKNs and CNs (reviewed by Gheysen and Mitchum, 2009), as they are key integrators modifying downstream gene expression to generate diverse plant cell responses. The expression profiles of genes encoding Arabidopsis TFs in galls induced by RKNs could be easily obtained in NEMATIC. As a first step, we selected the option 'Transcriptional Regulation//All (2620)' in the

dropdown menu and, in a few seconds, the expression values in 3-, 7-, 14- and 21-dpi galls of the 2620 TFs from the four different databases compiled (RARTF, AGRIS, DATF and PlnTFDB) were obtained.

Many plant TFs are usually regulated by phytohormones. Among all the phytohormones described, auxin has been the most studied in the interaction between Arabidopsis and plant-endoparasitic nematodes, particularly centred on auxin transport and accumulation in NFCs (Grunewald *et al.*, 2009; reviewed by Goverse and Bird, 2011). However, very few examples of auxin-regulated TFs are known, and the knowledge of their putative functions in either galls or syncytia formation or maintenance is still scarce (J. Cabrera *et al.*, unpublished data; Grunewald *et al.*, 2008). Therefore, in this context, as a second step, those TFs that were up-regulated in response to exogenous auxin in the study of Nemhauser *et al.* (2006) were selected after filtering by 'up-regulated by auxins'.

In the third step, among those TFs up-regulated by auxins filtered previously, only TFs up-regulated in any of the developmental stages of galls recorded were subsequently selected. From the initial 2620 TFs, a list of 13 genes was generated (Table 2). Among them are *LBD16* and *WRKY23* (Table 2), shown to be induced in galls using reporter lines (J. Cabrera *et al.*, unpublished data; Grunewald *et al.*, 2008, respectively). Moreover, functional studies showed that the suppression of *LBD16* function resulted in infection resistance to *M. javanica* in Arabidopsis, similar to the reduction in infection observed for *H. schachtii* in loss-of-function lines of *WRKY23* when compared with the wild-type (J. Cabrera *et al.*, unpublished data; Grunewald *et al.*, 2008). These experimental results validate the output obtained after application of the different set-up filters from NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>), as experimental data demonstrated that both TFs are induced by auxins, and during plant–nematode interactions, reinforcing the utility of the selection. In addition, RAP2.6L, an ethylene responsive factor homologue of RAP2.6, with a functional role in the *H. schachtii*–Arabidopsis interaction (Ali *et al.*, 2013), was detected by NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>) as also induced in galls.

Other TFs up-regulated by auxins and in galls from the output list, such as *LBD33* and *WRKY45*, belong to the same families as *LBD16* and *WRKY23*, respectively (Table 2). In parallel with *LBD16* function, *LBD33* has been shown to mediate lateral root organogenesis in combination with *LBD18* (Berckmans *et al.*, 2011). Another TF in the list, *LAX2*, regulates auxin influx into the cell, together with *LAX1* and *LAX3* (Yang *et al.*, 2006), and is expressed in lateral root primordia and emerging lateral root, but not in syncytia at any time during infection (Lee *et al.*, 2011). However, a related member, *LAX3*, interacts with 19C07, an effector from CNs, to control feeding site development. In addition to these TFs, seven other interesting TFs selected by our search have not yet been related, either directly or indirectly, to the plant–

	Log ₂ ratio in gall transcriptomes			
	3-dpi galls	7-dpi galls	14-dpi galls	21-dpi galls
AT1G29950	–	–	–	0.7
AT2G21050 (LAX2)	–	–	–	1.4
AT2G42430 (LBD16)	–	1.1	–	–
AT2G47260 (WRKY23)	1.4	–	–	–
AT3G01970 (<i>WRKY45</i>)	–	0.9	–	–
AT3G60530 (<i>GATA4</i>)	–	0.7	–	–
AT4G37790 (<i>HAT22</i>)	–	–	0.8	–
AT5G06080 (<i>LBD33</i>)	1.4	–	–	–
AT5G13330 (<i>Rap2.6L</i>)	1.1	–	–	0.8
AT5G25190 (<i>ESE3</i>)	–	–	–	0.7
AT5G47370 (<i>HAT2</i>)	–	1.2	1.2	1.8
AT5G48150 (<i>PAT1</i>)	–	–	–	0.9
AT5G65670 (<i>IAA9</i>)	–	–	–	0.6

dpi, days post-inoculation.

nematode interaction (Table 2). For example, *HAT2* is a TF preferentially expressed in the early stages of lateral root formation, and has been suggested to be involved in auxin-induced morphogenesis (Sawa *et al.*, 2002). The roles of these TFs in galls induced by RKNs deserve further investigation.

Example of utility 2: phytohormone-related expression profiles in NFCs

Phytohormones are signalling molecules that regulate plant development and cell differentiation, active processes occurring during NFC formation (reviewed in Goverse and Bird, 2011). Therefore, changes in hormone levels in NFCs are likely to mediate, in part, the drastic transcriptional changes observed that are necessary for the successive establishment of the nematode. The transcriptional data obtained by Nemhauser *et al.* (2006), i.e. those genes up- or down-regulated after the exogenous addition of ABA, GL, IAA, ACC, CK, BR and JA, obtained under the same experimental conditions and in the same laboratory, were compiled in NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>). These sets of genes can be selected in the dropdown menu, facilitating the visualization of hormone-regulated genes across the plant–nematode interactions. As an example, we can deepen our understanding of hormone transduction pathways and their regulatory networks underlying NFC formation (GCs and syncytia). First, we select from the dropdown menu the groups of up- or down-regulated genes for each hormone (Fig. 2). Second, making use of the filters placed above the GCs and syncytia transcriptomes, we filter by colour (red for up-regulated; green for down-regulated) those genes from the plant–nematode transcriptomes with the same regulation as the selected group of hormone-regulated genes (Fig. 2; Table S2, see Supporting Information). In addition, a summary table indicating the number of genes found in each NFC transcriptome is shown each time we introduce a set of genes from the dropdown menu.

Table 2 Genes filtered with the NEMATIC spreadsheet fulfilling three restrictions: (i) classified as plant transcription factors in any four different databases (RARTF, AGRIS, DATF and PlnTFDB); (ii) induced by auxins in Nemhauser *et al.* (2006); and (iii) up-regulated in any of the gall developmental stages recorded. In bold, those transcription factors (TFs) experimentally studied during plant–nematode interactions.

A bar chart summarizing the data obtained for each group of hormone-responsive genes is shown in Fig. 2. The predominant hormone-responsive genes from the up-regulated genes that are enriched in GCs relative to the exogenous application of phytohormones were those up-regulated by IAA, whereas those regulated by CK were enriched in the up-regulated genes of syncytia (Fig. 2). The proportion of ACC up-regulated genes was low among the up-regulated genes of syncytia and those responding to CK in GCs. Strikingly, IAA- and CK-repressed genes were also predominant in GCs and syncytia relative to the proportion of genes after exogenous treatment (Fig. 2). This suggests that the repression of auxin and/or CK-regulated genes may be crucial in both feeding site types. Most studies so far have focused on genes regulated by IAA or CK that are up-regulated in feeding sites. Therefore, NEMATIC is useful to glimpse the main differences found between the hormone transcriptional balances of the two types of NFC. We noted a high percentage of IAA up-regulated genes (26%) in GCs, but of CK up-regulated genes (21%) in syncytia (Fig. 2; Table S2). Although, in syncytia, there were more genes induced than repressed (121 and 84, respectively; Table S2), in GCs, there were 12.5 times more genes down-regulated than up-regulated by CK (25 and two genes, respectively; Table S2), therefore showing a clear repression (Fig. 2) in GCs. An interesting and novel finding was the observation that IAA- and CK-repressed genes are predominantly repressed respect to the experiment of Nemhauser *et al.* (2006) in both feeding sites.

Although further expression and functional analysis should be performed to validate these results, NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>) allowed us to obtain, in a rapid manner, a general view of hormone-related transcriptional regulation during the plant–nematode interaction. Moreover, the selection of genes responsive to hormones less studied in plant–nematode interactions, such as BR or GL, could also be of great interest.

Fig. 2 Right panel: percentage of hormone-responsive genes from each nematode feeding cell transcriptome responding to a specific hormone. Red bars indicate up-regulated genes and green bars indicate down-regulated genes. GCs, giant cells. Syncytia. Hormone treatments, transcript profile after exogenous treatment of the hormones indicated following Nemhauser *et al.* (2006). Left panel: flow chart showing the two-step procedure used to obtain the data represented in the right-hand graphs.

Example of utility 3: selection of 'a top list of genes' after sequential filtering

One of the main problems encountered after data analysis from holistic gene expression experiments is the huge amount of data obtained. There is an increased need to filter these output data in order to select some of these genes to raise hypotheses that could lead to direct biotechnological purposes. With NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>), it is possible to obtain a particular list of genes with a 'suitable selected' expression profile during plant–nematode interactions. These output data, followed by a detailed analysis of gene expression patterns during plant development and different biotic interactions, can be subsequently filtered, thus providing a sound aid to the selection and design of biotechnology-based tools for nematode control. For this purpose, we provided a set of independent filter-based tools that could be used for these multiple comparisons. The first step is to filter genes through comparisons with the plant–nematode interaction expression data available in Arabidopsis (Fig. 1). The

following steps can filter genes depending on their regulation in different Arabidopsis organs, developmental stages and/or cell types, together with their expression patterns during all biotic interactions contained in Genevestigator, hormonal regulation, etc. (Fig. 1). Finally, detailed and integrated information from different Arabidopsis databases can be obtained for each gene, allowing the selection of desired groups of genes, based on the information of transgenic and mutant lines available, orthologous genes, genes co-expressed in various biological processes, protein information, interactomes, references from functional analyses, etc., leading to the easy selection of a reduced number of genes (Fig. 1) for further experimental work.

To facilitate the understanding of this type of analysis, an example is given using NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>) applied to a particular comparison of genes up-regulated in GCs (Table S3, see Supporting Information) for a hypothetical biotechnological application. We follow the initial hypothesis: 'Genes fulfilling the following requirements: (i) up-regulated in GCs; (ii) with a fold change in GCs and syncytia

higher than 2.5; and (iii) barely expressed in selected root cell types and root parts'. Using the 310 up-regulated genes in GCs as the query list, obtained from the dropdown menu in the 'Plant–Nematode' sheet, we filtered (making use of the filter by text button above the column) those genes with a fold change ≥ 2.5 in GCs and syncytia (eight genes; Table S3). From these eight genes, those with very low expression (≤ 0.1) in roots were filtered (filtering by the colour code; in the 'Anatomy' sheet: roots, primary root, root tip, meristematic zone, elongation zone, root hair zone, stele, pericycle or lateral root; in the 'Cell types' sheet: protoplasts from any root tissue; two genes; Table S3). Therefore, from a list of 310 genes, only two genes fulfilled the desired expression requirements. Thereafter, we can consult the information provided for these two genes from the different databases compiled in the 'Gene Info' sheet (and, if necessary, apply new filters based on the MapMan category, hormone regulation, microRNA information, KEGG pathway, etc.). *At4g10270* encodes a wound-responsive protein and *At2g28950* encodes an expansin (*AtEXPA6*), whose expression in syncytia and GCs has been validated and confirmed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) by Wicczorek *et al.* (2006) and Barcala *et al.* (2010). These genes could possibly be used for biotechnological purposes in various ways. For example, their promoters, expected to be active in both feeding cell types, but with no or low activity in the rest of the root, could drive the expression of cytotoxic or anti-nematode proteins in rootstocks.

DISCUSSION

In the last decade, microarray technology has been used to reveal the molecular aspects underlying the interaction between the model plant *Arabidopsis thaliana* and the plant-endoparasitic nematodes *Meloidogyne* spp. and *Heterodera* spp. A large amount of data and extensive lists of DEGs have been generated (Escobar *et al.*, 2011; Kyndt *et al.*, 2012; Portillo *et al.*, 2013). These data are scattered in their corresponding publications or in databases, making it difficult to cross-compare the expression profiles among transcriptomes. It is also arduous to determine how a group of genes of interest is regulated in the different plant–nematode transcriptomes. Moreover, other transcriptomes have been released, such as those corresponding to cell cycle or hormone regulation, which could be of great interest for cross-comparison with those of plant–nematode interactions. However, the available tools for these holistic comparisons are not easy to handle.

Similar to the spreadsheet developed by Parizot *et al.* (2010) in the context of lateral root formation, we present here a simple and useful tool, NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>), which compiles transcriptomic data on plant–nematode interactions and adds other interesting transcriptomes and groups of genes, facilitating rapid data handling. With NEMATIC, it is possible to obtain the expression profiles of a list of

genes in the plant–nematode transcriptomes in a rapid manner. The query list can be either a personal set of genes of interest or a list of those predefined in the spreadsheet tool, e.g. those regulated by hormones (Nemhauser *et al.*, 2006), those preferentially expressed in each cell type of the root (Brady *et al.*, 2007), those from different cell cycle phases (Menges *et al.*, 2003), those from each MapMan category or those encoding TFs (Mitsuda and Ohme-Takagi, 2009). The output list shows the expression profiles of the selected genes in the Arabidopsis–nematode transcriptomes, as well as hormone regulation, cell cycle information, TF family and the specific root cell type for the DEGs. The compilation of these transcriptomes and the use of filters in the spreadsheet output, with their multiple combinations, becomes a powerful and useful tool to select candidate genes within the vast amount of existing data. In an easy manner, genes fulfilling different criteria across several transcriptomes can be extracted. It is important to note that all query and filter steps can be performed in a user-friendly, easy and intuitive way, making this tool useful for all scientists in the field with no bioinformatics background.

NEMATIC is centred in the model plant *Arabidopsis thaliana*. However, microarray experiments have been performed to study plant–nematode interactions in other plant species, such as *Glycine max* (Alkharouf *et al.*, 2006; Ithal *et al.*, 2007; Khan *et al.*, 2004; Klink *et al.*, 2007, 2009), tomato (Portillo *et al.*, 2013) and rice (Kyndt *et al.*, 2012). We have focused NEMATIC exclusively in Arabidopsis as a starting point, because there were accessible data from two different plant–nematode groups (RKNs and CNs), with specific information at the tissue and cellular level, and the great majority of the input data came from our own group. In addition, Arabidopsis is a model for dicotyledonous plant species with easy access to different databases providing massive complementary information. However, NEMATIC can be edited easily to introduce new transcriptomes obtained from other plant systems if the user is interested. NEMATIC aims to be the starting point for the construction of a full database with all transcriptomic (currently available or future) data related to plant–nematode interactions in different plant species, such as rice and several legumes.

The examples provided in the present work highlight the potential of the NEMATIC spreadsheet tool. In a few seconds, it is possible to select a short list of TFs induced by auxins and up-regulated in galls (Example 1, Table 2). Among them, we detected genes already validated in galls (*WRKY23* and *LBD16*) or studied in the context of CNs (*LAX2*), but we also extracted a new set of candidate TF-coding genes with similar characteristics, from the vast amount of existing data, paving the way for future studies on the molecular basis of the plant–nematode interaction (Table 2).

NEMATIC is also a good option for a general overview of how genes from particular functional categories perform during the plant–nematode interaction. In the second example provided, we

showed the trends of genes regulated by hormones in RKN and CN feeding cells (Fig. 2); a similar analysis could be performed with genes from other categories of interest, such as the cell wall, cell cycle or secondary metabolism. Novel results could also be obtained easily, although they require further validation, such as the large proportion of auxin down-regulated genes in NFCs (Fig. 2; Table S2). The proportion of auxin-regulated genes in NFCs is important with regard to both up- and down-regulated genes, but 136 and 54 auxin down-regulated genes (Nemhauser *et al.*, 2006) were also down-regulated in syncytia and GCs, respectively, whereas 61 and 20 were up-regulated (Table S3). As the study of this hormone in relation to the plant–nematode interaction has been centred in the up-regulated genes, the study of the many down-regulated genes identified using NEMATIC may change the view of auxin-regulated genes during plant–nematode interactions.

In the third example, we showed that the use of conventional Excel filters opens up a pathway to obtain many different combinations of genes fulfilling various requirements. For instance, it is possible to identify rapidly genes oppositely regulated in RKNs and CNs, those unique for each interaction or those commonly regulated. Although the genes and the results obtained with this tool must be studied functionally, it may represent a good starting point to select candidate genes for future validation (Table S3).

In conclusion, databases compiling data on transcriptomics from plant–nematode interactions are still lacking. The friendly and visual spreadsheet tool NEMATIC contains the data available in complex datasets from the main plant-endoparasitic nematodes interacting with the model plant *Arabidopsis*. This is the first time that a genome-wide resource for data mining, which facilitates efficient *in silico* studies on plant–nematode biology, has been made available. The main utilities of the tool are rapid cross-comparisons with other complex datasets and the acquisition of customized gene selections through sequential comparative and filtering steps. Therefore, NEMATIC is an efficient and easy to handle instrument for the selection of genes with biotechnological potential in the control of plant-endoparasitic nematodes, and in the basic molecular understanding of plant–nematode interactions, accelerating collective progress in the functional genomics of plant–nematode interactions.

EXPERIMENTAL PROCEDURES

NEMATIC (<http://www.uclm.es/grupo/gbbmp/english/nematic.asp>) has been constructed using Microsoft Excel 2007 and can also be opened using Microsoft Excel 2010 for Windows XP, Windows 7 or Mac Os X. The spreadsheet has been saved in the 'Office Excel 2007 Binary' file format (.xlsb extension), which minimizes its size. The .xlsb extension format is suitable to store files that contain a large amount of data and can be read rapidly. More detailed information on the use and content of NEMATIC is described in Experimental Procedures S1.

ACKNOWLEDGEMENTS

This work was supported by the Spanish Government (AGL2010-17388 to CE and CSD2007-057 to CF) and the Castilla-La Mancha Government (PCI08-0074-0294 to CF and CE). JC was supported by a fellowship from the Ministry of Education, Spain. The authors have no conflicts of interest to declare.

REFERENCES

- Ali, M.A., Abbas, A., Kreil, D.P. and Bohlmann, H. (2013) Overexpression of the transcription factor RAP2.6 leads to enhanced callose deposition in syncytia and enhanced resistance against the beet cyst nematode *Heterodera schachtii* in *Arabidopsis* roots. *BMC Plant Biol.* **13**, 47.
- Alkharouf, N.W., Klink, V.P., Chouikha, I.B., Beard, H.S., MacDonald, M.H., Meyer, S., Knap, H.T., Khan, R. and Matthews, B.F. (2006) Timecourse microarray analyses reveal global changes in gene expression of susceptible *Glycine max* (soybean) roots during infection by *Heterodera glycines* (soybean cyst nematode). *Planta*, **224**, 838–852.
- de Almeida Engler, J. and Favery, B. (2011) The plant cytoskeleton remodelling in nematode induced feeding sites. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J., Gheysen, G. and Fenoll, C., eds), pp. 369–394. Dordrecht: Springer.
- de Almeida Engler, J., Kyndt, T., Vieira, P., Van Cappelle, E., Boudolf, V., Sanchez, V., Escobar, C., De Veylder, L., Engler, G., Abad, P. and Gheysen, G. (2012) CCS52 and DEL1 genes are key components of the endocycle in nematode-induced feeding sites. *Plant J.* **72**, 185–198.
- Barcala, M., Garcia, A., Cabrera, J., Casson, S., Lindsey, K., Favery, B., Garcia-Casado, G., Solano, R., Fenoll, C. and Escobar, C. (2010) Early transcriptomic events in microdissected *Arabidopsis* nematode-induced giant cells. *Plant J.* **61**, 698–712.
- Barthels, N., van der Lee, F.M., Klap, J., Goddijn, O.J., Karimi, M., Puzio, P., Grundler, F.M., Ohl, S.A., Lindsey, K., Robertson, L., Robertson, W.M., Van Montagu, M., Gheysen, G. and Sijmons, P.C. (1997) Regulatory sequences of *Arabidopsis* drive reporter gene expression in nematode feeding structures. *Plant Cell*, **9**, 2119–2134.
- Berckmans, B., Vassileva, V., Schmid, S.P.C., Maes, S., Parizot, B., Naramoto, S., Magyar, Z., Alvim Kamei, C.L., Koncz, C., Bögre, L., Persiau, G., De Jaeger, G., Friml, J., Simon, R., Beeckman, T. and De Veylder, L. (2011) Auxin-dependent cell cycle reactivation through transcriptional regulation of *Arabidopsis* E2Fa by lateral organ boundary proteins. *Plant Cell*, **23**, 3671–3683.
- Bird, D.M. (1996) Manipulation of host gene expression by root-knot nematodes. *J. Parasitol.* **82**, 881–888.
- Brady, S.M., Orlando, D.A., Lee, J.Y., Wang, J.Y., Koch, J., Dinneny, J.R., Mace, D., Ohler, U. and Benfey, P.N. (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science*, **318**, 801–806.
- Caillaud, M.C., Dubreuil, G., Quentin, M., Perfus-Barbeoch, L., Lecomte, P., de Almeida Engler, J., Abad, P., Rosso, M.N. and Favery, B. (2008) Root-knot nematodes manipulate plant cell functions during a compatible interaction. *J. Plant Physiol.* **165**, 104–113.
- Damiani, I., Baldacci-Cresp, F., Hopkins, J., Andrio, E., Balzergue, S., Lecomte, P., Puppo, A., Abad, P., Favery, B. and Hérouart, D. (2012) Plant genes involved in harbouring symbiotic rhizobia or pathogenic nematodes. *New Phytol.* **194**, 511–522.
- Davuluri, R.V., Sun, H., Palaniswamy, S.K., Matthews, N., Molina, C., Kurtz, M. and Grotewold, E. (2003) AGRIS: *Arabidopsis* gene regulatory information server, an information resource of *Arabidopsis* cis-regulatory elements and transcription factors. *BMC Bioinformatics*, **4**, 25.
- Escobar, C., Brown, S. and Mitchum, M.G. (2011) Transcriptomic and proteomic analysis of the plant response to nematode infection. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J., Gheysen, G. and Fenoll, C., eds), pp. 157–174. Dordrecht: Springer.
- Fuller, V.L., Lilley, C.J., Atkinson, H.J. and Urwin, P.E. (2007) Differential gene expression in *Arabidopsis* following infection by plant-parasitic nematodes *Meloidogyne incognita* and *Heterodera schachtii*. *Mol. Plant Pathol.* **8**, 2–4.
- Gheysen, G. and Fenoll, C. (2002) Gene expression in nematode feeding sites. *Annu. Rev. Phytopathol.* **40**, 191–219.
- Gheysen, G. and Mitchum, M.G. (2009) Molecular insights in the susceptible plant response to nematode infection. In: *Cell Biology of Plant Nematode Parasitism* (Berg, R.H. and Taylor, C.G., eds), pp. 45–82. Heidelberg: Springer.

- Goddijn, O.J., Lindsey, K., van der Lee, F.M., Klap, J.C. and Sijmons, P.C. (1993) Differential gene expression in nematode-induced feeding structures of transgenic plants harbouring promoter-gusA fusion constructs. *Plant J.* **4**, 863–873.
- Goverse, A. and Bird, D. (2011) The role of plant hormones in nematode feeding cell formation. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J., Gheysen, G. and Fenoll, C., eds), pp. 325–348. Dordrecht: Springer.
- Grunewald, W., Karimi, M., Wieczorek, K., Van de Cappelle, E., Wischnitzki, E., Grundler, F., Inzé, D., Beeckman, T. and Gheysen, G. (2008) A role for AtWRKY23 in feeding site establishment of plant-parasitic nematodes. *Plant Physiol.* **148**, 358–368.
- Grunewald, W., van Noorden, G., Van Isterdael, G., Beeckman, T., Gheysen, G. and Mathesius, U. (2009) Manipulation of auxin transport in plant roots during Rhizobium symbiosis and nematode parasitism. *Plant Cell*, **21**, 2553–2562.
- Guo, A., He, K., Liu, D., Bai, S., Gu, X., Wei, L. and Luo, J. (2005) DATF: a database of Arabidopsis transcription factors. *Bioinformatics*, **21**, 2568–2569.
- Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Oertle, L., Oertle, L., Widmayer, P., Gruissem, W. and Zimmermann, P. (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinformatics*, **2008**, 420747.
- Hussey, R.S. and Grundler, F.M. (1998) Nematode parasitism of plants. In: *Physiology and Biochemistry of Free-living and Plant Parasitic Nematodes* (Perry, R.N. and Wright, J., eds), pp. 213–243. Oxford: CAB International Press.
- Iida, K., Seki, M., Sakurai, T., Satou, M., Akiyama, K., Toyoda, T., Konagaya, A. and Shinozaki, K. (2005) RARTF: database and tools for complete sets of Arabidopsis transcription factors. *DNA Res.* **12**, 247–256.
- Ithal, N., Recknor, J., Nettleton, D., Maier, T., Baum, T.J. and Mitchum, M.G. (2007) Developmental transcript profiling of cyst nematode feeding cells in soybean roots. *Mol. Plant–Microbe Interact.* **20**, 510–525.
- Jammes, F., Lecomte, P., de Almeida-Engler, J., Bitton, F., Martin-Magniette, M.L., Renou, J.P., Abad, P. and Favery, B. (2005) Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. *Plant J.* **44**, 447–458.
- Jones, M.G.K. (1981) Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. *Ann. Appl. Biol.* **97**, 353–372.
- Jones, M.G.K. and Goto, D.B. (2011) Root-knot nematodes and giant cells. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J., Gheysen, G. and Fenoll, C., eds), pp. 83–102. Dordrecht: Springer.
- Khan, R., Alkharouf, N., Beard, H., MacDonald, M., Chouikha, I., Meyer, S., Grefenstette, J., Knap, H. and Matthews, B. (2004) Microarray analysis of gene expression in soybean roots susceptible to the soybean cyst nematode two days post invasion. *J. Nematol.* **36**, 241–248.
- Klink, V.P., Overall, C.C., Alkharouf, N.W., MacDonald, M.H. and Matthews, B.F. (2007) Laser capture microdissection (LCM) and comparative microarray expression analysis of syncytial cells isolated from incompatible and compatible soybean (*Glycine max*) roots infected by the soybean cyst nematode (*Heterodera glycines*). *Planta*, **226**, 1389–1409.
- Klink, V.P., Hosseini, P., Matsye, P., Alkharouf, N.W. and Matthews, B.F. (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.
- Kyndt, T., Denil, S., Haegeman, A., Trooskens, G., Bauters, L., Van Crielinge, W., De Meyer, T. and Gheysen, G. (2012) Transcriptional reprogramming by root knot and migratory nematode infection in rice. *New Phytol.* **196**, 887–900.
- Lee, C., Chronis, D., Kenning, C., Peret, B., Hewezi, T., Davis, E.L., Baum, T.J., Hussey, R., Bennett, M. and Mitchum, M.G. (2011) The novel cyst nematode effector protein 19C07 interacts with the Arabidopsis auxin influx transporter LAX3 to control feeding site development. *Plant Physiol.* **155**, 866–880.
- Mathesius, U. (2003) Conservation and divergence of signalling pathways between roots and soil microbes—the Rhizobium–legume symbiosis compared to the development of lateral roots, mycorrhizal interactions and nematode-induced galls. *Plant Soil*, **255**, 105–119.
- Menges, M., Hennig, L., Gruissem, W. and Murray, J.A. (2003) Genome-wide gene expression in an Arabidopsis cell suspension. *Plant Mol. Biol.* **53**, 423–442.
- Mitsuda, N. and Ohme-Takagi, M. (2009) Functional analysis of transcription factors in Arabidopsis. *Plant Cell Physiol.* **50**, 1232–1248.
- Nemhauser, J.L., Hong, F. and Chory, J. (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell*, **126**, 467–475.
- Nicol, J.M., Turner, S.J., Coyne, D.L. and Nijs, L. (2011) Current nematode threats to world agriculture. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J., Gheysen, G. and Fenoll, C., eds), pp. 21–45. Dordrecht: Springer.
- Ogata, Y., Sakurai, N., Aoki, K., Suzuki, H., Okazaki, K., Saito, K. and Shibata, D. (2009) KAGIANA: an excel-based tool for retrieving summary information on Arabidopsis genes. *Plant Cell Physiol.* **50**, 173–177.
- Parizot, B., De Rybel, B. and Beeckman, T. (2010) VisualLRTC: a new view on lateral root initiation by combining specific transcriptome data sets. *Plant Physiol.* **153**, 34–40.
- Portillo, M., Cabrera, J., Lindsey, K., Topping, J., Andrés, M.F., Emiliozzi, M., Oliveros, J.C., García-Casado, G., Solano, R., Koltai, H., Resnick, N., Fenoll, C. and Escobar, C. (2013) Distinct and conserved transcriptomic changes during nematode-induced giant cell development in tomato compared with Arabidopsis: a functional role for gene repression. *New Phytol.* **197**, 1276–1290.
- Puthoff, D.P., Nettleton, D., Rodermeil, S.R. and Baum, T.J. (2003) Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. *Plant J.* **33**, 911–921.
- Riano-Pachon, D.M., Ruzicic, S., Dreyer, I. and Mueller-Roeber, B. (2007) PlnTFDB: an integrative plant transcription factor database. *BMC Bioinformatics*, **8**, 42.
- Sawa, S., Ohgishi, M., Goda, H., Higuchi, K., Shimada, Y., Yoshida, S. and Koshiba, T. (2002) The HAT2 gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in Arabidopsis. *Plant J.* **32**, 1011–1022.
- Sobczak, M. and Golinowski, W. (2011) Cyst nematodes and syncytia. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J., Gheysen, G. and Fenoll, C., eds), pp. 61–82. Dordrecht: Springer.
- Szakasits, D., Heinen, P., Wieczorek, K., Hofmann, J., Wagner, F., Kreil, D.P., Sykacek, P., Grundler, F.M. and Bohlmann, H. (2009) The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots. *Plant J.* **57**, 771–784.
- Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Müller, L.A., Rhee, S.Y. and Stitt, M. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.* **37**, 914–939.
- Wieczorek, K., Golecki, B., Gerdes, L., Heinen, P., Szakasits, D., Durachko, D.M., Cosgrove, D.J., Kreil, D.P., Puzio, P.S., Bohlmann, H. and Grundler, F.M. (2006) Expansins are involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. *Plant J.* **48**, 98–112.
- Yang, Y., Hammes, U.Z., Taylor, C.G., Schachtman, D.P. and Nielsen, E. (2006) High-affinity auxin transport by the AUX1 influx carrier protein. *Curr. Biol.* **16**, 1123–1127.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Experimental conditions and statistical analysis used in the experiments on plant–nematode interactions compiled in NEMATIC as described by the authors in the reference papers.

Table S2 Genes differentially expressed under seven different hormone treatments of Arabidopsis plants (Nemhauser *et al.*, 2006), which are also differentially expressed in plant–nematode feeding cells, listed in 14 independent sheets (up- and down-regulated). Arabidopsis Genome Initiative (AGI) codes for the genes from each described treatment are provided.

Table S3 Flow chart and details of the filtering steps to obtain genes fulfilling the following criteria: (i) up-regulated in giant cell (GCs); (ii) with a fold change in GCs and syncytia of greater than 2.5; and (iii) barely expressed in selected root cell types and root parts.

Experimental Procedures S1 Document containing extended and more detailed information on the use, content and data sources of NEMATIC.