PLOS | PATHOGENS

PEARLS

# "Cyst-ained" research into *Heterodera* parasitism

**Parijat S. Juvale, Thomas J. Baum***

Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, United States of America

* tbaum@iastate.edu

Nematodes are roundworms that constitute the phylum Nematoda. Only a small fraction of nematode genera contains plant-parasitic or animal-parasitic species, while the majority of nematodes are free-living [1]. *Heterodera glycines*, the soybean cyst nematode, is a plant-parasitic nematode causing major damage to soybean production worldwide. Annual United States yield loss estimates due to *H. glycines* range up to $1.2 billion, likely making this nematode the most serious pathogen threat to sustainable soybean production [2]. While cyst nematode-resistant soybean cultivars are available, they do not control all *H. glycines* biotypes present in a given field and, therefore, select for virulent nematode populations that can overcome available resistance genes, leading to a slow but steady erosion of resistance efficacy [3]. Clearly, long-term management of the soybean cyst nematode in modern soybean production will need additional tools, and it is likely that such new tools will be developed from detailed molecular knowledge of the complex *Heterodera* cyst nematode-plant interactions. This short review provides a snapshot of currently unfolding research discoveries from the genus *Heterodera*, which also includes other cyst nematodes, particularly the sugar beet cyst nematode *H. schachtii*, which can infect *Arabidopsis* and therefore has been used as a model system. Since nematode effectors (the proteins delivered into host plant tissues to mediate parasitism) are at the forefront of nematode–plant interactions, their identification and functional characterization are heavily emphasized in this manuscript.

*Heterodera* cyst nematodes are soil-borne pathogens. Infective juveniles (Fig 1A) hatch from eggs that are mostly contained in the hardened body wall of the previous generation's female, which represents the cyst structure giving this nematode group its name (Fig 1B). Infective juveniles migrate toward roots of host plants and penetrate intracellularly into and through the root tissue using mechanical force and cell wall-degrading enzymes delivered through a hollow mouth spear: the stylet (Fig 1A). Interestingly, during their intracellular migration toward the root's central cylinder, nematodes do not feed but select an initial feeding cell only at the end of their migration, when they become sedentary. At this point, the cyst nematode–plant interactions enter into a new, complex molecular phase of signal exchange, at the end of which the nematode will have reprogrammed a group of several hundred root cells to redifferentiate, partially dissolve their cell walls, and fuse to form a feeding structure: the syncytium (Fig 1C). This new plant organ is the evolutionary advancement that enabled the sedentary parasitic lifestyle of cyst nematodes, as the growing cyst nematodes now require intense levels of nourishment in one single location without the nematodes' ability to move to new food sources. This also means that a cyst nematode's survival and reproduction is tightly linked to the proper development and function of the syncytium.

At the heart of syncytium induction and formation are signals sent by the nematode to the initial feeding cell, and the most obvious candidates for such signals are effector proteins

Check for updates

OPEN ACCESS

**Citation:** Juvale PS, Baum TJ (2018) "Cyst-ained" research into *Heterodera* parasitism. PLoS Pathog 14(2): e1006791. https://doi.org/10.1371/journal.ppat.1006791

**Editor:** Cyril Zipfel, THE SAINSBURY LABORATORY, UNITED KINGDOM

**Published:** February 1, 2018

**Copyright:** © 2018 Juvale, Baum. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The authors were supported by the Iowa Agriculture and Home Economics Experiment Station and by Hatch Act and State of Iowa funds. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

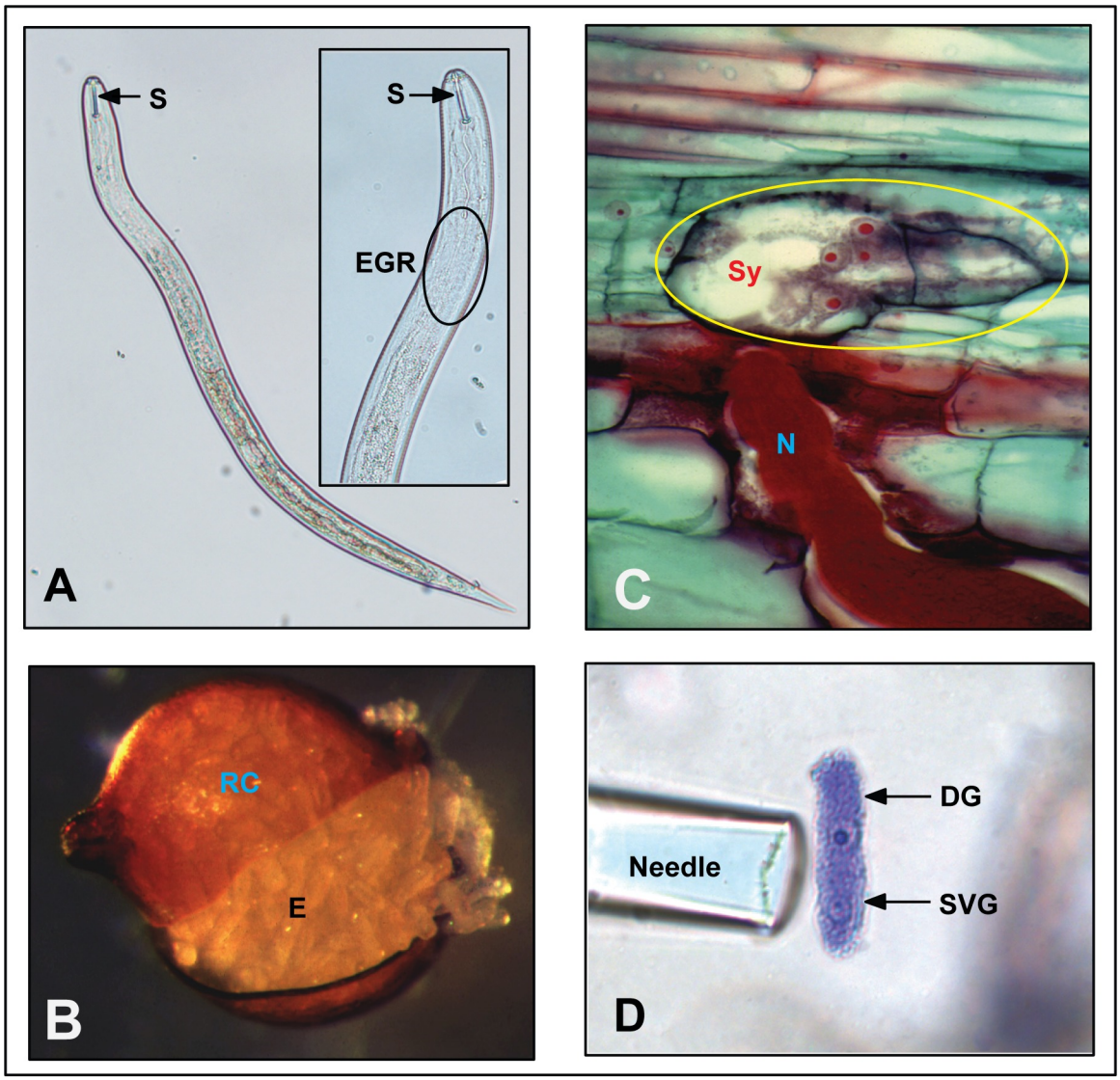


**Fig 1.** **A**. Infective *Heterodera glycines* juvenile with a close-up of the anterior region showing the stylet (S) and the EGR. **B**. Ruptured RC displaying stored E. *Image by E. C. McGawley, Nemapix*. **C**. Cross section through a SY of a *H. glycines* N. *Image by Burton Y. Endo, Nemapix*. **D**. Microaspiration of isolated and stained DG and SVG gland cells of *H. glycines* showing prominently stained nuclei. *Image by Thomas R. Maier, Iowa State University*. DG, dorsal gland; E, eggs; EGR, esophageal gland region; N, nematode; RC, ruptured *H. glycines* cyst; S, stylet; SVG, subventral gland; Sy, syncytium.

https://doi.org/10.1371/journal.ppat.1006791.g001

produced in three nematode esophagus-associated secretory cells, termed the dorsal and subventral esophageal glands (Fig 1A and 1D), and delivered through the stylet [4]. Identification and characterization of such effectors is thus of prime interest when exploring cyst nematode parasitism.

Microaspiration of esophageal gland-enriched tissues and the construction and sequencing of gland cDNA libraries has identified more than 80 bona fide esophageal gland-produced effector candidates from *H. glycines* [5,6]. With the goal of achieving stringent gland cell specificity, the latest efforts to identify additional gland–expressed secretory proteins (i.e., candidate effectors) rely on specifically purifying gland cells (Fig 1D) and using next-generation sequencing technologies [7]. This approach has the potential to unravel the complete effectorome of

plant-parasitic nematodes in general and of different strains exhibiting a wide range of virulence phenotypes in particular. Developmental stage- and population-specific effector identification efforts using this approach and bioinformatic prediction pipelines, combined with the development of a comprehensive and well-annotated genome resource (see below), are now unraveling the entire effector repertoire of *H. glycines*.

Effector identification has to be followed by functional characterization in order to advance our understanding of parasitism. As in other pathosystems, effectors play critical roles in the suppression of host defenses. In cyst nematode parasitism, however, effectors also are key contributors to reprogramming of plant cells to form syncytia [8–10]. Understanding the mechanisms deployed by *Heterodera* cyst nematodes to suppress or circumvent host defense responses is important, as it will identify vulnerable points for intervention that can aid in devising novel cyst nematode management strategies. Sensing nematode invasion, host plants initiate defense responses ranging all the way to programmed cell death at the infection site [11]. While the mechanisms of triggering defenses are still poorly understood, recent research has revealed that plants are able to detect ascarosides (conserved nematode pheromones) and up-regulate defenses in response to these chemicals [12]. Another mechanistic insight is provided by the recent discovery that *Arabidopsis* receptor kinase NEMATODE-INDUCED LRR-RLK 1 (NILR1) is specifically up-regulated in response to nematode infection and triggers basal immune responses [13]. Naturally, cyst nematodes have evolved the ability to counteract such mechanisms by deploying a suite of effectors. A number of *Heterodera* cyst nematode effector proteins already have been determined to modulate host defense responses. Most of these effectors have been shown to accomplish this feat by physically interacting with and modulating the functions of host proteins involved in different defense response mechanisms.

Cyst nematodes migrate intracellularly through host root tissues causing extensive damage and defense signaling. Delivery of "venom allergen-like protein effectors" (VAPs) during their migratory phase counteracts this defense response. Transgenic Arabidopsis lines expressing a VAP coding sequence showed impaired defense responses to elicitor peptides such as flg22 and were hypersusceptible to various pathogens [14].

One of the first mechanistic insights into defense suppression by *Heterodera* cyst nematodes is the effector-mediated manipulation of polyamine biosynthesis in the host plant through the targeting of plant spermidine synthase. Plants generate reactive oxygen species (ROS) as a response to nematode infection [15]. As a result, syncytia are at risk from the detrimental effects of ROS. Polyamines such as spermidine can effectively scavenge ROS [16,17], thus manipulating the polyamine biosynthetic pathway in the syncytium to generate elevated levels of spermidine could be an effective strategy to protect the syncytium from oxidative stress. *Heterodera* cyst nematodes appear to exploit this mechanism by deploying effector 10A06, which interacts with and activates spermidine synthase, a key enzyme in the polyamine biosynthetic pathway [18].

Elevated expression levels of pathogenesis-related (PR) proteins are primary defense responses observed in host plants under cyst nematode attack [11,19,20]. Growing evidence suggests that cyst nematodes have evolved effectors to counteract PR proteins. For example, Hamamouch et al. [21] described that the elevated expression level of β-1,3-endoglucanase, a PR2 protein family member, is counteracted by *Heterodera* effector 30C02, which physically interacts with this protein. Transgenic plants constitutively expressing a PR2 coding sequence were more resistant, while knock-out mutants were less resistant to the cyst nematodes. On the other hand, plants expressing the effector coding sequence were more susceptible, while plants expressing an RNA interference (RNAi) construct to downregulate effector expression in infecting cyst nematodes were less susceptible.

Another defense-suppressing effector is of interest for a different reason. *H. glycines* effector HgGland18 is a strong suppressor of basal as well as hypersensitive cell death immune responses. Bioinformatic and phylogenetic analyses of this effector protein revealed close sequence as well as functional similarity with a nonhomologous effector of the malaria parasite *Plasmodium* that also has immunosuppressive function in its animal host. HgGland18 represents an intriguing example of the convergent evolution of two effectors that arrived at similar structure and function [22].

In addition to effectors with known mechanistic understanding of their function, we also have observed strong defense suppression by a number of *Heterodera* effectors that still await in-depth functional characterization and publication. In fact, most known *H. glycines* effectors currently are being assessed for their ability to suppress pattern-triggered and effector-triggered immunity. At the time of writing, we are aware of 11 additional *H. glycines* effectors that are able to suppress defenses. It is of particular interest to note that different effector variants, i.e., highly similar proteins within a certain effector group, can exhibit profoundly different activities in such assays. These findings again emphasize the importance of discovering all variants of effectors and detecting correlations among such variants and nematode virulence phenotypes.

As mentioned above, along with robust defense suppression, cyst nematodes also orchestrate massive alterations and reprogramming of host cells at the infection site, which causes these cells to fuse and develop into a syncytium [23]. Not surprisingly, a number of *Heterodera* effectors have been shown to alter cellular structures and developmental pathways in the host plant. Massive modifications in cell wall architecture are essential for the fusion and expansion of root cells at the sites of syncytium formation. *Heterodera* cyst nematodes secrete a suite of cell wall-modifying enzymes such as cellulases and pectinases that can directly modify cell walls. However, their expression profiles show that their main functions lie in the weakening of cell wall barriers during the intracellular nematode migration [24–26]. Instead, cyst nematode infection alters the expression of endogenous cell wall-modifying plant enzymes at the infection sites, which then are responsible for the cell wall modifications during syncytium formation [27]. However, it is likely that cyst nematode effectors have a key role in triggering and modulating the expression changes of these plant genes. In addition, *Heterodera* nematodes also secrete a cellulose-binding protein effector that physically interacts with the host cell wall enzyme pectin methyl esterase and modulates its activity to aid parasitism by influencing cell wall changes [28].

Important functions in host cell modifications observed during cyst nematode infection can also be attributed to phytohormones. These signaling molecules are involved in many facets of plant development, and not surprisingly, they—especially auxin and cytokinin—have also been implicated as key players during syncytium development [19, 29–32]. It should therefore come as no surprise that studies discovered that host proteins involved in auxin functions are targeted by cyst nematode effectors [33, 34]. In addition, there is mounting evidence that *Heterodera* cyst nematodes possess genes essential for cytokinin biosynthesis, which suggests that they have the potential to produce and secrete active phytohormone analogs to induce changes at the infection site [35].

Similar to delivering nematode-produced phytohormones that could alter the endogenous hormone response, *H. glycines* secretes effectors with similarity to endogenous plant-signaling peptides. This "ligand mimicry" is best illustrated by the CLE-type effectors of *H. glycines*, which dramatically alter soybean cell-to-cell signaling and root development [36–38]. *Heterodera*-produced annexin-like effectors represent another example of mimicry. Cyst nematodes secrete annexin-like effectors to mimic plant annexins, which are involved in a variety of developmental and defense response pathways [39,40]. The constitutive expression of the

*Heterodera* annexin-like effector in an annexin knock-out *Arabidopsis* mutant restored the mutant, underscoring this effector's function [39].

Syncytium development is associated with a massive change in transcript abundances at the infection site [41–44]. Recent studies have begun to describe how *Heterodera* cyst nematodes exploit intrinsic plant mechanisms to control transcription on a global scale. For example, *H. glycines* infection results in large-scale methylation changes in the soybean genome [45]. In other words, *H. glycines* parasitism triggers significant epigenetic changes in its host, which influences transcript abundances. Furthermore, work in our laboratory produced functional data for an effector that causes DNA methylation changes. Other examples of cyst nematodes hijacking endogenous plant response mechanisms to amplify local nematode stimuli into large-scale systemic responses in the host plant are the reported changes in plant microRNAs during nematode infection [46, 47]. For example, miRNA396 abundance changes dramatically following nematode infection, and miR396 modulations alter the mRNA abundance of approximately half of the *Arabidopsis* genes reported to change in the syncytium [48]. Similarly, expression of miR827and miR858 are altered by cyst nematode infection and serve as amplification mechanisms to cause critical plant gene expression changes [49,50].

Although above-mentioned studies show that *Heterodera* cyst nematodes utilize various strategies and deploy specific effectors to target and modulate cellular pathways, our mechanistic knowledge of cyst nematode parasitism is still at an early stage. Unraveling the entire effector repertoire and functional characterizations are important steps toward developing exhaustive knowledge about cyst nematode parasitism. In this regard, one of the most important resources is a fully sequenced and annotated genome, which is not yet publicly available for any *Heterodera* species at the time of this writing, while high-quality genome assemblies of several *Globodera* cyst nematode species already are available and have significantly benefited researchers [51,52]. A multidisciplinary research team has recently resolved this bottleneck and has produced a high-quality *H. glycines* genome assembly that will be released shortly. Already, this genome assembly has allowed comprehensive secretome analyses that promise to expand the currently known effector repertoire considerably. Furthermore, this genome sequence resource is shedding light on the mechanisms of effector evolution and the proliferation of effector variants. Along with the actual annotated *H. glycines* genome, work is progressing to create an online web presence that should serve as a comprehensive resource for all *H. glycines* genome, transcriptome, effectorome, and proteome data.

While having a high-quality *H. glycines* reference genome is critically important, it is only a first step in capturing *H. glycines* genomic variability. As a part of the *H. glycines* genome project, multiple populations collected from diverse geographical locations or inbred for virulence traits have been and are being sequenced. In short, a boon of novel genomic information is due to be released in the near future, and *H. glycines* 'omics' research is entering into a new phase of discovery. Such resources will be instrumental in targeting ambitious research goals such as unraveling the genotypic bases of *H. glycines* virulence and the potential for molecular diagnoses of *H. glycines* strains that can overcome host resistance genes. Collectively, these advances in basic research will enable university and industry researchers to develop and implement novel control mechanisms.

*Heterodera*–host plant interactions are complex, and the recalcitrant nature of *Heterodera* nematodes toward any reverse genetic modifications have long complicated basic research into the molecular mechanisms of cyst nematode parasitism. However, recent advances in conjunction with novel 'omics' resources are changing the landscape and give rise to a promising outlook, particularly for *H. glycines* research projects with the ultimate goal of resolving this impediment to soybean production. "Cyst-ained," multipronged, and well-funded

research efforts, as described above, are required to develop novel, broad spectrum, and long-lasting resistance resources against this devastating pathogen.

## Author Contributions

**Conceptualization:** Thomas J. Baum.

**Writing – original draft:** Parijat S. Juvale, Thomas J. Baum.

**Writing – review & editing:** Parijat S. Juvale, Thomas J. Baum.

## References


1. van Megen H, van den Elsen S, Holterman M, Karssen G, Mooyman P, Bongers T, et al. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology. 2009; 11: 927–950.
2. Koenning SR, Wrather JA. Suppression of soybean yield potential in the continental United States from plant diseases estimated from 2006 to 2009. Plant Health Prog. 2010; https://doi.org/10.1094/PHP-2010-1122-01-RS
3. Niblack T, Lambert KN, Tylka GL. A model plant pathogen from the kingdom Animalia: *Heterodera glycines*, the soybean cyst nematode. Annu Rev Phytopathol. 2006; 44: 283–303. https://doi.org/10.1146/annurev.phyto.43.040204.140218 PMID: 16704359
4. Hussey R. Disease inducing secretions of plant parasitic nematodes. Annul Rev Phytopathol. 1989; 27: 123–141.
5. Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS. The parasitome of the phytonematode *Heterodera glycines*. Mol Plant Microbe Interact. 2003; 16(8): 720–726. https://doi.org/10.1094/MPMI.2003.16.8.720 PMID: 12906116
6. Noon JB, Hewezi T, Maier TR, Simmons C, Wei JZ, Wu G, et al. Eighteen new candidate effectors of the phytonematode *Heterodera glycines* produced specifically in the secretory esophageal gland cells during parasitism. New Phytol. 2015; 105(10): 1362–1372.
7. Maier TR, Hewezi T, Peng J, Baum TJ. Isolation of whole esophageal gland cells from plant-parasitic nematodes for transcriptome analyses and effector identification. Mol Plant Microbe Interact. 2013; 26 (1): 31–35. https://doi.org/10.1094/MPMI-05-12-0121-FI PMID: 22876962
8. Mitchum MG, Hussey RS, Baum TJ, Wang X, Elling AA, Wubben M, et al. Nematode effector proteins: an emerging paradigm of parasitism. New Phytol. 2013; 199(4): 879–894. https://doi.org/10.1111/nph.12323 PMID: 23691972
9. Hewezi T, Baum TJ. Manipulation of plant cells by cyst and root-knot nematode effectors. Mol Plant Microbe Interact. 2013; 26(1): 9–16. https://doi.org/10.1094/MPMI-05-12-0106-FI PMID: 22809272
10. Hewezi T. Cellular Signaling Pathways and Posttranslational Modifications Mediated by Nematode Effector Proteins. Plant Physiol. 2015; 169(2): 1018–1026. https://doi.org/10.1104/pp.15.00923 PMID: 26315856
11. Kandoth PK, Ithal N, Recknor J, Maier TR, Nettleton D, Baum TJ, et al. The soybean Rhg1 locus for resistance to the soybean cyst nematode Heterodera glycines regulates expression of a large number of stress- and defense-related genes in degenerating feeding cells. Plant Physiol. 2011; 155(4): 1960–1975. https://doi.org/10.1104/pp.110.167536 PMID: 21335526
12. Manosalva P, Manohar M, von Reuss SH, Chen S, Koch A, Kaplan F, et al. Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. Nat. Commun. 2015. https://doi.org/10.1038/ncomms8795 PMID: 26203561
13. Mendy B, Wang'ombe MW, Radakovic ZS, Holbein J, Ilyas M, Chopra D, et al. Arabidopsis leucine-rich repeat receptor–like kinase NILR1 is required for induction of innate immunity to parasitic nematodes. PLoS Pathog. 2017; 13(4): e1006284. https://doi.org/10.1371/journal.ppat.1006284 PMID: 28406987
14. Lozano-Torres JL, Wilbers RH, Warmerdam S, Finkers-Tomczak A, Diaz-Granados A, van Schaik CC, et al. Apoplastic venom allergen-like proteins of cyst nematodes modulate the activation of basal plant innate immunity by cell surface receptors. PLoS Pathog. 2014; 10(12): e1004569. https://doi.org/10.1371/journal.ppat.1004569 PMID: 25500833
15. Siddique S, Matera C, Radakovic ZS,Hasan MS, Gutbrod P, Rozanska E, et al. Parasitic worms stimulate host NADPH oxidases to produce reactive oxygen species that limit plant cell death and promote infection. Sci. Signal. 2014; 7: ra33. https://doi.org/10.1126/scisignal.2004777 PMID: 24714570

16. Groppa MD, Tomaro ML, Benavides MP. Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. Plant Sci. 2001; 161: 481–488.
17. Groppa MD, Benavides MP. Polyamines and abiotic stress: recent advances. Amino Acids. 2008; 34 (1): 35–45. https://doi.org/10.1007/s00726-007-0501-8 PMID: 17356805
18. Hewezi T, Howe PJ, Maier TR, Hussey RS, Mitchum MG, Davis EL, et al. Arabidopsis spermidine synthase is targeted by an effector protein of the cyst nematode *Heterodera schachtii*. Plant Physiol. 2010; 152(2): 968–984. https://doi.org/10.1104/pp.109.150557 PMID: 19965964
19. Mazarei M, Puthoff DP, Hart JK, Rodermel SR, Baum TJ. Identification and characterization of a soybean ethylene-responsive element-binding protein gene whose mRNA expression changes during soybean cyst nematode infection. Mol Plant Microbe Interact. 2002; 15(6): 577–586. https://doi.org/10.1094/MPMI.2002.15.6.577 PMID: 12059106
20. Hamamouch N, Li C, Seo PJ, Park CM, Davis EL. Expression of Arabidopsis pathogenesis-related genes during nematode infection. Mol Plant Pathol. 2011; 12(4): 355–364. https://doi.org/10.1111/j.1364-3703.2010.00675.x PMID: 21453430
21. Hamamouch N, Li C, Hewezi T, Baum TJ, Mitchum MG, Hussey RS, et al. The interaction of the novel 30C02 cyst nematode effector protein with a plant β-1,3-endoglucanase may suppress host defense to promote parasitism. J Exp Bot. 2012; 63(10): 3683–3695. https://doi.org/10.1093/jxb/ers058 PMID: 22442414
22. Noon JB, Qi M, Sill DN, Muppirala U, Eves-van den Akker S, Maier TR, et al. A Plasmodium-like virulence effector of the soybean cyst nematode suppresses plant innate immunity. New Phytol 2016; 212 (2): 444–460. https://doi.org/10.1111/nph.14047 PMID: 27265684
23. Endo BY. Penetration and development of *Heterodera glycines* in soybean and related anatomical changes. Phytopathology. 1964; 55: 375–381.
24. Wang X, Meyers D, Yan Y, Baum T, Smant G, Hussey R, et al. In planta localization of a *beta*-1,4-endoglucanase secreted by *Heterodera glycines*. Mol Plant Microbe Interact. 1999; 12(1): 64–67. https://doi.org/10.1094/MPMI.1999.12.1.64 PMID: 9885195
25. Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS. Identification of a new 1,4-endoglucanase gene expressed in the esophageal subventral gland cells of *Heterodera glycines*. J Nematol. 2002; 34 (1): 12–15. PMID: 19265901
26. Gao B, Allen R, Davis EL, Baum TJ, Hussey RS. Developmental expression and biochemical properties of a beta-1,4-endoglucanase family in the soybean cyst nematode, *Heterodera glycines*. Mol Plant Pathol. 2005; 5(2): 93–104.
27. Goellner M, Wang X, Davis EL. Endo-β-1,4-Glucanase Expression in Compatible Plant–Nematode Interactions. Plant Cell. 2001; 13 (10): 2241–2255. https://doi.org/10.1105/tpc.010219 PMID: 11595799
28. Hewezi T, Howe P, Maier TR, Hussey RS, Mitchum MG, Davis EL, et al. Cellulose binding protein from the parasitic nematode *Heterodera schachtii* interacts with Arabidopsis pectin methylesterase: cooperative cell wall modification during parasitism. Plant Cell. 2008; 20(11): 3080–3093. https://doi.org/10.1105/tpc.108.063065 PMID: 19001564
29. Goverse A, Overmars H, Engelbertink J, Schots A, Bakker J, Helder J. Both induction and morphogenesis of cyst nematode feeding cells are mediated by auxin. Mol Plant Microbe Interact. 2000; 13(10): 1121–1129. https://doi.org/10.1094/MPMI.2000.13.10.1121 PMID: 11043473
30. Wubben MJ II, Su H, Rodermel SR, Baum TJ. Susceptibility to the sugar beet cyst nematode is modulated by ethylene signal transduction in *Arabidopsis thaliana*. Mol Plant Microbe Interact. 2001; 14(10): 1206–1212. https://doi.org/10.1094/MPMI.2001.14.10.1206 PMID: 11605960
31. Wubben MJ, Jin J, Baum TJ. Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. Mol Plant Microbe Interact. 2008; 21(4): 424–432. https://doi.org/10.1094/MPMI-21-4-0424 PMID: 18321188
32. Dowd CD, Chronis D, Radakovic ZS, Siddique S, Schmülling T, Werner T, et al. Divergent expression of cytokinin biosynthesis, signaling and catabolism genes underlying differences in feeding sites induced by cyst and root-knot nematodes. Plant J. 2017; https://doi.org/10.1111/tpj.13647 PMID: 28746737
33. Lee C, Chronis D, Kenning C, Peret B, Hewezi T, Davis EL, et al. The novel cyst nematode effector protein 19C07 interacts with the Arabidopsis auxin influx transporter LAX3 to control feeding site development. Plant Physiol. 2011; 155(2): 866–880. https://doi.org/10.1104/pp.110.167197 PMID: 21156858
34. Hewezi T, Juvale PS, Piya S, Maier TR, Rambani A, Rice JH, et al. The cyst nematode effector protein 10A07 targets and recruits host posttranslational machinery to mediate its nuclear trafficking and to promote parasitism in Arabidopsis. Plant Cell. 2015; 27(3): 891–907. https://doi.org/10.1105/tpc.114.135327 PMID: 25715285

35. Siddique S, Radakovic ZS, De La Torre CM, Chronis D, Novák O, Ramireddy E, et al. A parasitic nematode releases cytokinin that controls cell division and orchestrates feeding site formation in host plants. Proc Natl Acad Sci U S A. 2015; 112(41): 12669–12674. https://doi.org/10.1073/pnas.1503657112 PMID: 26417108

36. Wang X., Mitchum MG, Gao B, Li C, Diab H, Baum TJ, et al. A parasitism gene from a plant-parasitic nematode with function similar to CLAVATA3/ESR (CLE) of *Arabidopsis thaliana*. Molecular Plant Path. 2005; 6(2): 187–191.

37. Mitchum MG, Wang X, Wang J, Davis EL. Role of nematode peptides and other small molecules in plant parasitism. Annu Rev Phytopathol. 2013; 50: 175–195.

38. Guo X, Wang J, Gardner M, Fukuda H, Kondo Y, Etchells JP, et al. Identification of cyst nematode B-type CLE peptides and modulation of the vascular stem cell pathway for feeding cell formation. PLoS Pathog. 2017; 13(2): e1006142. https://doi.org/10.1371/journal.ppat.1006142 PMID: 28158306

39. Patel N, Hamamouch N, Li C, Hewezi T, Hussey RS, Baum TJ, et al. A nematode effector protein similar to annexins in host plants. J Exp Bot. 2010; 61(1): 235–248. https://doi.org/10.1093/jxb/erp293 PMID: 19887499

40. Chen C, Liu S, Liu Q, Niu J, Liu P, Zhao J, et al. An ANNEXIN-like protein from the cereal cyst nematode *Heterodera avenae* suppresses plant defense. PLoS ONE. 2015; 10(4): e0122256. https://doi.org/10.1371/journal.pone.0122256 PMID: 25849616

41. Hermsmeier D, Hart JK, Byzova M, Rodermel SR, Baum TJ. Changes in mRNA abundance within *Heterodera schachtii*-infected roots of *Arabidopsis thaliana*. Mol Plant Microbe Interact. 2000; 13(3): 309–315. https://doi.org/10.1094/MPMI.2000.13.3.309 PMID: 10707356

42. Puthoff DP, Nettleton D, Rodermel SR, Baum TJ. Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant J. 2003; 33(5): 911–921. PMID: 12609032

43. Ithal N, Recknor J, Nettleton D, Maier T, et al. Developmental transcript profiling of cyst nematode feeding cells in soybean roots. Mol Plant Microbe Interact. 2007; 20: 510–525. https://doi.org/10.1094/MPMI-20-5-0510 PMID: 17506329

44. Szakasits D, Heinen P, Wieczorek K, Hofmann J, Wagner F,Kreil DP, et al. The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots. Plant J. 2009; 57(5): 771–784. https://doi.org/10.1111/j.1365-313X.2008.03727.x PMID: 18980640

45. Rambani A, Rice JH, Liu J, Lane T, Ranjan P, Mazarei M, et al. The methylome of soybean roots during the compatible interaction with the soybean cyst nematode. Plant Physiol. 2015; 168(4): 1364–1377. https://doi.org/10.1104/pp.15.00826 PMID: 26099268

46. Hewezi T, Howe P, Maier TR, Baum TJ. Arabidopsis small RNAs and their targets during cyst nematode parasitism. Mol Plant Microbe Interact. 2008; 21(12): 1622–1634. https://doi.org/10.1094/MPMI-21-12-1622 PMID: 18986258

47. Hewezi T, Baum T. Chapter Nine—Gene Silencing in Nematode Feeding Sites. Adv Bot Res. 2015; 73: 221–239.

48. Hewezi T, Maier TR, Nettleton D, Baum TJ. The Arabidopsis microRNA396-GRF1/GRF3 regulatory module acts as a developmental regulator in the reprogramming of root cells during cyst nematode infection. Plant Physiol. 2012; 159(1): 321–335. https://doi.org/10.1104/pp.112.193649 PMID: 22419826

49. Hewezi T, Piya S, Qi M, Balasubramaniam M, Rice JH, Baum TJ. Arabidopsis miR827 mediates post-transcriptional gene silencing of its ubiquitin E3 ligase target gene in the syncytium of the cyst nematode *Heterodera schachtii* to enhance susceptibility. Plant J. 2016; 88(2): 179–192. https://doi.org/10.1111/tpj.13238 PMID: 27304416

50. Piya S, Kihm C, Rice JH, Baum TJ, Hewezi T. Cooperative Regulatory Functions of miR858 and MYB83 during cyst nematode parasitism. Plant Physiol. 2017; 174(3): 1897–1912. https://doi.org/10.1104/pp.17.00273 PMID: 28512179

51. Cotton JA, Lilley CJ, Jones LM, Kikuchi T, Reid AJ, Thorpe P, et al. The genome and life-stage specific transcriptomes of Globodera pallida elucidate key aspects of plant parasitism by a cyst nematode. Genome Biol. 2014; 15: R43. https://doi.org/10.1186/gb-2014-15-3-r43 PMID: 24580726

52. Eves-van den Akker S, Laetsch DR, Thorpe P, Lilley CJ, Danchin EG, Da Rocha M, et al. The genome of the yellow potato cyst nematode, Globodera rostochiensis, reveals insights into the basis of parasitism and virulence. Genome Biol. 2016; 17: 124. https://doi.org/10.1186/s13059-016-0985-1 PMID: 27286965