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Gall-forming root-knot nematodes hijack key plant cellular functions to induce multinucleate and hypertrophied feeding cells

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ABSTRACT

Among plant-parasitic nematodes, the root-knot nematodes (RKNs) of the *Meloidogyne* spp. are the most economically important genus. RKN are root parasitic worms able to infect nearly all crop species and have a wide geographic distribution. During infection, RKNs establish and maintain an intimate relationship with the host plant. This includes the creation of a specialized nutritional structure composed of multinucleate and hypertrophied giant cells, which result from the redifferentiation of vascular root cells. Giant cells constitute the sole source of nutrients for the nematode and are essential for growth and reproduction. Hyperplasia of surrounding root cells leads to the formation of the gall or root-knot, an easily recognized symptom of plant infection by RKNs. Secreted effectors produced in nematode salivary glands and injected into plant cells through a specialized feeding structure called the stylet play a critical role in the formation of giant cells. Here, we describe the complex interactions between RKNs and their host plants. We highlight progress in understanding host plant responses, focusing on how RKNs manipulate key plant processes and functions, including cell cycle, defence, hormones, cellular scaffold, metabolism and transport.

1. Introduction

Plants have antagonistic associations with a wide range of parasitic biotrophic organisms. A common feature of biotrophs is that they extract their nutrients only from living plant tissues. Therefore, it is quite conceivable that during evolution these interactions might have evolved certain common core components affecting cellular functions such as suppression of plant defence, cytoskeleton rearrangements, cell-wall reorganisation, membrane synthesis or metabolite fluxes (Parniske, 2000). Plant parasitic nematodes predominantly exploit the root as their only source of nutrients. These microscopic worms may be ectoparasitic spending their whole life cycle outside the root and feeding from the surface or deeper tissues, or may be endoparasitic invading the root tissues. Among endoparasitic nematodes, sedentary nematodes have a highly evolved association with their hosts and include the most economically important group of plant-parasitic nematodes worldwide, the root-knot nematodes (RKNs) *Meloidogyne* species (Trudgill and Blok, 2001). Three remarkable features of RKNs are (i) their enormous host range that encompasses most flowering plants, (ii) their worldwide distribution and (iii) their diversity in term of modes of reproduction. The most important RKN species in terms of geographic distribution and agronomic impact (*Meloidogyne incognita*, *Meloidogyne arenaria*, and *Meloidogyne javanica*), reproduce exclusively by mitotic (apomictic) parthenogenesis. The lack of sexual reproduction means that classical genetics approach cannot be used to study important traits of these apomictic RKN species. Some species (e.g., *Meloidogyne chitwoodi*, *Meloidogyne fallax*, most *Meloidogyne hapla* populations) can reproduce by cross-fertilization or, when males are absent, meiotic (automictic) parthenogenesis. Finally, a few RKN species only reproduce sexually. These amphimictic species (*Meloidogyne carolinensis*, *Meloidogyne megatyla*, *Meloidogyne microtyla*, *Meloidogyne pini*) are considered as minor RKN species because of their very restricted distribution, host range and economic impact (Castagnone-Sereno et al., 2013). Thus, marked differences in host preference occur among the 80 RKN species currently described. Although notable exceptions do occur and information about the mode of reproduction is sometimes lacking, it is generally admitted that apomictic species have wider host ranges than automictic or amphimictic species (Castagnone-Sereno et al., 2013). Trudgill and Blok (2001) highlighted the evolutionary paradox of the

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extreme parasitic success of these apomictic species that have representative hosts in every plant family and are considered as true polyphagous species. In contrast, at least 32 sexually reproducing species are limited to a single plant family, genus, or species and thus are considered to be host specialists.

The most important means of RKN control were the nematicides, but since they threatened environment and human health they were banned. Natural plant resistance is an available, safe and efficient option. Major RKN resistance genes have been described and two have been cloned (Milligan et al., 1998; Claverie et al., 2011). However the use of resistant varieties is limited by the number of available genotypes and the occurrence of resistance breaking nematode populations. Some families have no nematode resistance genes identified (i.e., *Cucurbitaceae*). In addition, monogenic resistances, such as the tomato *Mi* gene, have been shown to be overcome by "virulent" nematode populations, and especially by new emerging *Meloidogyne* species e.g., *Meloidogyne enterolobii*, leading to ineffectiveness of the developed elite crop lines (Castagnone-Sereno, 2006; Abad et al., 2009). New technologies and practices are required to offer alternatives to producers. An increase of fundamental knowledge on the plant–nematode interaction, especially on plant cellular functions hijacked during disease development, should lead to the discovery of novel targets and strategies to control RKN. Finally, study of plant-parasitic nematodes biology benefits little from being related to the model nematode *Caenorhabditis elegans*. Although nematode developmental biology and general physiology have been shown to be conserved, essential differences in lifestyle (bacteriophagous free living vs obligate plant parasite) make *C. elegans* not so helpful for studies on the biology of plant parasitism (Blok et al., 2008).

2. Life cycle and infection of plants by RKNs

The life cycle of RKNs spans 3–8 weeks, depending on the nematode species and environmental conditions. There are five stages separated by moults, during which the cuticle is replaced, including four juvenile stages and an adult stage. Eggs are laid by the female in the soil into a protective gelatinous matrix. J1 (first-stage juvenile) resides entirely inside the translucent egg, where it molts into a J2 (second-stage juvenile). J2, the only infective stage, hatches when environmental conditions are suitable and cannot persist for extended periods without a new host plant. Attracted by the plant root (Curtis et al., 2009), the J2 burrows into the host root close to the growing tip, and migrates through intercellular spaces to reach the vascular cylinder (Caillaud et al., 2008b). After an initial penetration and migratory phase, RKNs adopt a sedentary lifestyle involving the transformation of few root cells into a highly specialized feeding structure from which nematodes withdraw water and nutrients. The feeding structure comprises a group of hypertrophied and multinucleate "giant cells" (GC) (Fig. 1). The J2 chooses the cells that will be redifferentiated into GCs, these being 5–7 root vascular cells close to the xylem cells (Bird, 1961; Jones and Payne, 1978). Secretions from the salivary glands of the nematode are injected into selected root cells via the stylet, leading to their transformations into GCs (Fig. 1A and B). Once they have established their feeding sites, the J2 become sedentary, starts to feed from the GCs moving only the head end (Sijmons et al., 1991), and then undergoes three moults to mature into adults. Sexual dimorphism with rotund females and vermiform males is associated with the sedentary lifestyle. The female is always sedentary, whereas the male becomes vermiform and motile again during the third moult, and then leaves the root. Sex is determined by environmental conditions and the frequency of males increased in conditions of crowding or poor nutrition (Papadopoulou and Triantaphyllou, 1982). At the end of the cycle, the female produces eggs that are released onto the root surface in a gelatinous matrix.

The feeding site produced by RKN plays an essential role in sustaining nematode development. Giant cells result from repeated nuclear divisions without cell division (cytokinesis) and isotropic plant cell growth (Caillaud et al., 2008b). The first sign of the nematodeã s manipulation of cell development is the appearance of a second nucleus in the cell. This is initially accompanied by a sign that the cell will eventually divide into two. Thus, cell plate vesicles line up between the two daughter nuclei. Subsequent dispersal of these vesicles disrupts the formation of the cell plate and a cell with two nuclei is created (Caillaud et al., 2008c). This process continues until the GC contains up to 100 nuclei. GCs also have a dense cytoplasm and a high level of metabolic activity. They can be up to 400 times larger than a normal root vascular cell (Abad et al., 2009). Solutes uptake from the vascular system is enhanced by the development of numerous ingrowths of cell walls of the GC in contact with the xylem. The constant withdrawal of cytoplasm by the nematode converts the feeding cells into metabolic sinks for the host plant. Interestingly and simultaneously, cells surrounding RKN and GCs start to divide, and this proliferation gives rise to the formation of a typical root knot or gall (Fig.ô∂1C). These extreme modifications of root architecture result in devastating effects of RKNs on the quality and yield of crops. Although, many other organisms induce feeding tissues in their host plants, including insects (e.g., cynipid gall wasps and hemipterans), mites,

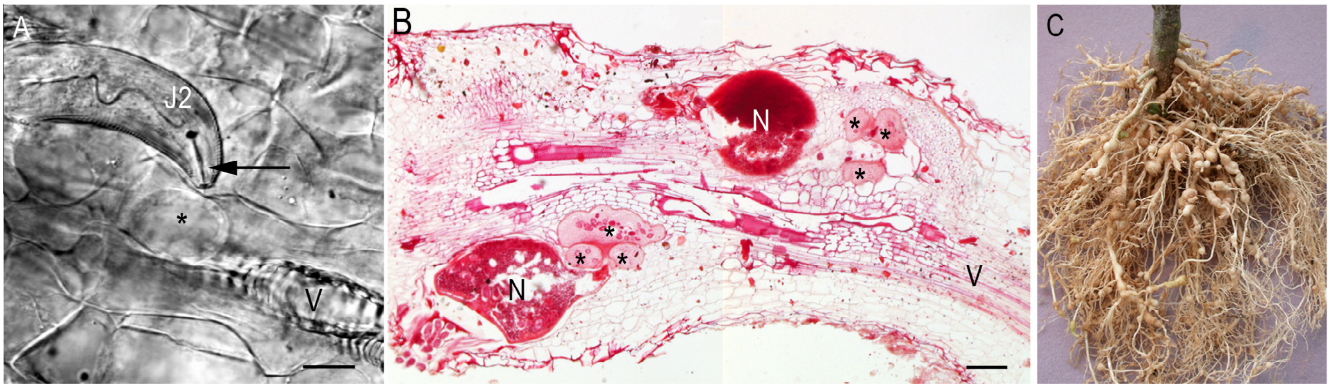

Fig. 1. Galls and giant cells induced by root-knot nematodes. (A) *Meloidogyne incognita* second-stage juvenile (J2) injecting saliva through its stylet (arrow) into a vascular cell (∗) that will become a giant cell. Section through a gall in Arabidopsis, 12 hours post infection. V, vessels. Scale bar = 10 μm. (B) Section through a gall in tomato, 15 days post infection, containing two nematode feeding sites. Asterisks, giant cells; N, female root-knot nematode; V, vessels. Scale bar = 40 μm. (C) Root galls of tomato roots infected by *M. incognita*.