Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2017) 18(1), 3–15 DOI: 10.1111/mpp.12394

Pathogen profile

Meloidogyne graminicola: a major threat to rice agriculture

SOPHIE MANTELIN[1], STÉPHANE BELLAFIORE[2,3] AND TINA KYNDT[4,*]

[1]*The James Hutton Institute, Dundee Effector Consortium, Invergowrie, Dundee DD2 5DA, UK*
[2]*IRD-CIRAD-Université Montpellier II, UMR Interactions Plantes Microorganismes Environnement (IPME), 34394 Montpellier, France*
[3]*LMI-RICE, Hanoi, Vietnam*
[4]*Department of Molecular Biotechnology, Ghent University, 9000 Ghent, Belgium*

SUMMARY

Taxonomy: Superkingdom Eukaryota; Kingdom Metazoa; Phylum Nematoda; Class Chromadorea; Order Tylenchida; Suborder Tylenchina; Infraorder Tylenchomorpha; Superfamily Tylenchoidea; Family Meloidogynidae; Subfamily Meloidogyninae; Genus *Meloidogyne*.

Biology: Microscopic non-segmented roundworm. Plant pathogen; obligate sedentary endoparasitic root-knot nematode. Reproduction: facultative meiotic parthenogenetic species in which amphimixis can occur at a low frequency (*c.* 0.5%); relatively fast life cycle completed in 19–27 days on rice depending on the temperature range.

Host range: Reported to infect over 100 plant species, including cereals and grass plants, as well as dicotyledonous plants. Main host: rice (*Oryza sativa*).

Symptoms: Characteristic hook-shaped galls (root swellings), mainly formed at the root tips of infected plants. Alteration of the root vascular system causes disruption of water and nutrient transport, stunting, chlorosis and loss of vigour, resulting in poor growth and reproduction of the plants with substantial yield losses in crops.

Disease control: Nematicides, chemical priming, constant immersion of rice in irrigated fields, crop rotation with resistant or non-host plants, use of nematode-free planting material. Some sources of resistance to *Meloidogyne graminicola* have been identified in African rice species (*O. glaberrima* and *O. longistaminata*), as well as in a few Asian rice cultivars.

Agronomic importance: Major threat to rice agriculture, particularly in Asia. Adapted to flooded conditions, *Meloidogyne graminicola* causes problems in all types of rice agrosystems.

Keywords: effectors, nematode control, *Oryza sativa*, root-knot nematode.

* *Correspondence*: Email: tina.kyndt@ugent.be

INTRODUCTION TO THE RICE ROOT-KNOT NEMATODE AND DAMAGE TO CROP PRODUCTION

Nematodes are among the most widespread organisms on Earth. They are capable of colonizing any ecosystem, including extreme environments, such as deserts, hot spring waters, arctic lands and polar seas (Yeates, 2004). Many species of nematode are free-living, but some nematodes have developed the ability to parasitize other organisms. The evolution of plant parasitism in nematodes has occurred independently on several occasions (van Megen *et al.*, 2009), giving rise to at least four different groups of plant-feeding nematodes, which include over 4100 species (Decraemer and Hunt, 2013). Plant-parasitic nematodes (PPNs) are responsible for more than $US80 billion losses in worldwide agriculture annually (Nicol *et al.*, 2011). The most economically important species are the sedentary endoparasitic nematodes, including the root-knot nematodes (RKNs) and the cyst nematodes of the genera *Meloidogyne* and *Heterodera/Globodera*, respectively (Jones *et al.*, 2013; Fig. 1). At least 20% of the total estimated economic losses inflicted by nematodes derive from rice alone (Sasser and Freckman, 1987). In rice, the environmental conditions and type of agrosystem determine the community of nematodes present and, consequently, the species profile of parasitic nematodes can differ from region to region (Prot and Rahman, 1994). Many nematode species have been described in association with rice, but only a few of these have significant detrimental effects (Kyndt *et al.*, 2014; Fig. 1). *Meloidogyne graminicola* (*Mg*), commonly named as the rice RKN, is one of the most prevalent PPNs in rice agrosystems. It is considered to be a major threat to rice agriculture, particularly in Asia, where changes in agricultural practices in response to environmental (climate change) and socioeconomic conditions have led to a dramatic increase in *Mg* populations (De Waele and Elsen, 2007). Asia is the main rice-growing region of the world, responsible for about 90% of global rice production, which is estimated at around 740 million tons of paddy rice annually (FAOSTAT, 2013). In addition, rice is the staple food for more than 50% of the population in Asia, a figure that can reach up to 70% for southern regions of this continent (Muthayya *et al.*, 2014). Rice production in Asia is therefore of essential importance for both local and global food

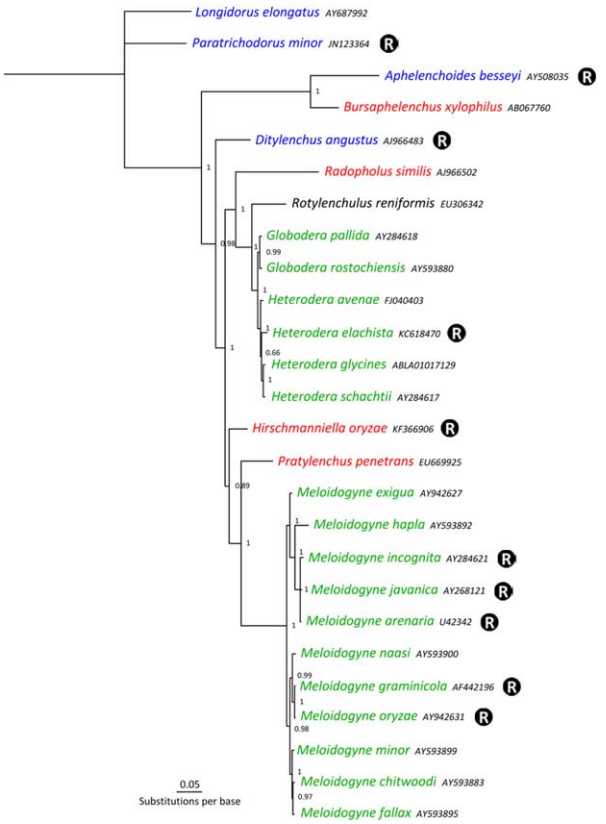

Fig. 1 Phylogenetic position of *Meloidogyne graminicola* based on the small subunit ribosomal DNA (18S). The tree represents a Bayesian phylogeny of 26 plant-parasitic nematodes, including the rice root-knot nematode *M. graminicola*, nine other rice-pathogenic nematodes (R) and some related species. Several important rice-pathogenic cyst nematodes are absent from the phylogeny (e.g. *Heterodera oryzae*, *H. oryzicola* and *H. sacchari*) as no 18S rDNA sequence information is currently available for these species. Nematode species life strategies are colour coded: green, sedentary endoparasite; black, sedentary semi-endoparasite; red, migratory endoparasite; blue, migratory ectoparasite. One representative 18S sequence for each species was used (GenBank accession number indicated in the phylogenetic tree). The 18S sequences were aligned and refined using MUSCLE v3.8.31 (Edgar, 2004). The selection of the best fitted model of DNA evolution and Bayesian phylogenetic construction (MrBayes with model selection SYM + Gamma) were carried out in TOPALi v2 (Milne *et al.*, 2009). The resulting phylogeny was re-routed by known outgroup (*Longidorus elongatus* and *Paratrichodorus minor*) and formatted in FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/). Bootstrap values for one million iterations are indicated for each node. Sequence alignment (.ALN) and phylogenetic distance (.TRE) files are provided as Files S1 and S2 in Supporting Information.

security and its production is threatened by the increasing prevalence of *Mg*.

Meloidogyne graminicola was first described by Golden and Birchfield (1965) and was isolated from the roots of a barnyard grass in the American state of Louisiana. Since then, *Mg* has been identified in many countries, revealing a distribution in rice-growing areas throughout South and South-East Asia as well as in the USA and Latin America (*Mg* distribution map: http://www.cabi.org/isc/datashe/33243). This nematode may be absent from Africa as only one technical report exists that mentions the presence of *Mg* in South Africa (Kleynhans, 1991). Similarly, to our knowledge, *Mg* has not yet been identified in Australia or Europe. *Mg* is a devastating plant pathogen, and is therefore classified as a quarantine pest in many countries. Rice is the most important host for *Mg*, but the nematode has a wide range of alternative hosts (Bridge *et al.*, 2005). This nematode is frequently found associated with other cereals, as well as dicotyledonous and grass plants, including many weeds commonly found in rice fields that may constitute a major reservoir of nematodes (Rich *et al.*, 2009).

Meloidogyne graminicola is an obligate sedentary endoparasite adapted to flooded conditions. It is found in both upland (rainfed) and lowland (irrigated) rice, as well as in deepwater ecosystems, where *Mg* is one of the three predominant pathogenic nematode species (Prot and Rahman, 1994). Infection by *Mg* in rice induces the formation of galls, mainly at the root tips with a characteristic hook shape (see inset in Fig. 2), that strongly impair root development and physiology. The disruption of water and nutrient transport by the alteration of the root vascular system leads to above-ground symptoms, such as stunting, chlorosis and loss of vigour, which ultimately result in poor growth of the crop and substantial yield losses that can represent up to 87% of production (Netscher and Erlan, 1993; Fig. 2). Losses in flooded rice fields occur by drowning when infected seedlings fail to elongate above the rising flood water, leaving patches of open water in flooded fields (Bridge and Page, 1982; Fig. 2). Under simulated upland or intermittently flooded conditions, yield losses caused by *Mg* range from 20% to 80% and 11% to 73%, respectively (Plowright and Bridge, 1990; Soriano *et al.*, 2000). In the field, these losses may be exacerbated in combination with other biotic or abiotic stresses, such as drought. *Mg* is thus a severe constraint to productivity in rice-growing countries and is likely to be an underestimated pathogen because of the lack of specific above-ground symptoms that can lead growers to wrongly attribute the damage to nutritional and water-associated disorders or to secondary diseases.

LIFE CYCLE, BIOLOGY AND DIVERSITY OF *MELOIDOGYNE GRAMINICOLA*

Like other RKNs, *Mg* infective juvenile stage 2 (J2) nematodes can be found in soil samples as small filiform roundworms (length, 350–510 μm), where they are part of the mesofauna. Preparasitic J2s cannot feed until they enter a host and become parasitic. As free-living worms, their entire metabolism is dependent on lipid reserves laid down during embryonic development. Spatio-temporal studies have shown that densities of *Mg* J2s in the soil fluctuate throughout the year. Factors such as soil

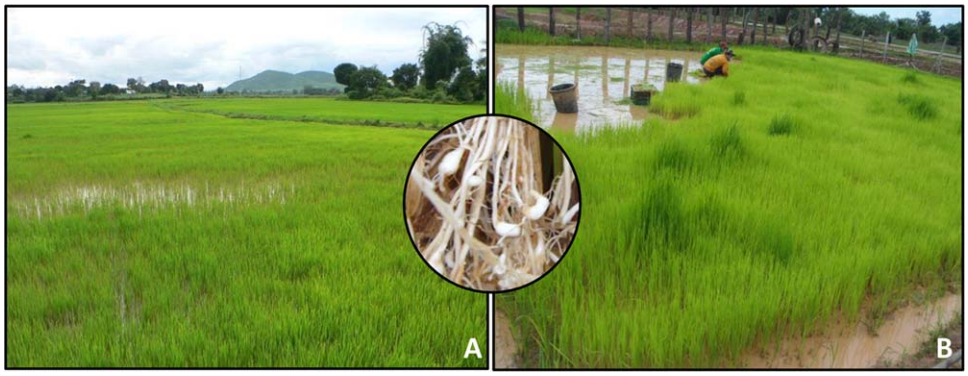

Fig. 2 Rice field and nursery in Asia severely infested with *Meloidogyne graminicola*. (A) A typical patch of open water in *M. graminicola*-infected lowland rice flooded field in Vietnam. (B) Infected plants in a rice nursery in Laos showing stunted growth with an apparent chlorosis (Picture courtesy of Phetsamone Songvilay, MOAF Laos). The middle inset illustrates *M. graminicola*-infected root systems showing gall formations with typical hook-shaped root tips.

structure, temperature, pH, redox state and moisture, as well as the host plant growth stage and crop cycle duration, can affect the capacity of the nematode to survive in the ecosystem and, consequently, its ability to infect plants (Soriano *et al.*, 2000; Win *et al.*, 2011, 2013).

Compared with other *Meloidogyne* species, *Mg* has a relatively fast life cycle on rice; this is completed in 19–27 days depending on the soil temperature, which usually ranges from 22 to 29 °C in the areas in which *Mg* is found (Bridge and Page, 1982; Yik and Birchfield, 1979). Infection studies using freshly hatched J2s show that female nematodes will develop within 14 days of infection and start to lay eggs in the root cortex (Fig. 3A–F). Then, 18–20 days post-infection (dpi), abundant juvenile stage 1 (J1) nematodes can be observed within the eggs (Bellafiore *et al.*, 2015; Fig. 3F). Like other *Meloidogyne* species, *Mg* J1s undergo their first moult in the egg to become pre-parasitic J2s. After hatching, if the nematodes are released in soil, they locate roots by chemotaxis (Reynolds *et al.*, 2011) and invade the root at the elongation zone (Fig. 3A). The newly parasitic J2s migrate intercellularly in the rice root cortex towards the root tip, where they invade the vascular cylinder. Unlike other *Meloidogyne* species, which will subsequently move up the vascular cylinder for a long distance, *Mg* establishes its feeding site in the stele close to the root meristem, forming five to eight giant cells (Cabasan *et al.*, 2014; Jena and Rao, 1977; Fig. 3B).

Once established in the root, the nematode becomes sedentary, feeds from the giant cells and, after three moults, reaches the adult stage, taking a pyriform or filiform shape for the female (Fig. 3C) or male, respectively. In other *Meloidogyne* species, female development can result in tearing of the root and the subsequent release of egg masses through this aperture into the soil. By contrast, the *Mg* adult female remains inside the root, where it is protected from the external environment by several layers of plant cells, and releases eggs inside the cortex (Fig. 3D,E). This unusual method of egg-laying is an advantage when the host is in flooded conditions. The next generation of nematodes, developing inside these egg masses (Fig. 3F), are consequently more likely to make new feeding sites within the same root. It is also worth noting that plant sedentary endoparasitic nematodes, such as *Mg*, remain within their host for most of their life cycle, and are thus protected from predators and potential pathogens by the immune systems of the host. One consequence of this parasitic lifestyle is the reduced immune gene complement found in the genome of these nematodes compared with free-living nematodes (e.g. Abad *et al.*, 2008; Cotton *et al.*, 2014; Ghedin *et al.*, 2007).

Meloidogyne graminicola is a facultative meiotic parthenogenetic species in which amphimixis can occur at a low frequency (approximately 0.5%; Triantaphyllou, 1969). Oogenesis and spermatogenesis studies have revealed that the haploid chromosome number ($n = 18$) is determined during the first and second maturation divisions without any variation in number (Triantaphyllou, 1969). It is assumed that, when males are present and in contact with females, classical amphimixis happens, whereas, in their absence, parthenogenesis takes over. For this asexual reproduction mode, the egg nucleus undergoes classical meiosis, in which the first polar body degenerates and fusion of the second polar body with the egg pronucleus restores the somatic chromosome number.

Several *Mg* populations have been isolated and examined for differences in morphology, DNA sequence, virulence and aggressiveness. Quantitative characters, such as the length of the body and stylet, were used to reveal intraspecific diversity between populations isolated from Vietnam (Bellafiore *et al.*, 2015), Nepal, India, Bangladesh, Thailand and the USA (Jepson, 1983; Pokharel *et al.*, 2010). Interestingly, the aggressiveness (defined here as the quantification of parasite fitness on a susceptible host), evaluated under controlled conditions, revealed significant differences between populations, again suggesting intraspecific diversity (Bellafiore *et al.*, 2015; Pokharel *et al.*, 2007). In addition to 'quantitative' differences, a 'qualitative' difference was observed between

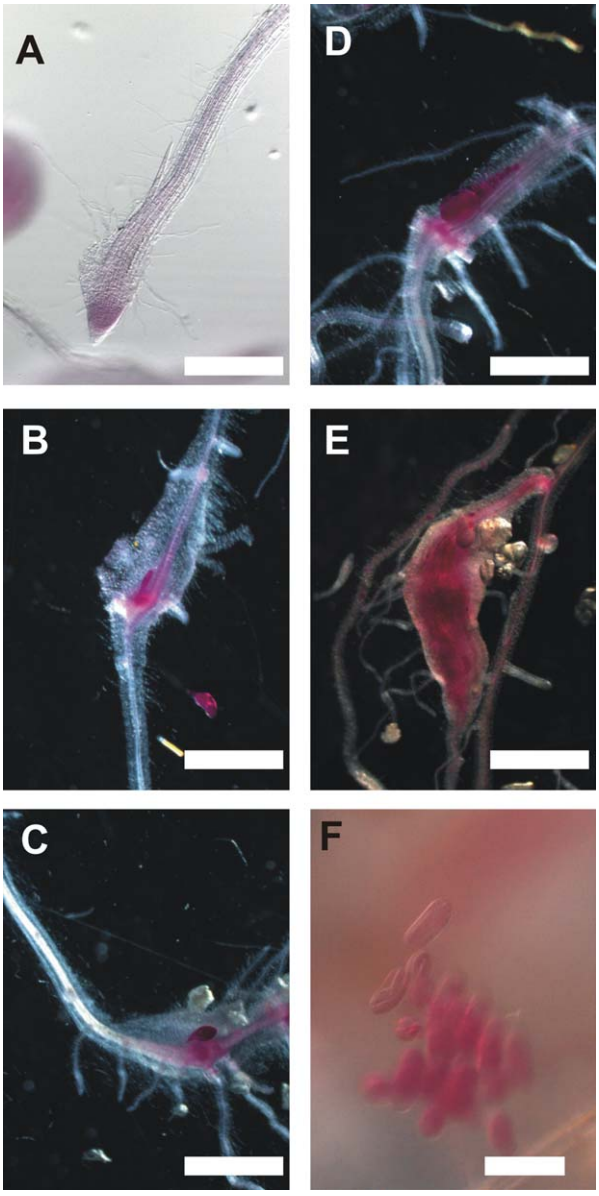

Fig. 3 *Meloidogyne graminicola* (*Mg*) development in rice root of susceptible *Oryza sativa*. (A) *Mg* juvenile stage 2 (J2) invading the root at the elongation zone 18 h after infection. (B) J2 feeding inside a gall at 3 days post-infection (dpi). (C) J3/J4 in roots at 10 dpi. (D) Female of *Mg* with eggs at 15 dpi. (E) Typical mature gall, including many developing *Mg* nematodes. (F) Eggs containing developing *Mg* J1, extracted by puncturing a gall at 17 dpi. Nematodes were stained inside the rice roots using acid fuchsin (Nahar *et al.*, 2011). Scale bar in (A)–(E), 1 mm. Scale bar in (F), 100 μm.

populations isolated from North America and Asia. Despite a similar host range, the American population of *Mg* failed to propagate on several rice cultivars compared with the Asian populations (Pokharel *et al.*, 2010). Other independent pathogenicity studies on a variety of hosts suggest that *Mg* consists of more than one race. For example, *Mg* populations show variable ability to parasitize tomato and maize (Bellafiore *et al.*, 2015; Pokharel *et al.*, 2010; Yik and Birchfield, 1979). The compatibility observed between *Mg* and its hosts is cultivar dependent. These data are consistent with both the existence of different races of *Mg* and with the concept that some cultivars in a plant species are resistant to some *Mg* populations. Another explanation for the apparent difference in virulence of some *Mg* populations on the same plant species could be the misidentification of the nematode species. For example, *Mg* and *M. oryzae* are closely related (Fig. 1), are morphologically very much alike, have a similar life cycle and cause very similar symptoms on plants. However, *M. oryzae* has a slightly different host range (Maas *et al.*, 1978). This demonstrates the importance of the development of reliable methods of identification for the rice RKNs.

Nematode species identification based on enzymatic phenotypes may be misleading unless several enzymes are used, as the enzyme profiles can be almost identical between species such as *Mg* and *M. oryzae*, with only malate dehydrogenase and esterase that can distinguish between them (Carneiro *et al.*, 2000). In order to facilitate the identification of *Mg*, several mitochondrial and nuclear DNA markers have been designed (Bellafiore *et al.*, 2015; Besnard *et al.*, 2014). Nucleotide polymorphisms between populations have been evaluated using the internal transcribed spacer (ITS) region (Bellafiore *et al.*, 2015; Pokharel *et al.*, 2007). Different haplotypes were observed, but without a clear pattern of geographical distribution, suggesting a recent expansion of the species. The whole mitochondrial genomes of two *Mg* populations isolated from the Philippines and China have been sequenced recently (Besnard *et al.*, 2014; Sun *et al.*, 2014), providing molecular information potentially useful for population genetic studies and life history reconstruction. Mitochondrial DNA evolves more rapidly in Nematoda than in other taxa, and gene duplication, gene order rearrangement and DNA recombination in the variable number of tandem repeat (VNTR) regions are reminiscent of this rapid evolution in the *Meloidogyne* genus (Humphreys-Pereira and Elling, 2015; Lunt and Hyman, 1997). Therefore, the mitochondrial genome and, more specifically, the VNTR regions (Lunt *et al.*, 1998) have been successfully used to explore the population dynamics of other *Meloidogyne* species (Whipple *et al.*, 1998). *Mg* mitochondrial DNA analysis revealed unexpected transfer RNA duplications and the presence of two VNTR regions (111R and 94R), one of which (the 111R VNTR region) contained polymorphisms that are potentially useful for further population genetic studies (Besnard *et al.*, 2014; Humphreys-Pereira and Elling, 2015; Sun *et al.*, 2014). One potential drawback of evolutionary studies on mitochondrial DNA comes from the fact that a nematode is made up of approximately 1000 cells and each could contain up to 1000 mitochondria (Rokas *et al.*, 2003). Each nematode could therefore contain approximately one million mitochondria with a potential heteroplasmic status that would make the analysis more

complex (Lunt *et al.*, 1998; Okimoto *et al.*, 1991; Whipple *et al.*, 1998). The use of mitochondrial VNTR markers to address the genetic structure can therefore be challenging. The potential use of nuclear genetic markers [i.e. microsatellites, indels and single nucleotide polymorphisms (SNPs)] is currently being tested (G. Besnard, personal communication). Such tools may allow the exploration of the diversity of the species and the reconstruction of its evolutionary history.

MELOIDOGYNE GRAMINICOLA: A MANIPULATOR OF PLANT CELL METABOLISM AND IMMUNITY

The RKNs penetrate their host at the root elongation zone, after which they migrate through the cortex to the root tip. In contrast with most migratory and cyst nematodes, the movement of RKNs inside the roots does not cause extensive damage to the cells, as the juveniles migrate between cells. However, the cells do respond to the nematodes, either by sensing this movement or by responding to minor plant damage and recognition mediated by pathogen-associated molecular patterns (PAMPs). For example, susceptible tomato roots have been shown to respond to RKN attack by producing reactive oxygen species (ROS) 12 h after inoculation (Melillo *et al.*, 2006). Excessive production of ROS, such as H_2O_2, can ultimately lead to cell death—the hypersensitive response (HR)—but, when present in minute amounts, ROS act as signalling molecules that trigger the induction of defence genes in infected and neighbouring cells (Mellersh *et al.*, 2002). The typical feeding sites established by RKNs are multinucleate, hypertrophied cells—formed by repeated rounds of nuclear division and cell growth in the absence of cytokinesis—called giant cells (Kyndt *et al.*, 2013; Fig. 4A). Importantly, 48 h after RKN inoculation, concomitant with giant cell induction, ROS levels are lower in infected than in uninfected tomato roots and become cytologically undetectable in infected cells (Melillo *et al.*, 2006). Similar observations have been made in the rice–*Mg* interaction: although H_2O_2 accumulates at 1 dpi in *Mg*-infected rice roots, its level is slightly lower or equal to the basal levels at 3 and 5 dpi (Ji *et al.*, 2015b). As a burst of ROS occurs during the initial migratory phase of *Mg* in the rice root, an active suppression mechanism is most probably used by the nematode to prevent defence activation by ROS once the nematode has started to establish its feeding site.

In order to sustain the intimate relationship with their host, biotrophic parasites such as *Mg* must suppress the plant defence system, which is responsible for the production of many defence metabolites and cell death-related proteins. This is observed in young *Mg*-induced feeding sites (Ji *et al.*, 2013; Kyndt *et al.*, 2012), with the repression of jasmonate (JA)-related genes in giant cells (Ji *et al.*, 2013) and genes of the PR13/thionin gene family in gall tissues (Ji *et al.*, 2015a). The JA pathway indeed plays a determinant role in rice basal immunity against *Mg* (Nahar *et al.*, 2011). Genes involved in the phenylpropanoid pathway, such as *OsPAL*, *OsC4H*, *OsCOMT* and *OsCAD*, are also strongly repressed in established giant cells and slightly repressed in whole galls induced by *Mg* (Ji *et al.*, 2013, 2015b; Kyndt *et al.*, 2012), a phenomenon which has also been observed in giant cells induced by *M. javanica* in tomato and *Arabidopsis* plants (Portillo *et al.*, 2013). In addition, callose deposition, which is a typical hallmark of enhanced plant defence (Jacobs *et al.*, 2003; Luna *et al.*, 2011), is barely detectable in *Mg*-induced gall tissue at 4 dpi. Although the regulation of callose biosynthesis genes was not affected, *Mg* feeding led to a significant increase in the levels of transcripts encoding OsGNS5, a callose-degrading enzyme (Ji *et al.*, 2015b).

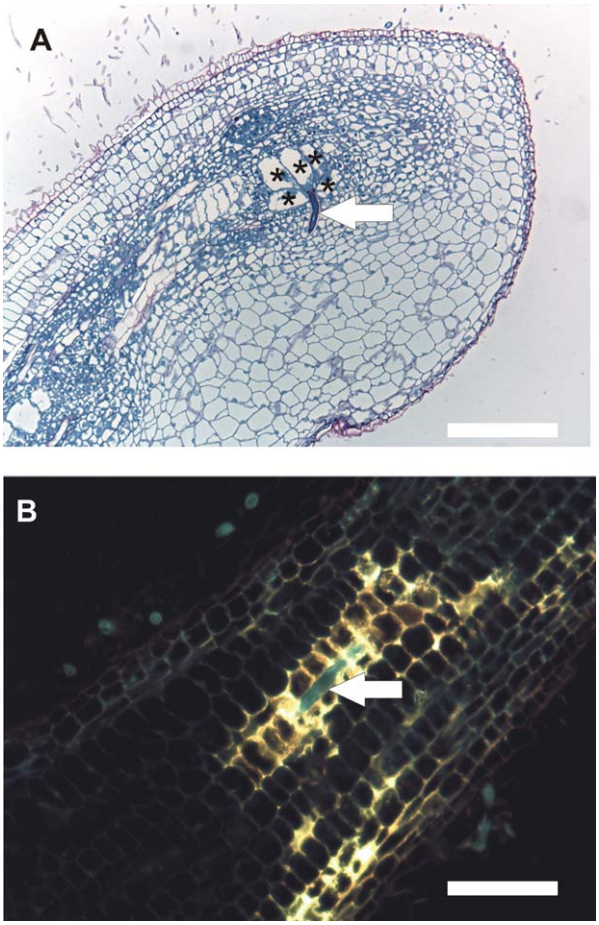

Fig. 4 Longitudinal section of *Meloidogyne graminicola*-infected roots. (A) Susceptible *Oryza sativa* 'Nipponbare' rice root at 4 days post-infection (dpi). Longitudinal root sections (4 μm) stained with 0.05% toluidine blue. (B) Invading nematode in resistant *Oryza glaberrima* at 6 dpi observed under UV light (UV filter set A2, Carl Zeiss AXIO Imager Microscopy GmbH, Jena, Germany), showing the accumulation of phenolic compounds around the nematode. Scale bar, 100 μm. The white arrow points to the nematode. *Giant cell.

Recent research highlights the importance of hormone balances at the frontline in the battle between *Mg* and rice (reviewed in Kyndt *et al.*, 2014). For example, brassinosteroid biosynthesis and signalling genes are generally activated in gall tissue (Nahar *et al.*, 2013), where the brassinosteroid pathway seems to negatively cross-talk with the JA pathway, which is a key determinant of rice root immunity against *Mg* (Nahar *et al.*, 2011). Similarly, the abscisic acid (ABA) signalling pathway may be involved, as *in planta* induction of the ABA pathway has been shown to antagonize the salicylic acid/JA/ethylene-dependent core rice root defence system, leading to increased susceptibility to the migratory rice root nematode *Hirschmanniella oryzae* (Nahar *et al.*, 2012).

In contrast with the suppression of secondary metabolism, transcriptomics of galls and isolated giant cells from *Mg*-infected rice roots has revealed a general induction of primary metabolism (Ji *et al.*, 2013; Kyndt *et al.*, 2012). For example, genes involved in nucleotide synthesis, starch production, phospholipid production, protein and sucrose biosynthesis, transporters, photosynthesis and glycolysis are generally activated in galls and giant cells, indicating that the heterotrophic RKN stimulates the cells within the gall to be more active and to produce energy and nutrients to the benefit of the nematode (reviewed in Fernandez *et al.*, 2015).

This transcriptional reprogramming observed in RKN-infected plant tissues could potentially be caused by epigenetic mechanisms, such as DNA methylation, histone modifications or RNA interference (RNAi)-based gene silencing. Indeed, genes involved in chromatin remodelling, DNA methylation, small RNA formation and histone modifications are all highly expressed inside giant cells induced by *Mg* in rice (Ji *et al.*, 2013), as well as in *M. incognita*-induced giant cells in tomato (Portillo *et al.*, 2013). How these epigenetic mechanisms are activated and which genes/pathways they are targeting remains unknown.

GENOMICS AND TRANSCRIPTOMICS: A DEVELOPING RESOURCE FOR *MELOIDOGYNE GRAMINICOLA*

The *Mg* sequence information currently present in public databases is very limited. The genome of *Mg* has not been published to date. Although this nematode species is not yet listed as proposed under the 959 nematode genomes initiative project (Kumar *et al.*, 2012; http://www.nematodes.org/nematodegenomes), sequencing of several genomes from different *Mg* populations is underway (S. Bellafiore, unpublished data). The size of the *Mg* genome has been estimated at around 30 Mb by Feulgen densitometry (56% $\pm$ 7.6% relative DNA content of hypodermal nuclei of J2 using *M. incognita* as reference, equivalent to a C-value of 0.03 pg gametic nuclear DNA content; Lapp and Triantaphyllou, 1972), an estimate that seems to hold based on the current draft genome (S. Bellafiore, unpublished data). If true, the genome of *Mg* would be much more compact than those of its closest sequenced relatives *M. incognita* (*c.* 86 Mb; Abad *et al.*, 2008) and *M. hapla* (*c.* 54 Mb; Opperman *et al.*, 2008). *Mg* is also poorly represented in other DNA sequence databases. Entries for *Mg* are absent from the two main nematode databases, NEMBASE4 and the nematode.net portal (Elsworth *et al.*, 2011; http://www.nematodes.org/nembase4; Wylie *et al.*, 2004; http://nematode.net), and only 124 nucleotide sequences are present in GenBank (Benson *et al.*, 2013; http://www.ncbi.nlm.nih.gov/genbank), mostly ribosomal RNA genes used in phylogenetic studies, as well as cell wall-modifying enzymes and the complete mitochondrial genome.

Recently, two studies have explored the transcriptome of *Mg* across several life stages of the nematode, from pre-parasitic J2s to established females in roots of rice (Haegeman *et al.*, 2013; Petitot *et al.*, 2015). Almost 52 000 gene loci represented by over 66 000 transcripts were predicted that could encode putative proteins, about 50% of which contain known functional domains. Relatively little investigation has been performed into the overall changes in gene expression profile that occur between life stages in relation to the general biology of the nematode life cycle and development. Instead, the focus for these studies has been the mining of the transcriptome, emphasizing the importance of the putative secretome of *Mg* and the identification of candidate parasitism genes.

In order to successfully establish parasitism, PPNs secrete a cocktail of effectors, thought to be mainly proteins, into their host plant (Haegeman *et al.*, 2012; Hewezi and Baum, 2013). These effectors facilitate the migration of the nematode inside the plant roots and are required to initiate and maintain their feeding site (Fig. 4A). These proteins can be secreted into the apoplast through the cuticle or the stylet, as well as from the amphids and the phasmids of the nematode. The majority of the best characterized effectors are produced in the nematode oesophageal gland cells, from where they are thought to be directly injected into the plant cells through the stylet. These proteins can be identified on the basis of the presence of a predicted signal peptide for secretion and the absence of a transmembrane domain, as well as being preferentially expressed in invasive and early parasitic stages. The transcriptome sets generated from *Mg* were screened for putative effectors (Haegeman *et al.*, 2013; Petitot *et al.*, 2015). Unsurprisingly, these studies revealed the presence of many peptidases and plant cell wall-modifying enzymes, including a pectate lyase and a poly-α-D-galacturonosidase for pectin degradation, β-1,4-endoglucanases and a xylanase for cellulose and hemicellulose degradation, as well as cellulose-binding proteins and expansin-like proteins, which are not enzymes *per se*, but which disrupt non-covalent bonds between cell wall components. These proteins are commonly secreted by PPNs to soften and degrade the plant cell wall, thus facilitating the invasion of the root tissues by nematodes and the movement of the worms within the roots.

Most of these genes are strongly expressed in *Mg* infective juveniles but are down-regulated at later stages of parasitism, correlating with the transition to the sedentary phase for the nematode. However, some of these enzymes may be expressed in later stages, when they may have a role in processes such as egg laying or migration of the adult male from the roots (Danchin *et al.*, 2010; Thorpe *et al.*, 2014).

As the nematode progresses through the root, its migration, as well as the by-products of the peptidases and cell wall-modifying enzyme activity, are likely to cause the production of damage-associated molecular patterns (DAMPs). In addition, nematode secretions and surface factors may be recognized as PAMPs by plant cell receptors (Manosalva *et al.*, 2015). Both DAMPs and PAMPs may thus elicit plant defence responses, such as the production of ROS, which must be suppressed by the invader to succeed in parasitism. Consistent with this hypothesis, transcriptome analyses from *Mg* identified antioxidant proteins that may be involved in the detoxification of host-derived ROS, including several glutathione peroxidases, glutathione-*S*-transferases, thioredoxins and peroxiredoxins. A metallothionein is also highly expressed in early parasitic stages that could have a protective role in cells exposed to ROS (Petitot *et al.*, 2015). In addition, a series of transcripts similar to nematode venom allergen-like proteins (VAP) has been identified in *Mg* (Haegeman *et al.*, 2013). It has been proposed recently that the VAPs may suppress the activation of defences triggered by DAMPs that would be generated in the plant during nematode infection (Lozano-Torres *et al.*, 2014).

Other candidate effectors of *Mg*, which are similar to described and/or characterized genes from other PPNs, may be involved in the recognition between plant and nematode or in host manipulation and suppression of plant defences. At least 17 contigs containing *map-1* homologues (corresponding to the *Meloidogyne* avirulence protein MAP-1) have been identified in *Mg* (Haegeman *et al.*, 2013). In *Meloidogyne* species, *map-1* is only expressed in the infective juveniles of avirulent lines of the RKNs controlled by *Mi-1* in tomato, and therefore it was speculated that MAP-1 may be involved in the early recognition steps between the resistant host and avirulent nematodes (Semblat *et al.*, 2001). Furthermore, *map-1* belongs to a small gene family and variation in the number/arrangement of internal repeats in the genes correlates with the (a)virulence profile of *M. incognita* lines, suggesting a role in the specificity of the plant–nematode interaction (Castagnone-Sereno *et al.*, 2009). The possibility of *Mg* MAP-1 homologues being recognized by Mi-1 and/or Mi homologues to explain the failure of the nematode to establish in tomato has not yet been investigated.

Effectors have been identified which may play a role in the suppression of plant defences by interfering with hormonal pathways involved in plant immunity. Consistent with JA-related genes being suppressed in giant cells induced by *Mg* in rice (Ji *et al.*, 2013), several transcripts encoding fatty acid and retinol-binding proteins (FAR), proteins which can bind JA precursors (Prior *et al.*, 2001), have been identified in the *Mg* transcriptome (Haegeman *et al.*, 2013; Petitot *et al.*, 2015). Collectively, functional analysis of FAR-1 homologues in cyst nematodes and RKNs indeed supports a role for these effectors in suppressing the JA pathway (Mantelin *et al.*, 2015).

The secretion of effectors that mimic plant proteins or signalling peptides is a common strategy observed in PPNs to interfere with plant signalling pathways to either block, modulate or hijack host cellular processes (Haegeman *et al.*, 2012). Many putatively secreted 14-3-3 proteins have been identified in *Mg* (Haegeman *et al.*, 2013). 14-3-3 proteins are major regulatory proteins highly conserved in eukaryotes. In plants, they regulate the activities of a plethora of proteins involved in a wide range of cellular and physiological processes, such as the regulation of transcription, coordination of mitosis, modulation of phosphatase- and kinase-dependent signalling pathways (including pathogen-elicited and hormone signalling pathways), primary metabolism, global stress responses and organelle trafficking (Denison *et al.*, 2011; Roberts, 2003); many of these processes constitute key host targets to be hijacked by the nematode. In addition, several annexin-like proteins are expressed in the infective juveniles that are involved in protection against environmental stresses. As observed for *Heterodera schachtii* Hs4F01, *Mg* annexin-like effectors may mimic plant annexin function during the parasitic interaction in order to promote host susceptibility (Patel *et al.*, 2010), possibly by suppressing plant defence, as described for the cereal cyst nematode Ha-ANNEXIN (Chen *et al.*, 2015).

Three sequences of predicted secreted C-type lectins have also been identified in the *Mg* transcripts (Haegeman *et al.*, 2013). Many of these proteins produced in nematodes have a role in innate immune defences against bacterial infection and in the regulation of interactions with other organisms (De Schutter and Van Damme, 2015). In animal-parasitic nematodes, C-type lectins homologous to some key receptors of the mammalian immune system are released into the host by infective juveniles, possibly to neutralize host defence mechanisms (Harcus *et al.*, 2009). Similarly, the C-type lectins from *Mg* may interfere with plant defence. Indeed, lectins are involved in plant immunity (De Schutter and Van Damme, 2015) and, to some extent, may confer protection against nematodes (Burrows *et al.*, 1998; Ripoll *et al.*, 2003). Moreover, a small number of lectin domain-containing calreticulins similar to *M. incognita* Mi-CRT are also present in *Mg* (Haegeman *et al.*, 2013). Functional analysis of secreted Mi-CRT established that it suppresses plant defence in *Arabidopsis* (Jaouannet *et al.*, 2013).

Finally, one CLE-like peptide gene is expressed early in *Mg* during infection (Petitot *et al.*, 2015). The transcript identified is

incomplete, but the analysis of the deduced protein sequence indicates that it contains at least nine CLE-like motifs. This structure of CLE motifs in tandem repeats has been found in wheat, rice and *Medicago truncatula* (Oelkers *et al.*, 2008), as well as in cyst nematodes (Lu *et al.*, 2009) and, more recently, was reported in *Meloidogyne* species, where it was identified by bio-informatics in the sequence of the *map* avirulence gene family (Rutter *et al.*, 2014). Plant CLE ligands are involved in a multitude of growth and developmental processes which may be targeted by the nematode for the development of its feeding site (Miyawaki *et al.*, 2013).

A large proportion of *Mg* candidate effectors represent pioneer genes, encoding proteins of unknown function and with no similarity to other proteins in databases other than homologues in other nematodes. Surprisingly, only four of 35 *M. incognita* pioneers (Huang *et al.*, 2003) originally found a match in the *Mg* transcriptome (Haegeman *et al.*, 2013). Screening the draft genome of *Mg* for homologues of these pioneers indicated that most are present (S. Mantelin and S. Bellafiore, unpublished data). Only 10 did not hit any genomic contig (AF531163, AF531164, AF531169, AY134435, AY134441, AY134443, AY134444, AY142117, AY142118, AY142120). However, most of these sequences also did not find a match when BLASTed against *M. incognita* and/or *M. hapla* genomes. Any genome assembly is, however, only a hypothesis and it is not unusual to find expressed sequence tags (ESTs) that cannot be mapped onto a genome. Collectively, this suggests that some pioneer effector genes may be conserved among *Meloidogyne* species, but their expression in time or place might vary between different nematodes.

In conclusion, a significant number of candidate effectors have been identified that may be deployed by *Mg* in order to promote its biotic interaction with the host. Many of the putative effectors identified from *Mg* transcriptomes were tested for their expression in the nematode by *in situ* hybridization (Haegeman *et al.*, 2013; Petitot *et al.*, 2015). Most of the predicted effectors were expressed in the nematode dorsal or subventral gland cells, or in the amphids, indicating that the corresponding proteins would probably be secreted into the plant tissue, thus supporting a role in parasitism. However, functional studies of effectors in rice, and in rice roots, remain more difficult than studies on model plant systems, such as *Arabidopsis*, meaning that these areas are not as fully developed to date.

CURRENT METHODS AND FUTURE PROSPECTS FOR THE CONTROL OF *MELOIDOGYNE GRAMINICOLA* IN RICE

The efficient control of a pest such as *Mg* requires a combination of different methods. Here, we describe some of the current or prospective approaches that could be used in infested fields to reduce yield losses. The use of nematicides has been the most efficient way to control PPNs in the field for many years, but at a substantial cost to both farmers and the environment. Most of the chemicals used to control nematodes are extremely toxic. Therefore, many effective nematicides, including methyl bromide, have already been banned from most countries as a result of United Nations protocols (MBTOC, 2010). Local legislative pressures from transnational (e.g. European Union) and national authorities are also removing active ingredients from permitted lists, and the uptake of more restrictive methods of assessment of potential toxicity means that active ingredients will continue to be withdrawn in the future. These legislative pressures are thus prompting the development of alternative strategies to control PPNs. Constant immersion of rice in irrigated fields allows better control of infection by RKNs than is possible in any other rice agrosystem. Under flooding conditions, *Mg* can still propagate, but the damage induced by the nematode is not as severe as in shallow intermittently flooded lands. The Asia Pacific region currently produces about 90% of the rice consumed worldwide. When including rice grown in upland conditions under the rainfall system, the irrigated rice area in Asia encompasses up to 50% of the total growing area and contributes to 75% of the total production (GRiSP, 2013). However, rice-producing countries are being urged to change their water management practices in an effort to limit their impact on global warming caused by methane production in rice fields. In addition, as a result of socio-economic and environmental changes, these regions are starting to suffer from water shortages. Responding to the need to reduce water demand and chemical inputs simultaneously, whilst increasing crop yield, is becoming a serious challenge in rice production. Although further development of aerobic field conditions in Asia has been proposed as a solution to water shortages, the adverse effects on the sustainability of rice production also need to be considered (Belder *et al.*, 2004), as emerging studies have suggested a considerable negative impact of *Mg* on both growth and yield in aerobic rice. Rotating cropping systems, in which farmers introduce non-host plants, such as mustard, sesame, millet (Rahman, 1990) or mung bean (Ventura *et al.*, 1981), can reduce the occurrence of *Mg* and therefore yield losses in rice. Soriano and Reversat (2003) demonstrated that rice yield could be improved by up to 85% in upland rice when grown under crop rotation with cowpea, which minimizes nematode populations. However, in addition to the socio-economic impacts related to changes in farmers' crop production habits, the efficiency of crop rotation could be compromised by the relatively wide host range of *Mg*, which is able to propagate in reservoir weeds commonly found in tropical fields, such as several *Cyperus* and *Echinochloa* species. Moreover, although the *Mg* population declines rapidly after 4 months of crop rotation, some eggs can remain viable for up to 14 months in water-logged soils (Bridge and Page, 1982; Roy, 1982), indicating that crop rotations must include a long sequence without rice for greater efficiency. This may be unacceptable for growers who depend on rice.

Studies from other PPN–plant systems suggest that screening of germplasm for genotypes that are resistant or tolerant to *Meloidogyne* species could offer a promising genetic alternative for the control of *Mg*. For example, several resistance genes, such as *Mi1.2* found in the wild relative of tomato (*Solanum peruvianum*) and the *Ma* gene from Myrobalan plum (*Prunus cerasifera*), provide specific resistance against some *Meloidogyne* species, and have been cloned (Claverie *et al.*, 2011; Milligan *et al.*, 1998). *Mi1.2* was introgressed in tomato (*S. lycopersicum*) and is now present in many cultivars. This genetic strategy offers an effective and economic alternative to other nematode controlling practices. Screening for *Mg* resistance in *Oryza* germplasm has already been successful. Lines of African rice from *O. glaberrima*, *O. longistaminata* and *O. rufipogon* (Plowright *et al.*, 1999; Soriano *et al.*, 1999), as well as several *Oryza* landraces, have been described as fully or partially resistant to *Mg* (Gergon and Prot, 1993; Plowright *et al.*, 1999; Soriano *et al.*, 1999) and have been used as resistance donors for interspecific crosses with the Asian cultivated rice *O. sativa*. Interestingly, several cultivars from *O. glaberrima* are highly resistant to *Mg*, and studies on the kinetics of infection using histological analysis of infected CG14 cultivar suggest that HR-like reactions may occur (Cabasan *et al.*, 2014; Fig. 4B). Unfortunately, several genes causing an interspecific *O. sativa* × *O. glaberrima* sterility barrier were also identified and, among them, the S1 locus had the strongest effect (Garavito *et al.*, 2010). The hybrid non-viability and sterility compromised the full exploitation of the genetic diversity found within *O. glaberrima*, despite the presence of remarkable traits that would have been useful to overcome biotic and abiotic stresses in rice. However, successive backcrosses, followed by selfing, have allowed the development of fertile plants derived from *O. sativa* × *O. glaberrima* crosses and the screening for nematode resistance of backcross lines is possible (Ghesquière *et al.*, 1997). Resistance in Asian rice has been actively sought for several decades (Bridge *et al.*, 2005) and, very recently, three cultivars of *O. sativa* resistant to *Mg* have been identified (S. Bellafiore, unpublished data; Dimkpa *et al.*, 2015): two fully resistant *O. sativa* accessions, Khao Pahk Maw and LD24, as well as one accession from China which shows HR-like responses on *Mg* infection. This Chinese resistant accession has been crossed with the susceptible *O. sativa* IR64 line and the progeny will be tested for the segregation of resistance(s). Currently, all F1 plants are resistant to the nematode at a similar level to the resistant donor, suggesting dominant resistance gene(s). These three accessions will be extremely valuable for future breeding programmes aimed at producing resistance against *Mg* in Asian rice cultivars.

Induced resistance and chemical priming represent complementary approaches to rice genetics in the control of *Mg*. Some chemical and biological compounds are able to trigger the plant defence machinery, leading to induced resistance. For example, compounds such as β-aminobutyric acid (BABA) or benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methylester (BTH) are capable of inducing rapid and effective defence responses to ward off various invading pathogens in different plant species (Ton and Mauch-Mani, 2004). Induced resistance to *Mg* in rice has been shown to be feasible, where foliar sprays with 250 μM BTH, 500 μM ethephon or 100 μM methyl-JA provide systemic defence activation, reducing gall formation by 24% (BTH) to 64% (methyl-JA) (Nahar *et al.*, 2011). A 3.5 mM BABA soil drench efficiently protects the roots from infection by enhancing the basal plant defence system, as exemplified by potentiated H_2O_2 generation, lignin formation and callose deposition in the galls of BABA-treated plants (Ji *et al.*, 2015b). Nevertheless, defence activators also tend to have negative effects on plant growth, because of the trade-off between growth and defence. Priming is a more efficient type of induced resistance, where defence responses are not directly activated, but only induced when plants are subsequently challenged by a pathogen or pest. Consequently, the metabolic investment of the plant is reduced compared with constitutive defence activation. Recently, priming against *Mg* has been tested using thiamine treatment (Huang *et al.*, 2015b). After thiamine root drenching, the number of nematodes in rice roots was slightly but significantly reduced, and the development of the nematodes was delayed, whereas no negative effects of thiamine on nematode viability and infectivity or on plant growth were observed. Molecular and biochemical studies have shown that thiamine-induced priming against *Mg* involves H_2O_2 and phenylpropanoid-mediated lignin production, whereas giant cell development and callose deposition are not affected (Huang *et al.*, 2015b).

Another option that may induce resistance in plants is the addition of organic compounds to the substrate. Soil amended with biochar, a solid coproduct of biomass pyrolysis, has previously been shown to enhance the productivity of various crops and to induce systemic plant resistance to fungal pathogens (Elad *et al.*, 2011). Similarly, a 1.2% concentration of biochar added to the potting medium of rice enhances plant tolerance to *Mg* infection and slightly reduces nematode development in the roots, whereas direct toxic effects of biochar exudates on nematode viability, infectivity or development were not observed (Huang *et al.*, 2015a). The increased plant resistance was associated with primed H_2O_2 accumulation in the infected root system, as well as the transcriptional enhancement of genes involved in the ethylene signalling pathway.

Altogether, these data show that priming against rice RKNs is feasible, although the current effects with thiamine and biochar amendments are rather minor. Further investigations into more potent defence elicitors are needed. Biological control of *Mg* by antagonistic activities of endophytic and rhizosphere fungi may also be worth investigating, as inoculation a rice field with *Fusarium* isolates and *Trichoderma* species can reduce root gall severity

in rice by 29%–42% and 38%, respectively, in controlled conditions (Le *et al.*, 2009).

Finally, transgenic approaches using genetically modified (GM) plants may be considered to protect crops against *Mg*. The inhibition of nematode infestation has been successful using the ectopic expression of cystatin protease inhibitors (reviewed by Lilley *et al.*, 2011). Transgenic plants of four African rice varieties with *Cauliflower mosaic virus* (CaMV) 35S-driven expression of a modified rice cystatin OcIΔD86 displayed 55% resistance to *M. incognita* (Vain *et al.*, 1998). This work was extended by introducing the same gene in rice variety Nipponbare under the control of a feeding site-specific root promoter from Arabidopsis (*TUB-1*) (Green *et al.*, 2002). The best transgenic lines displayed 91% ± 7% resistance to *M. incognita* (Lilley *et al.*, 2011). Whether these plants are also resistant to other PPNs, such as *Mg*, is not known.

In another GM-based approach, a rice thionin gene *OsThi7*, belonging to the pathogenesis-related *PR13* gene family, was overexpressed in rice plants (Ji *et al.*, 2015a). Under normal growth conditions, *OsTHI7* shows a root tip-specific expression pattern, and its expression was found to be lower in galls than in non-infected root tips. *OsTHI7* promoter activity was not detectable inside 7-dpi giant cells induced by *Mg*. Whether this low expression is caused by an active suppression of the immune system by the nematode remains to be determined. When overexpressing this gene in the susceptible cultivar Nipponbare, the transgenic lines had a normal growth phenotype and similar gall numbers as in wild-type plants. However, there was a 40% reduction in the number of females and total nematodes per plant in the overexpressing plants, in comparison with control lines. The enhanced resistance of the transgenic lines is probably a result of a direct toxic effect of OsTHI7 on *Mg*. Interestingly, these plants also showed a significantly lower disease score and reduced *Pythium* colonization in roots than in control roots. The *Pythium* hyphae in the overexpressing line were largely restricted to the root epidermis and occasionally occurred in a few cortical and vascular cells, in contrast with dense hyphal networks formed in epidermis, cortical cells and vascular bundles of control plants. As *Pythium* species and RKNs frequently co-occur in rice fields, where they cause so-called 'soil sickness' syndrome (De Waele and Elsen, 2007; Peng *et al.*, 2006; Van Buyten and Höfte, 2013), this transgenic approach could provide options for future field applications. Nevertheless, thionins have been shown to be toxic to mammalian cells (Stec, 2006), and hence tissue-specific expression needs to be targeted in future research.

Specific knock-down of nematode effectors or other essential genes by host-mediated gene silencing is frequently suggested as another promising GM strategy for nematode control (Danchin *et al.*, 2013). For example, some of the identified transcripts in Haegeman *et al.* (2013) were targeted for silencing by RNAi in *Mg*, resulting in high nematode mortality, and hence represent candidate targets for host-induced gene silencing against nematodes. The emerging possibilities offered by CRISPR-Cas9-based targeted genome editing might provide a fast and specific way of modifying susceptibility genes in host plants to increase crop resistance to nematodes. However, although putative effectors of *Mg* have been identified (Haegeman *et al.*, 2013; Petitot *et al.*, 2015), we are still awaiting the first publication on the discovery of a rice interactor for any of these candidates.

ACKNOWLEDGEMENTS

This work benefited from interactions funded through COST Actions 872 and FA1208, as well as a Researcher Links grant (ID-127419762), awarded under the Newton-Vietnam Fund partnership (S.M.). The James Hutton Institute receives funding from the Scottish Government. T.K. was supported by an FWO postdoctoral fellowship. S.B. was supported by GRiSP (Menergep) and the French Ministry of Foreign Affairs and International Development (#4764 - BIOASIA). The authors thank Dr S. Eves-van den Akker for assistance with the phylogeny, and Dr V. C. Blok and G. Gheysen for comments on the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Abad, P., Gouzy, J., Aury, J.-M., Castagnone-Sereno, P., Danchin, E.G.J., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V.C., Caillaud, M.-C., Coutinho, P.M., Dasilva, C., De Luca, F., Deau, F., Esquibet, M., Flutre, T., Goldstone, J.V., Hamamouch, N., Hewezi, T., Jaillon, O., Jubin, C., Leonetti, P., Magliano, M., Maier, T.R., Markov, G.V., McVeigh, P., Pesole, G., Poulain, J., Robinson-Rechavi, M., Sallet, E., Segurens, B., Steinbach, D., Tytgat, T., Ugarte, E., van Ghelder, C., Veronico, P., Baum, T.J., Blaxter, M.L., Bleve-Zacheo, T., Davis, E.L., Ewbank, J.J., Favery, B., Grenier, E., Henrissat, B., Jones, J.T., Laudet, V., Maule, A.G., Quesneville, H., Rosso, M.-N., Schiex, T., Smant, G., Weissenbach, J. and Wincker, P. (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nat. Biotechnol.* **26**, 909–915.

Belder, P., Bouman, B.A.M., Cabangon, R., Guoan, L., Quilang, E.J.P., Yuanhua, L., Spiertz, J.H.J. and Tuong, T.P. (2004) Effect of water-saving irrigation on rice yield and water use in typical lowland conditions in Asia. *Agric. Water Manage.* **65**, 193–210.

Bellafiore, S., Jougla, C., Chapuis, É., Besnard, G., Suong, M., Vu, P.N., De Waele, D., Gantet, P. and Thi, X.N. (2015) Intraspecific variability of the facultative meiotic parthenogenetic root-knot nematode (*Meloidogyne graminicola*) from rice fields in Vietnam. *C. R. Biol.* **338**, 471–483.

Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Sayers, E.W. (2013) GenBank. *Nucleic Acids Res.* **41**, D36–D42.

Besnard, G., Jühling, F., Chapuis, É., Zedane, L., Lhuillier, É., Mateille, T. and Bellafiore, S. (2014) Fast assembly of the mitochondrial genome of a plant parasitic nematode (*Meloidogyne graminicola*) using next generation sequencing. *C. R. Biol.* **337**, 295–301.

Bridge, J. and Page, S.L. (1982) The rice root-knot nematode, *Meloidogyne graminicola*, on deep water rice (*Oryza sativa* subsp. *indica*). *Rev. Nematol.* **5**, 225–232.

Bridge, J., Plowright, R.A. and Peng, D. (2005) Nematode parasites of rice. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (Luc, M., Sikora, R.A. and Bridge, J., eds), pp. 87–130. Wallingford, Oxfordshire: CABI Bioscience.

Burrows, P.R., Barker, A.D.P., Newell, C.A. and Hamilton, W.D.O. (1998) Plant-derived enzyme inhibitors and lectins for resistance against plant-parasitic nematodes in transgenic crops. *Pestic. Sci.* **52**, 176–183.

Cabasan, M.T.N., Kumar, A., Bellafiore, S. and De Waele, D. (2014) Histopathology of the rice root-knot nematode, *Meloidogyne graminicola*, on *Oryza sativa* and *O. glaberrima*. *Nematology*, **16**, 73–81.

Carneiro, R.M.D.G., Almeida, M.R. and Quénéhervé, P. (2000) Enzyme phenotypes of *Meloidogyne* spp. populations. *Nematology*, **2**, 645–654.

Castagnone-Sereno, P., Semblat, J.-P. and Castagnone, C. (2009) Modular architecture and evolution of the *map-1* gene family in the root-knot nematode *Meloidogyne incognita*. *Mol. Genet. Genomics*, **282**, 547–554.

Chen, C., Liu, S., Liu, Q., Niu, J., Liu, P., Zhao, J. and Jian, H. (2015) An ANNEXIN-Like protein from the cereal cyst nematode *Heterodera avenae* suppresses plant defense. *PLoS One*, **10**, e0122256.

Claverie, M., Dirlewanger, E., Bosselut, N., Van Ghelder, C., Voisin, R., Kleinhentz, M., Lafargue, B., Abad, P., Rosso, M.-N., Chalhoub, B. and Esmenjaud, D. (2011) The *Ma* gene for complete-spectrum resistance to *Meloidogyne* species in Prunus is a TNL with a huge repeated C-terminal post-LRR region. *Plant Physiol*, **156**, 779–792.

Cotton, J., Lilley, C.J., Jones, L., Kikuchi, T., Reid, A., Thorpe, P., Tsai, I., Beasley, H., Blok, V.C., Cock, P., Eves-van den Akker, S., Holroyd, N., Hunt, M., Mantelin, S., Naghra, H., Pain, A., Palomares-Rius, J.E., Zarowiecki, M., Berriman, M., Jones, J.T. and Urwin, P. (2014) The genome and life-stage specific transcriptomes of *Globodera pallida* elucidate key aspects of plant parasitism by a cyst nematode. *Genome Biol.* **15**, R43.

Danchin, E.G.J., Rosso, M.-N., Vieira, P., de Almeida-Engler, J., Coutinho, P.M., Henrissat, B. and Abad, P. (2010) Multiple lateral gene transfers and duplications have promoted plant parasitism ability in nematodes. *Proc. Natl. Acad. Sci. USA*, **107**, 17 651–17 656.

Danchin, E.G.J., Arguel, M.-J., Campan-Fournier, A., Perfus-Barbeoch, L., Magliano, M., Rosso, M.-N., Da Rocha, M., Da Silva, C., Nottet, N., Labadie, K., Guy, J., Artiguenave, F. and Abad, P. (2013) Identification of novel target genes for safer and more specific control of root-knot nematodes from a pan-genome mining. *PLoS Pathog.* **9**, e1003745.

Decraemer, W. and Hunt, D.J. (2013) Structure and classification. In: *Plant Nematology* (**Perry, R.N.** and **Moens, M.**, eds), pp. 3–39. Wallingford, Oxfordshire: CABI Publishing.

Denison, F.C., Paul, A.-L., Zupanska, A.K. and Ferl, R.J. (2011) 14-3-3 proteins in plant physiology. *Semin. Cell Dev. Biol.* **22**, 720–727.

De Schutter, K. and Van Damme, E. (2015) Protein–carbohydrate interactions as part of plant defense and animal immunity. *Molecules*, **20**, 9029.

De Waele, D. and Elsen, A. (2007) Challenges in tropical plant nematology. *Annu. Rev. Phytopathol.* **45**, 457–485.

Dimkpa, S.O.N., Lahari, Z., Shrestha, R., Douglas, A., Gheysen, G. and Price, A.H. (2015) A genome-wide association study of a global rice panel reveals resistance in *Oryza sativa* to root-knot nematodes. *J. Exp. Bot.* **37**, 1191–1200.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797.

Elad, Y., Cytryn, E., Harel, Y.M., Lew, B. and Graber, E.R. (2011) The biochar effect: plant resistance to biotic stresses. *Phytopathol. Mediterr.* **50**, 335–349.

Elsworth, B., Wasmuth, J. and Blaxter, M.L. (2011) NEMBASE4: the nematode transcriptome resource. *Int. J. Parasitol.* **41**, 881–894.

FAOSTAT. (2013) Food and Agriculture Organization of the United Nations. FAOSTAT Database. Available at: http://faostat3.fao.org.

Fernandez, D., Petitot, A.-S., Grossi de Sa, M., Nguyen, V.P., de Almeida-Engler, J. and Kyndt, T. (2015) Recent advances in understanding plant–nematode interactions in monocots. In: *Advances in Botanical Research* (**Escobar, C.** and **Fenoll, C.**, eds), pp. 189–219. Oxford: Elsevier.

Garavito, A., Guyot, R., Lozano, J., Gavory, F., Samain, S., Panaud, O., Tohme, J., Ghesquière, A. and Lorieux, M. (2010) A genetic model for the female sterility barrier between Asian and African cultivated rice species. *Genetics*, **185**, 1425–1440.

Gergon, E.B. and Prot, J.-C. (1993) Susceptibility of wild rice species to the nematode *Meloidogyne graminicola*. *Int. Rice Res. Notes*, **18**, 16.

Ghedin, E., Wang, S., Spiro, D., Caler, E., Zhao, Q., Crabtree, J., Allen, J.E., Delcher, A.L., Guiliano, D.B., Miranda-Saavedra, D., Angiuoli, S.V., Creasy, T., Amedeo, P., Haas, B., El-Sayed, N.M., Wortman, J.R., Feldblyum, T., Tallon, L., Schatz, M., Shumway, M., Koo, H., Salzberg, S.L., Schobel, S., Pertea, M., Pop, M., White, O., Barton, G.J., Carlow, C.K.S., Crawford, M.J., Daub, J., Dimmic, M.W., Estes, C.F., Foster, J.M., Ganatra, M., Gregory, W.F., Johnson, N.M., Jin, J., Komuniecki, R., Korf, I., Kumar, S., Laney, S., Li, B.-W., Li, W., Lindblom, T.H., Lustigman, S., Ma, D., Maina, C.V., Martin, D.M.A., McCarter, J.P., McReynolds, L., Mitreva, M.D., Nutman, T.B., Parkinson, J., Peregrín-Alvarez, J.M., Poole, C., Ren, Q., Saunders, L., Sluder, A.E., Smith, K., Stanke, M., Unnasch, T.R., Ware, J., Wei, A.D., Weil, G., Williams, D.J., Zhang, Y., Williams, S.A., Fraser-Liggett, C., Slatko, B., Blaxter, M.L. and Scott, A.L. (2007) Draft genome of the filarial nematode parasite *Brugia malayi*. *Science*, **317**, 1756–1760.

Ghesquière, A., Séquier, J., Second, G. and Lorieux, M. (1997) First steps towards a rational use of African rice, *Oryza glaberrima*, in rice breeding through a 'contig line' concept. *Euphytica*, **96**, 31–39.

Golden, A.M. and Birchfield, W. (1965) *Meloidogyne graminicola* (Heteroderidae), a new species of root-knot nematode from grass. *Proc. Helminthol. Soc. Wash.* **32**, 228–231.

Green, J., Vain, P., Fearnehough, M.T., Worland, B., Snape, J.W. and Atkinson, H.J. (2002) Analysis of the expression patterns of the *Arabidopsis thaliana* tubulin-1 and *Zea mays* ubiquitin-1 promoters in rice plants in association with nematode infection. *Physiol. Mol. Plant Pathol.* **60**, 197–205.

GRiSP (Global Rice Science Partnership) (2013) *Rice Almanac – Source Book for the Most Important Economic Activity on Earth*. Los Baños: International Rice Research Institute (IRRI).

Haegeman, A., Mantelin, S., Jones, J.T. and Gheysen, G. (2012) Functional roles of effectors of plant-parasitic nematodes. *Gene*, **492**, 19–31.

Haegeman, A., Bauters, L., Kyndt, T., Rahman, M.M. and Gheysen, G. (2013) Identification of candidate effector genes in the transcriptome of the rice root knot nematode *Meloidogyne graminicola*. *Mol. Plant Pathol.* **14**, 379–390.

Harcus, Y., Nicoll, G., Murray, J., Filbey, K., Gomez-Escobar, N. and Maizels, R.M. (2009) C-type lectins from the nematode parasites *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis*. *Parasitol. Int.* **58**, 461–470.

Hewezi, T. and Baum, T.J. (2013) Manipulation of plant cells by cyst and root-knot nematode effectors. *Mol. Plant–Microbe Interact.* **26**, 9–16.

Huang, G., Gao, B., Maier, T.R., Allen, R., Davis, E.L., Baum, T.J. and Hussey, R.S. (2003) A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode *Meloidogyne incognita*. *Mol. Plant–Microbe Interact.* **16**, 376–381.

Huang, W.-K., Ji, H.-L., Gheysen, G., Debode, J. and Kyndt, T. (2015a) Biochar-amended potting medium reduces the susceptibility of rice to root-knot nematode infections. *BMC Plant Biol.* **15**, 267.

Huang, W.-K., Ji, H.-L., Gheysen, G. and Kyndt, T. (2015b) Thiamine-induced priming against root-knot nematode infection in rice involves lignification and hydrogen peroxide generation. *Mol. Plant Pathol.* **17**, 614–624.

Humphreys-Pereira, D.A. and Elling, A.A. (2015) Mitochondrial genome plasticity among species of the nematode genus *Meloidogyne* (Nematoda: Tylenchina). *Gene*, **560**, 173–183.

Jacobs, A.K., Lipka, V., Burton, R.A., Panstruga, R., Strizhov, N., Schulze-Lefert, P. and Fincher, G.B. (2003) An *Arabidopsis* callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell*, **15**, 2503–2513.

Jaouannet, M., Magliano, M., Arguel, M.-J., Gourgues, M., Evangelisti, E., Abad, P. and Rosso, M.-N. (2013) The root-knot nematode calreticulin Mi-CRT is a key effector in plant defense suppression. *Mol. Plant–Microbe Interact.* **26**, 97–105.

Jena, R.N. and Rao, Y.S. (1977) Nature of resistance in rice (*Oryza sativa* L.) to the root knot nematode (*Meloidogyne graminicola*) II. Histopathology of nematode infection in rice varieties. *Proc. Indian Acad. Sci. B*, **86**, 87–91.

Jepson, S.B. (1983) Identification of *Meloidogyne*: a general assessment and a comparison of male morphology using light microscopy, with a key to 24 species. *Rev. Nematol.* **6**, 291–309.

Ji, H., Gheysen, G., Denil, S., Lindsey, K., Topping, J.F., Nahar, K., Haegeman, A., De Vos, W.H., Trooskens, G., Van Criekinge, W., De Meyer, T. and Kyndt, T. (2013) Transcriptional analysis through RNA sequencing of giant cells induced by *Meloidogyne graminicola* in rice roots. *J. Exp. Bot.* **64**, 3885–3898.

Ji, H., Gheysen, G., Ullah, C., Verbeek, R., Shang, C., De Vleesschauwer, D., Hofte, M. and Kyndt, T. (2015a) The role of thionins in rice defence against root pathogens. *Mol. Plant Pathol.* **16**, 870–881.

Ji, H., Kyndt, T., He, W., Vanholme, B. and Gheysen, G. (2015b) β-Aminobutyric acid-induced resistance against root-knot nematodes in rice is based on increased basal defense. *Mol. Plant–Microbe Interact.* **28**, 519–533.

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L. and Perry, R.N. (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathol.* **14**, 946–961.

Kleynhans, K.P.N. (1991) *The Root-Knot Nematodes of South Africa*. Pretoria: Department of Agricultural Development.

Kumar, S., Schiffer, P.H. and Blaxter, M.L. (2012) 959 Nematode genomes: a semantic wiki for coordinating sequencing projects. *Nucleic Acids Res.* **40**, D1295–D1300.

Kyndt, T., Denil, S., Haegeman, A., Trooskens, G., Bauters, L., Van Criekinge, W., De Meyer, T. and Gheysen, G. (2012) Transcriptional reprogramming by root knot and migratory nematode infection in rice. *New Phytol.* **196**, 887–900.

Kyndt, T., Vieira, P., Gheysen, G. and Almeida-Engler, J. (2013) Nematode feeding sites: unique organs in plant roots. *Planta*, **238**, 807–818.

Kyndt, T., Fernandez, D. and Gheysen, G. (2014) Plant-parasitic nematode infections in rice: molecular and cellular insights. *Annu. Rev. Phytopathol.* **52**, 7.1–7.19.

Lapp, N.A. and Triantaphyllou, A.C. (1972) Relative DNA content and chromosomal relationships of some *Meloidogyne*, *Heterodera*, and *Meloidodera* spp. (*Nematoda*: *Heteroderidae*). *J. Nematol.* **4**, 287–291.

Le, H.T.T., Padgham, J.L. and Sikora, R.A. (2009) Biological control of the rice root-knot nematode *Meloidogyne graminicola* on rice, using endophytic and rhizosphere fungi. *Int. J. Pest Manage.* **55**, 31–36.

Lilley, C.J., Kyndt, T. and Gheysen, G. (2011) Nematode resistant GM crops in industrialised and developing countries. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J.T., Gheysen, G. and Fenoll, C., eds), pp. 517–541. London: Springer.

Lozano-Torres, J.L., Wilbers, R.H.P., Warmerdam, S., Finkers-Tomczak, A., Diaz-Granados, A., van Schaik, C.C., Helder, J., Bakker, J., Goverse, A., Schots, A. and Smant, G. (2014) Apoplastic venom allergen-like proteins of cyst nematodes modulate the activation of basal plant innate immunity by cell surface receptors. *PLoS Pathog.* **10**, e1004569.

Lu, S.-W., Chen, S., Wang, J., Yu, H., Chronis, D., Mitchum, M.G. and Wang, X. (2009) Structural and functional diversity of CLAVATA3/ESR (CLE)-like genes from the potato cyst nematode *Globodera rostochiensis*. *Mol. Plant–Microbe Interact.* **22**, 1128–1142.

Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B. and Ton, J. (2011) Callose deposition: a multifaceted plant defense response. *Mol. Plant–Microbe Interact.* **24**, 183–193.

Lunt, D.H. and Hyman, B.C. (1997) Animal mitochondrial DNA recombination. *Nature*, **387**, 247.

Lunt, D.H., Whipple, L.E. and Hyman, B.C. (1998) Mitochondrial DNA variable number tandem repeats (VNTRs): utility and problems in molecular ecology. *Mol. Ecol.* **7**, 1441–1455.

Maas, P.W.T., Sanders, H. and Dede, J. (1978) *Meloidogyne oryzae* N. Sp. (*Nematoda, Meloidogynidae*) infesting irrigated rice in Surinam (South America). *Nematologica*, **24**, 305–311.

Manosalva, P., Manohar, M., von Reuss, S.H., Chen, S., Koch, A., Kaplan, F., Choe, A., Micikas, R.J., Wang, X., Kogel, K.-H., Sternberg, P.W., Williamson, V.M., Schroeder, F.C. and Klessig, D.F. (2015) Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. *Nat. Commun.* **6**, Article number 7795.

Mantelin, S., Thorpe, P. and Jones, J.T. (2015) Suppression of plant defences by plant-parasitic nematodes. In: *Advances in Botanical Research* (Escobar, C. and Fenoll, C., eds), pp. 325–337 Oxford: Elsevier.

MBTOC (2010) *Report of the Methyl Bromide Technical Options Committee (MBTOC) 2010 Assessment.* Montreal protocol on substances that deplete the ozone layer. Nairobi: United Nations Environment Programme (UNEP).

van Megen, H., van den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J. and Helder, J. (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology*, **11**, 927–950.

Melillo, M.T., Leonetti, P., Bongiovanni, M., Castagnone-Sereno, P. and Bleve-Zacheo, T. (2006) Modulation of reactive oxygen species activities and H_2O_2 accumulation during compatible and incompatible tomato–root-knot nematode interactions. *New Phytol.* **170**, 501–512.

Mellersh, D.G., Foulds, I.V., Higgins, V.J. and Heath, M.C. (2002) H_2O_2 plays different roles in determining penetration failure in three diverse plant–fungal interactions. *Plant J.* **29**, 257–268.

Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and Williamson, V.M. (1998) The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell*, **10**, 1307–1320.

Milne, I., Lindner, D., Bayer, M., Husmeier, D., McGuire, G., Marshall, D.F. and Wright, F. (2009) TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics*, **25**, 126–127.

Miyawaki, K., Tabata, R. and Sawa, S. (2013) Evolutionarily conserved CLE peptide signaling in plant development, symbiosis, and parasitism. *Curr. Opin. Plant Biol.* **16**, 598–606.

Muthayya, S., Sugimoto, J.D., Montgomery, S. and Maberly, G.F. (2014) An overview of global rice production, supply, trade, and consumption. *Ann. N. Y. Acad. Sci.* **1324**, 7–14.

Nahar, K., Kyndt, T., De Vleesschauwer, D., Höfte, M. and Gheysen, G. (2011) The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. *Plant Physiol.* **157**, 305–316.

Nahar, K., Kyndt, T., Nzogela, Y.B. and Gheysen, G. (2012) Abscisic acid interacts antagonistically with classical defense pathways in rice–migratory nematode interaction. *New Phytol.* **196**, 901–913.

Nahar, K., Kyndt, T., Hause, B., Höfte, M. and Gheysen, G. (2013) Brassinosteroids suppress rice defense against root-knot nematodes through antagonism with the jasmonate pathway. *Mol. Plant–Microbe Interact.* **26**, 106–115.

Netscher, C. and Erlan (1993) A root-knot nematode, *Meloidogyne graminicola*, parasitic on rice in Indonesia. *Afro-Asia J. Nematol.* **3**, 90–95.

Nicol, J.M., Turner, S.J., Coyne, D.L., den Nijs, L., Hockland, S. and Tahna Maafi, Z. (2011) Current nematode threats to world agriculture. In: *Genomics and Molecular Genetics of Plant–Nematode Interactions* (Jones, J.T., Gheysen, G. and Fenoll, C., eds), pp. 21–43. London: Springer.

Oelkers, K., Goffard, N., Weiller, G., Gresshoff, P., Mathesius, U. and Frickey, T. (2008) Bioinformatic analysis of the CLE signaling peptide family. *BMC Plant Biol.* **8**, 1.

Okimoto, R., Chamberlin, H.M., Macfarlane, J.L. and Wolstenholme, D.R. (1991) Repeated sequence sets in mitochondrial DNA molecules of root knot nematodes (*Meloidogyne*): nucleotide sequences, genome location and potential for host-race identification. *Nucleic Acids Res.* **19**, 1619–1626.

Opperman, C.H., Bird, D.M., Williamson, V.M., Rokhsar, D.S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., Graham, S., Houfek, T.D., Liu, Q., Mitros, T., Schaff, J., Schaffer, R., Scholl, E., Sosinski, B.R., Thomas, V.P. and Windham, E. (2008) Sequence and genetic map of *Meloidogyne hapla*: a compact nematode genome for plant parasitism. *Proc. Natl. Acad. Sci. USA*, **105**, 14 802–14 807.

Patel, N., Hamamouch, N., Li, C., Hewezi, T., Hussey, R.S., Baum, T.J., Mitchum, M.G. and Davis, E.L. (2010) A nematode effector protein similar to annexins in host plants. *J. Exp. Bot.* **61**, 235–248.

Peng, S.B., Bouman, B.A.M., Visperas, R.A., Castaneda, A., Nie, L.X. and Park, H.K. (2006) Comparison between aerobic and flooded rice in the tropics: agronomic performance in an eight-season experiment. *Field Crop Res.* **96**, 252–259.

Petitot, A.-S., Dereeper, A., Agbessi, M., Da Silva, C., Guy, J., Ardisson, M. and Fernandez, D. (2015) Dual RNA-seq reveals *Meloidogyne graminicola* transcriptome and candidate effectors during the interaction with rice plants. *Mol. Plant Pathol.* **17**, 860–874.

Plowright, R.A. and Bridge, J. (1990) Effect of *Meloidogyne graminicola* (Nematoda) on the establishment, growth and yield of rice cv. *IR36. Nematologica*, **36**, 81–89.

Plowright, R.A., Coyne, D.L., Nash, P. and Jones, M.P. (1999) Resistance to the rice nematodes *Heterodera sacchari*, *Meloidogyne graminicola* and *M. incognita* in *Oryza glaberrima* and *O. glaberrima* × *O. sativa* interspecific hybrids. *Nematology*, **1**, 745–751.

Pokharel, R.R., Abawi, G.S., Zhang, N., Duxbury, J.M. and Smart, C.D. (2007) Characterization of isolates of *Meloidogyne* from rice–wheat production fields in Nepal. *J. Nematol.* **39**, 221–230.

Pokharel, R.R., Abawi, G.S., Duxbury, J.M., Smat, C.D., Wang, X. and Brito, J.A. (2010) Variability and the recognition of two races in *Meloidogyne graminicola*. *Australas. Plant Pathol.* **39**, 326–333.

Portillo, M., Cabrera, J., Lindsey, K., Topping, J.F., 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.

Prior, A., Jones, J.T., Blok, V.C., Beauchamp, J., McDermott, L., Cooper, A. and Kennedy, M.W. (2001) A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode *Globodera pallida*: lipid binding activities, structural analysis and expression pattern. *Biochem. J.* **356**, 387–394.

Prot, J.-C. and Rahman, M.L. (1994) Nematode ecology, economic importance, and management in rice ecosystems in South and Southeast Asia. In: *Rice Pest Science and Management* (Teng, P.S., Heong, K.L. and Moody, K., eds), pp. 129–144. Los Baños: International Rice Research Institute (IRRI).

Rahman, M.L. (1990) Effect of different cropping sequences on root-knot nematode, *Meloidogyne graminicola*, and yield of deepwater rice. *Nematol. Mediterr.* **18**, 213–217.

Reynolds, A.M., Dutta, T.K., Curtis, R.H.C., Powers, S.J., Gaur, H.S. and Kerry, B.R. (2011) Chemotaxis can take plant-parasitic nematodes to the source of a chemo-attractant via the shortest possible routes. *J. R. Soc. Interface*, **8**, 568–577.

Rich, J.R., Brito, J.A., Kaur, R. and Ferrell, J.A. (2009) Weed species as hosts of *Meloidogyne*: a review. *Nematropica*, **39**, 157–185.

Ripoll, C., Favery, B., Lecomte, P., Van Damme, E., Peumans, W., Abad, P. and Jouanin, L. (2003) Evaluation of the ability of lectin from snowdrop (*Galanthus nivalis*) to protect plants against root-knot nematodes. *Plant Sci.* **164**, 517–523.

Roberts, M.R. (2003) 14-3-3 Proteins find new partners in plant cell signalling. *Trends Plant Sci.* **8**, 218–223.

Rokas, A., Ladoukakis, E. and Zouros, E. (2003) Animal mitochondrial DNA recombination revisited. *Trends Ecol. Evol.* **18**, 411–417.

Roy, A.K. (1982) Survival of *Meloidogyne graminicola* eggs under different moisture conditions *in vitro*. *Nematol. Mediterr.* **10**, 221–222.

Rutter, W.B., Hewezi, T., Maier, T.R., Mitchum, M.G., Davis, E.L., Hussey, R.S. and Baum, T.J. (2014) Members of the *Meloidogyne* avirulence protein family contain multiple plant ligand-like motifs. *Phytopathology*, **104**, 879–885.

Sasser, J.N. and Freckman, D.W. (1987) A world prospective on nematology: the role of the society. In: *Vistas on Nematology* (Veech, J.A. and Dickson, D.W., eds), pp. 7–14. Hyattsville, MD: Society of Nematologists.

Semblat, J.-P., Rosso, M.-N., Hussey, R.S., Abad, P. and Castagnone-Sereno, P. (2001) Molecular cloning of a cDNA encoding an amphid-secreted putative avirulence protein from the root-knot nematode *Meloidogyne incognita*. *Mol. Plant–Microbe Interact.* **14**, 72–79.

Soriano, I.R.S. and Reversat, G. (2003) Management of *Meloidogyne graminicola* and yield of upland rice in South-Luzon, Philippines. *Nematology*, **5**, 879–884.

Soriano, I.R.S., Schmit, V., Brar, D.S., Prot, J.-C. and Reversat, G. (1999) Resistance to rice root-knot nematode *Meloidogyne graminicola* identified in *Oryza longistaminata* and *O. glaberrima*. *Nematology*, **1**, 395–398.

Soriano, I.R.S., Prot, J.-C. and Matias, D.M. (2000) Expression of tolerance for *Meloidogyne graminicola* in rice cultivars as affected by soil type and flooding. *J. Nematol.* **32**, 309–317.

Stec, B. (2006) Plant thionins – the structural perspective. *Cell Mol. Life Sci.* **63**, 1370–1385.

Sun, L., Zhuo, K., Lin, B., Wang, H. and Liao, J. (2014) The complete mitochondrial genome of *Meloidogyne graminicola* (Tylenchina): a unique gene arrangement and its phylogenetic implications. *PLoS One*, **9**, e98558.

Thorpe, P., Mantelin, S., Cock, P., Blok, V.C., Coke, M., Eves-van den Akker, S., Guzeeva, E., Lilley, C.J., Smant, G., Reid, A., Wright, K., Urwin, P. and Jones, J.T. (2014) Genomic characterisation of the effector complement of the potato cyst nematode *Globodera pallida*. *BMC Genomics*, **15**, 923.

Ton, J. and Mauch-Mani, B. (2004) beta-Amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J.* **38**, 119–130.

Triantaphyllou, A.C. (1969) Gametogenesis and the chromosomes of two root-knot nematodes, *Meloidogyne graminicola* and *M. naasi*. *J. Nematol.* **1**, 62–71.

Vain, P., Worland, B., Clarke, M.C., Richard, G., Beavis, M., Liu, H., Kohli, A., Leech, M., Snape, J.W., Christou, P. and Atkinson, H.J. (1998) Expression of an engineered cysteine proteinase inhibitor (Oryzacystatin-I Delta D86) for nematode resistance in transgenic rice plants. *Theor. Appl. Genet.* **96**, 266–271.

Van Buyten, E. and Höfte, M. (2013) *Pythium* species from rice roots differ in virulence, host colonization and nutritional profile. *BMC Plant Biol.* **13**, 203.

Ventura, W., Watanabe, I., Castillo, M.B. and De La Cruz, A. (1981) Involvement of nematodes in the soil sickness of a dryland rice-based cropping system. *Soil Sci. Plant Nutr.* **27**, 305–315.

Whipple, L.E., Lunt, D.H. and Hyman, B.C. (1998) Mitochondrial DNA length variation in *Meloidogyne incognita* isolates of established genetic relationships: utility for nematode population studies. *Fund. Appl. Nematol.* **21**, 265–271.

Win, P.P., Kyi, P.P. and De Waele, D. (2011) Effect of agro-ecosystem on the occurrence of the rice root-knot nematode *Meloidogyne graminicola* on rice in Myanmar. *Australas. Plant Pathol.* **40**, 187–196.

Win, P.P., Kyi, P.P., Maung, Z.T.Z. and De Waele, D. (2013) Population dynamics of *Meloidogyne graminicola* and *Hirschmanniella oryzae* in a double rice-cropping sequence in the lowlands of Myanmar. *Nematology*, **15**, 1–13.

Wylie, T., Martin, J.C., Dante, M., Mitreva, M.D., Clifton, S.W., Chinwalla, A., Waterston, R.H., Wilson, R.K. and McCarter, J.P. (2004) Nematode.net: a tool for navigating sequences from parasitic and free-living nematodes. *Nucleic Acids Res.* **32**, D423–D426.

Yeates, G.W. (2004) Ecological and behavioural adaptations. In: *Nematode Behaviour* (**Gaugler, R.** and **Bilgrami, A.L.**, eds), pp. 1–24. Wallingford, Oxfordshire: CABI Publishing.

Yik, C.P. and Birchfield, W. (1979) Host studies and reactions of rice cultivars to *Meloidogyne graminicola*. *Phytopathology*, **69**, 497–499.

SUPPORTING INFORMATION

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

File S1 Multiple sequences alignment file used for the 18S phylogeny (18S Alignment.ALN).

File S2 Phylogenetic distances used to build the 18S tree (18S Distance.TRE).