Reproductive, pathogenic and genotypic characterisation of five *Meloidogyne graminicola* populations from the Philippines on susceptible and resistant rice varieties

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Summary – Five populations of *Meloidogyne graminicola* isolated from different rice-growing areas in the Philippines were characterised. The populations showed little phenotypic variability of second-stage juveniles and female perineal pattern. Differences in reproduction among *M. graminicola* populations were not observed on mature resistant *Oryza glaberrima* varieties ‘TOG5674’, ‘TOG5675’, ‘RAM131’ and ‘CG14’, or on susceptible *O. sativa* varieties ‘IR64’ and ‘UPLRi-5’. In all infected rice varieties, plant growth and yield-contributing traits showed no differences among the populations. A search on *M. graminicola* populations from the Philippines for single-nucleotide polymorphism on the sequences of Internal Transcribed Spacer (ITS) of rDNA genes and mtDNA indicated only few points of heteroplasmy. Nematode reproduction and disease induction of the five *M. graminicola* populations in the Philippines exerted the same level of aggressiveness and virulence. The absence of resistance-breaking populations of *M. graminicola* is important for the maintenance of durability of resistance to this important rice pathogen.

Keywords – aggressiveness, heteroplasmy, ITS, morphology, mtDNA, *Oryza glaberrima*, *Oryza sativa*, root-knot nematode, virulence.

The use of a resistant or tolerant variety of an agricultural crop can be a suitable alternative for managing plant-parasitic nematodes. This is particularly relevant in farming systems with limited options for controlling nematodes, such as intensive rice-based production systems in which the efficacy of nematicides is often limited and only continuous flooding appears to be effective, which requires a constant and abundant water supply (De Waele *et al.*, 2013).

Populations of *Meloidogyne* spp. may differ in their ability to reproduce and to cause damage and yield loss. Several studies have reported variability in aggressive-

ness, virulence and pathogenicity on agricultural crops among populations of *Meloidogyne* spp. Examples are *Meloidogyne javanica* on tomato (Roberts & Thomason, 1986), *M. arenaria* on peanut, soybean and tomato (Carpenter & Lewis, 1991; Noe, 1992) and *M. incognita* on cotton (Zhou *et al.*, 2000; Anwar & McKenry, 2007).

Aggressiveness is a characteristic of a pathogen and it refers to the ability of a pathogen to attack and parasitise a host plant, while virulence refers to the ability of a pathogen to cause damage and/or induce disease in a host plant. The greater the aggressiveness and virulence of a pathogen, the greater is its pathogenicity (Bos

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& Parlevliet, 1995). In our study, aggressiveness of a nematode population is measured by the reproductive fitness (reproduction) of the nematode population on the host crop. Virulence will refer to the ability of the nematode population to cause damage and/or yield loss to the host crop. Yield is, of course, the best trait to measure the virulence of a pathogen on an agricultural crop variety.

It is common to identify varieties of an agricultural crop that are resistant to *Meloidogyne* spp. but resistance-breaking populations of several *Meloidogyne* species are challenging the use of *Meloidogyne*-resistant crop varieties. Virulent populations of *M. incognita*, *M. javanica* and *M. arenaria* were able to reproduce well on tomatoes 'Piersol' and 'Rossol' carrying the resistant *Mi*-gene (Netscher, 1976; Berthou *et al.*, 1989; Jarguin-Barberena *et al.*, 1991; Castagnone-Sereno *et al.*, 1993). A virulent population of *M. arenaria* that reproduced on susceptible tomato varieties also reproduced well on several *M. arenaria*-resistant tomato varieties (Prot, 1984) and the same observation, also on tomato, was made for several virulent populations of *M. incognita* (Roberts *et al.*, 1990). Resistance-breaking populations of *M. arenaria* were also observed on tobacco (Noe, 1992), *M. javanica* on tomato (Eddoudi *et al.*, 1997) and *M. incognita* on cowpea (Olowe, 2010).

The rice root-knot nematode, *M. graminicola*, is one of the most damaging nematode species affecting Asian rice (*Oryza sativa*). Almost every rice-based production system in Asia is vulnerable to this nematode species because it is well-adapted to the wide variety of agro-ecosystems in which rice is being produced from deepwater rice to irrigated lowland and rainfed upland rice. *Meloidogyne graminicola* can severely reduce rice yields (Soriano *et al.*, 2000; Padgham *et al.*, 2004; Win *et al.*, 2013; Patil & Gaur, 2014). This nematode species has also been identified as a major causal agent of yield decline and even yield failure in tropical aerobic rice (Kreye *et al.*, 2009; De Waele *et al.*, 2013). Because of its emergence as an important pathogen of rice, the search for resistance sources to manage *M. graminicola* has been intensified (De Waele, pers. comm.).

Oryza glaberrima varieties resistant to *M. graminicola* have been reported (Plowright *et al.*, 1999; Soriano *et al.*, 1999; Cabasan *et al.*, 2012, 2014). Although the mode of action of this resistance has been examined and illustrated (Jena & Rao, 1977a, b; Cabasan *et al.*, 2012, 2014), this resistance could be challenged with different populations of *M. graminicola*. It cannot be excluded that either more virulent *M. graminicola* populations than the populations

initially used in the host response studies exist or that resistance-breaking *M. graminicola* populations may have developed by selection of pre-existing variation among different populations (Dropkin, 1988). The existence or the emergence of virulent well-reproducing nematode populations on resistant agricultural crop varieties is of great concern as this could significantly reduce the durability of the natural resistance sources (Semblat *et al.*, 2000).

Pokharel *et al.* (2007) studied 33 *M. graminicola* populations isolated from the soil of rice-wheat fields in Nepal and observed that they were almost similar in phenotype (second-stage juvenile (J2) measurements, female perineal pattern, host range and gall shape) but that these populations showed differences in aggressiveness, measured on the basis of the nematode multiplication factor and severity of root galling on the susceptible Asian rice 'Labelle' and 'LA 110'. The authors also (partially) amplified and sequenced the internally transcribed spacer (ITS) region of the rRNA genes and found some intraspecific polymorphism but phylogenetic analyses indicated that all Nepalese *M. graminicola* populations examined formed a distinct clade with known populations of *M. graminicola* from Nepal. In 2010, Pokharel *et al.* reported a similar study, this time after having examined nine *M. graminicola* populations isolated from (Asian) rice in Nepal, India and Bangladesh, and one *M. graminicola* population isolated from the weed *Cyperus rotundus* in Florida, USA. Substantial variations in morphometrics of J2 among and within the populations were observed. All populations had the same host range, but the *M. graminicola* population isolated from *C. rotundus* in Florida did not infect the rice 'Labelle' and 'LA 110', suggesting that *M. graminicola* consists of more than one race/pathotype as based on the descriptions of race/pathotype by Triantaphyllou (1985) and van der Beek *et al.* (1999). The ITS sequences of all ten *M. graminicola* populations examined matched those of *M. graminicola* in GenBank and formed a single clade with minor variations among and within the populations. Bellafiore *et al.* (2015) studied 21 populations of *M. graminicola* isolated from the soil of rice fields in Vietnam and observed variability in the length of the body and stylet of J2 but without significant differences in the host range. These populations showed little intraspecific variability based on the ITS of rDNA genes but, as with the Nepalese populations (Pokharel *et al.*, 2007), no *M. graminicola* subclades were supported by a bootstrap value higher than 65%.

Because a sound knowledge of variation in pathogenicity among populations of the same species of plant-parasitic nematodes is important for management decisions and plant breeding programmes, the main objectives of our study were: *i*) to examine the phenotypic and genotypic variability among *M. graminicola* populations isolated from different rice-growing regions in the Philippines; and *ii*) to examine the pathogenicity of those *M. graminicola* populations from the Philippines on a selection of *M. graminicola*-resistant and susceptible rice varieties, and also to examine if (some of) these populations could break the natural resistance observed in resistant *O. glaberrima*.

Materials and methods

NEMATODE POPULATIONS

The *M. graminicola* populations were collected from five rice-growing areas in the Philippines differing in agro-ecological conditions (Table 1). These regions represent the major rice-growing regions in the Philippines. One third to more than half of the total land area is used for agriculture and rice is the main agricultural crop. Rainfed and irrigated rice cultivation is being practised in

Batangas, Bulacan, Laguna and Tarlac, whilst rainfed rice cultivation is practised in most parts of Cotabato. The *M. graminicola* populations were isolated from infected rice plants (unknown variety) collected in Batangas, Bulacan, Cotabato, Laguna and Tarlac (Fig. 1). Rice plants showing above-ground symptoms of *M. graminicola* infection (*i.e.*, yellowing of leaves and stunting) were carefully uprooted and the plant shoots cut off. The roots were placed in plastic bags and transported to the nematology laboratory at the International Rice Research Institute (IRRI) in the Philippines for nematode extraction. Roots were washed free of adhering soil particles and examined for the presence of hook-like galls on the root tips, typical of *M. graminicola* infection. Galled roots were cut into 5–10 mm pieces and placed in a mistifier for 48 h to extract J2 (Seinhorst, 1950). The nematode suspensions were examined for the presence of *M. graminicola* using a stereo microscope.

A culture was established for each *M. graminicola* population isolated from a single J2. J2 were handpicked and placed singly on a 5-day-old seedling of the susceptible upland rice ‘UPLRi-5’ planted in 30-mm-diam. transparent polystyrene (PS) tubes filled with 10 g of water-absorbent polymer (SAP: sand + absorbent polymer) as substrate (Reversat *et al.*, 1999). The seedlings were main-

Table 1. Agro-ecology of the sampled areas.

Area	Topography	Soil type	Temperature (°C)	Annual rainfall (mm)	Season
Batangas	rolling terrain, small low flat lands and several mountains	Sandy loam to clay loam	24–28	2175	rainy season lasts from June to November; dry season lasts from January to May
Bulacan	flat with mountains near its boundaries	Clay loam	23–32	2555	rainy season lasts from April to November, the rest of the year is dry; exposed to the southwest monsoon and occasional typhoons
Cotabato	upland ranging from level to gently sloping, to rolling, moderately steep to very steep	Clay loam to clay	18–30	875	rainy and dry seasons are not pronounced; rainfall is more or less evenly distributed throughout the year
Laguna	level and gently rolling plains	Clay loam	22–34	2548	rainfall is more or less evenly distributed throughout the year but nevertheless a rainy and a dry season can be recognised; dry from November to April and rainy during the rest of the year
Tarlac	flat lands and gently sloping plains punctuated by mountain ranges on its western boundary	Sandy loam to clay loam	21–34	2478	with two distinct seasons: a dry season from November to April and a rainy season from May to October

Fig. 1. Collection sites in the Philippines of the *Meloidogyne graminicola* populations studied.

tained in an indoor growth chamber (IGC) set at 29/26°C with a 12 h photoperiod. Seven days later the infected seedlings were transplanted into PS tubes each filled with 13 ml of diluted Hoagland's solution and grown hydroponically (Reversat & Fernandez, 2004) in the IGC for 2 weeks. To propagate the culture, J2 that developed in the rice roots grown in the hydroponic solution were extracted using the mistifier technique (Seinhorst, 1950), then an aqueous suspension containing 50 J2 was pipetted in 5-cm-deep holes around the base of 2-week-old seedlings of the rice 'UPLRi-5', each seedling growing in a 15-cm-diam. pot filled with a sterilised sandy loam soil. At the time of inoculation the soil in the pots was at field capacity (50% of the soil pore volume filled with water). The five pure cultures of *M. graminicola* were maintained in the glasshouse on 50 'UPLRi-5' plants, each as a source of inoculum, under upland conditions, *i.e.*, temperature above 24°C, well-drained sandy-loamy (52% sand, 21% loam, 27% clay), typically subjected to moisture stress, water added only when needed, with no surface water accumulation (*i.e.*, at field capacity).

Identification of the five *M. graminicola* populations was based on the perineal pattern and morphology of the mature females, and the morphology, morphometrics and molecular characterisation of the J2.

MORPHOLOGICAL AND MORPHOMETRIC CHARACTERISATION

Mature *M. graminicola* females (egg-laying females: ELF) were handpicked from dissected galled roots of 'UPLRi-5' and placed in a drop of water on a glass slide. The morphological features of the ELF were described as observed using a light microscope. Perineal patterns were prepared by cutting the posterior end of the ELF with a sharp razor blade and cleaning the end in a solution of 45% lactic acid. The cut end was trimmed and placed on a glass slide in glycerin (Hartman & Sasser, 1985). The perineal patterns of ten ELF per *M. graminicola* population were examined using a BX41 Olympus light microscope equipped with camera media cybernetics PL-A662 for photography.

J2 were also handpicked randomly from the extraction suspension, killed and fixed with dimethyl sulfoxide-salt solution (DESS). Eighteen juveniles were randomly selected per *M. graminicola* population and mounted on a glass slide in 100% glycerin. The morphological features observed and measured were based on the description by Mulk (1976). Measurements were taken using a BX41 light Olympus microscope equipped with a drawing tube. Measurements included were body length and maximum body width, stylet length, length from the anterior end to the end of the pharynx, tail length. The 'a' value (body length/maximum body width), 'b' value (body length/length from the anterior end to the end of the pharynx) and 'c' value (body length/tail length) were calculated for each J2.

MOLECULAR CHARACTERISATION

The molecular (genome) variability of the populations of *M. graminicola* was examined based on the comparison of partial DNA sequences of the ITS region from the rDNA (Pokharel *et al.*, 2007) and of three mitochondrial DNA (mtDNA) sequences: mtMg-1, mtMg-2 and mtMg-3. These mtDNA sequences use combined non-coding and coding sequences: mtMg-1 is a 1998 bp sequence including 3' part of *COX1* and 5' part of *ND1* genes; mtMg-2 is a 1891 bp sequence including 3' part of *NAD4* gene and the tRNA-Asp and finally mtMg-3 is a

1153 bp sequence including the *ATP6* and a 5' part of the *ND5* genes with the tRNA-S2.

In each population 1000 J2 were collected and preserved in 1 ml of DESS. Nematodes were rinsed twice in 1 ml of sterile distilled water before DNA extraction and PCR as described by Bellafiore *et al.* (2015). For the nuclear DNA marker, primers rDNA2 (5'-TTGATTACGTC CCTGCCCTTT-3') and rDNA1.58s (5'-ACGAGCCCGA GTGATCCACCG-3') were used to amplify 386-bp region including a portion of the 18S rDNA gene (151 bp), the complete ITS-1 and part of the 5.8S rDNA gene. PCR products were gel-purified and directly sent for sequencing using rDNA2 primer (Macrogen).

mtDNA markers were amplified by PCR in a final volume of 50 µl containing 5 µl of crude DNA extract, 500 nM of each PCR primer, 200 mM each dNTP, 50 mg of BSA, 10 µl 5× Phusion buffer and one unit of the proofreading enzyme, Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific Inc.). The following PCR program was applied: 98°C for 30 s; 35× (98°C, 10 s; 56°C, 30 s; 72°C, 90 s); 72°C for 10 min. The primer sequences used to amplify mtMg-1, mtMg-2 and mtMg-3 are shown in Table 2 together with the position and length of the amplified fragments. Ten independent PCR for each population and DNA marker were done. PCR products were gel-purified and directly sent for sequencing (Macrogen) using two specific primers for each amplicon. Heteroplasmy, defined as the co-existence of multiple mitochondrial DNA variants in a single nematode, was considered only if the nematode population's DNA presented

at the same position with two alternative nucleotides and thus were verified in at least two independently obtained sequences (PCR and sequencing).

ITS and mtDNA sequences were aligned and manually edited with the application Mega v.6 (Tamura *et al.*, 2013). As described in Bellafiore *et al.* (2015), Phylogeny.fr web platform (Dereeper *et al.*, 2008) was used with nematode ITS and mtDNA sequences either downloaded from GenBank or generated in this study. Phylogenetic trees were generated by the maximum-likelihood method and the phylogram was bootstrapped 400 times.

RICE VARIETIES

Two *M. graminicola*-susceptible *O. sativa* varieties ('IR64' and 'UPLRi-5') and four *M. graminicola*-resistant *O. glaberrima* varieties ('TOG5674', 'TOG5675', 'CG14' and 'RAM131') were included in the pathogenicity study. The host response to *M. graminicola* infection of these rice varieties has been identified previously by Soriano *et al.* (1999), Plowright *et al.* (1999) and Cabasan *et al.* (2012, 2014).

The rice seeds were obtained from the seed bank of IRRI, the Philippines. 'TOG5674' and 'TOG5675' are traditional varieties from Nigeria, whilst 'CG14' and 'RAM131' are from Senegal and Mali, respectively. The African rice species *O. glaberrima* represents a valuable source of genes potentially capable of alleviating many abiotic and biotic stresses (Sarila & Swamy, 2005) such as weed competitiveness, drought and water-lodging tolerance (Jones *et al.*, 1997; Futakuchi *et al.*, 2001) and

Table 2. List of mitochondrial DNA amplification and sequencing primers used in this study.

Primer name	Targeted DNA marker	Primer sequence (5' → 3')	Primer position	Fragment length (bp)
Fw-mtMg-1	mtMg-1	TTTTGGTCATCCAGAGGTTT	693	1998
Rev-mtMg-1		AGATGAAGATCGTAACCTACTAA	2680	
Fw-mtMg-2	mtMg-2	AGCTTTAATCTTTGGTGTTCAAGT	9041	1891
Rev-mtMg-2		AGTGACAGCCATTCCACAAA	10,931	
Fw-mtMg-3	mtMg-3	TAGTCTATAGAATTCATATTTCTAAGG	17,613	1153
Rev-mtMg-3		TAGGTGCAACCATAGCTTTA	18,765	
SeqFwmtMg-1	mtMg-1	GCACCAGCTTTTGGTTTAG	724	
Seq2FwmtMg-1	mtMg-1	TTACGTACCAGATAACTCGG	1632	
SeqRevmtMg-2	mtMg-2	GCAGTCAAAATCGCCTCCG	10,057	
Seq2RevmtMg-2	mtMg-2	GTTCTAAATCTACAACATATGG	10,723	
SeqRevmtMg-3	mtMg-3	CAAAAAACAGCAATTACTCATTAC	18,263	
Seq2RevmtMg-3	mtMg-3	GGAAACTGTGACCTTTAG	18,727	

Sequences of newly developed primers and binding position of the 5' extremity of these primers on the reference mitochondrial *Meloidogyne graminicola* coding sequence (NC_024275). The total length of the amplicon is given for the three DNA markers: mtMg-1, mtMg-2 and mtMg-3.

resistance to plant-parasitic nematodes (Soriano *et al.*, 1999; Plowright *et al.*, 1999; De Waele, pers. comm.). 'IR64' was developed by IRRI as an improved irrigated lowland variety, whilst 'UPLRi-5' was developed in the Philippines by the University of the Philippines in Los Baños (UPLB) as an improved upland variety. Both *O. sativa* varieties have a higher yield potential (George *et al.*, 2002) compared with the *O. glaberrima* varieties, with wide adaptability and good eating quality (Wu *et al.*, 2005), but are moderately sensitive to drought (Wade *et al.*, 1999).

NEMATODE INOCULUM

The nematode inoculum was prepared by uprooting 10-week-old 'UPLRi-5' plants inoculated separately with the five *M. graminicola* populations. Galled roots were washed thoroughly to remove any adhering soil particles, cut into 5–10 mm pieces and incubated in a mist chamber (Seinhorst, 1950). The *M. graminicola* J2 extracted after 24 h of incubation were used as the inoculum.

PATHOGENICITY STUDY

Rice seeds were pre-germinated in Petri dishes and two 5-day-old seedlings were transplanted into 20-cm-diam., 24-cm-high plastic pots each filled with 6 kg of a sterilised sandy loam soil. The soil was saturated at planting and at field capacity at the time of inoculation. One week later, the seedlings were thinned to one plant per pot. Two-week-old plants were inoculated with 3000 *M. graminicola* J2 by pipetting three aliquots of the same volume in three 5-cm-deep holes around the base of the seedlings. Two days later, the nematode inoculation was repeated so that the final pathogen pressure was *ca* 1 J2 (g soil)⁻¹. Non-inoculated plants served as control plants.

One day after the second inoculation water was added and the soil was maintained at field capacity up to plant maturity. The rice plants were grown in the glasshouse and fertilised three times (at transplanting, and at 30 and 60 days after transplanting (DAT)) at a rate of 90–60–60 NPK kg ha⁻¹. The experimental lay-out consisted of a randomised complete block design (RCB) in a factorial arrangement with eight replications for each rice variety, each *M. graminicola* population, inoculated and non-inoculated (control) plants.

The *O. glaberrima* varieties (both inoculated and non-inoculated plants) started to mature from 85 DAT onwards and at 101 DAT all the plants were harvested. The *O. sativa* varieties (both inoculated and non-inoculated

plants) started to mature at 98 DAT, and at 104 DAT all the plants were harvested. At harvest, the roots were carefully uprooted, excised from the shoots, washed with tap water and the severity of root galling determined. Assessment of the severity of root galling was based on the rating scale of 0–5 (Hussey & Janssen, 2002). After the fresh root weight of each plant was recorded, the roots were cut into 1-cm pieces and incubated for 2 weeks in a mistifier for nematode extraction (Seinhorst, 1950). After 2 weeks in the mistifier, the nematode suspension was collected and the final number of J2 per root system (P_f) counted using a stereo microscope. The nematode multiplication factor (MF) was calculated as $P_f/6000$ (6000 = the initial number of nematodes (P_i) inoculated in each pot).

At harvest, the following plant growth and yield contributing traits were measured for all treatments: fresh root and shoot weight, plant height (from the soil surface to the tip of the tallest panicle), number of tillers per plant at 30 DAT, number of panicles per plant, number of spikelets per panicle, percentage of filled grains per panicle, weight of 100 grains per plant and filled grain weight per plant (adjusted to 14% moisture content). The yield per plant was measured based on the weight of the filled grains produced per plant, not including the unfilled and partially filled grains.

DATA ANALYSIS

Nematode counts were subjected to $\log(x + 1)$ transformation prior to analysis to meet the assumptions of analysis of variance (ANOVA). When these assumptions were met (*i.e.*, normal distribution and homogeneity of variances), data were analysed using STATISTICA software (StatSoft). Factorial analysis of ANOVA was used to examine the effect of the treatments. In the case where an interaction was obtained between the two factors (nematode population and rice variety), individual comparisons were made for each rice variety separately. Tukey's HSD test ($P < 0.05$) was applied for comparisons of factor level means for nematode, plant growth and yield-contributing trait. Dunnett's test ($P < 0.05$) was applied to compare the means of the nematode-inoculated rice varieties with the non-inoculated control plants. When the assumptions for ANOVA were met, all data were analysed using ANOVA. When the assumptions for ANOVA were not met and after transformation of the data, the non-parametric Kruskal-Wallis Rank test was used to analyse the data and mean separation was performed with the Kruskal-Wallis-Bonferroni method (combined confidence coefficient $\infty = 0.05$). The linear relationship be-

tween the % yield reduction and number of J2 per root system was analysed using Pearson correlation analysis ($P < 0.05$).

Results

MORPHOLOGICAL OBSERVATIONS

In general, *M. graminicola* females of all five populations have a globular to pear-shaped body. The neck is small and the head is not distinctly set-off from the neck. The cephalic framework is inconspicuous. The stylet is small, slender and delicate, with rounded knobs sloping posteriorly. The cuticle is annulated and marked with irregular punctuations. The lateral field is obscure. The orifice of the dorsal pharyngeal gland is situated behind the stylet base. The excretory pore is conspicuous, anterior to the median pharyngeal gland, near to the stylet base. The pharynx is elongated with a cylindrical procorpus, large and rounded metacarpus, with heavily sclerotised

valve. The pharyngeal gland overlaps with the intestine. The nerve ring is obscure. The two ovaries are convoluted. The vulva and anus are terminally located. The tail tip is marked with prominent striations or patterns.

Very minor variations in the perineal pattern were observed among the different populations. In general, the females of all five populations have an oval or egg-shaped perineal pattern. The dorsal arches are moderately high. No lateral lines or gaps were observed. The cuticular striae are smooth in the outer region of the oval pattern. Wavy striae converge into a tetragonal or pyramidal web near the tail remnant. All vulval lips are smooth without invaginations (Fig. 2).

No distinct differences were observed in the morphology of the J2 of all five populations. In general, the J2 had a vermiform body tapering towards the posterior end. The cuticle has fine annulations, about $1\ \mu\text{m}$ apart. The lateral field is wide, about one-third of the mid-body width. The head is rounded, and the lip region is continuous with the body and weakly sclerotised. The stylet is

Fig. 2. Perineal patterns of mature females of *Meloidogyne graminicola* populations from the Philippines. A: Batangas; B: Bulacan; C: Cotabato; D: Laguna; E: Tarlac. Striae converging into a tetragonal web (w) near the tail (t) terminus dorsal to the vulva (v). (Scale bar = $20\ \mu\text{m}$.)

small and delicate with rounded basal knobs. The excretory pore is situated behind the nerve ring. The median pharyngeal bulb is rounded with a prominent refractive valve and almost spherical. The tail is long and tapering uniformly with a hyaline portion and rounded tail terminus.

MORPHOMETRICS

The morphometrics of the J2 of all five populations are presented in Table 3. The body length of the J2 ranged from 340 to 503 μm (mean: 408.2 μm). The population from Batangas had the longest ($P < 0.05$) average body length (446.6 μm). The length of the pharynx ranged from 84 to 158 μm (mean: 116.2 μm). Among the five *M. graminicola* populations no significant differences in tail length (range 57 to 97 μm ; mean: 80.5 μm), stylet length (range 9 to 15 μm ; mean: 13.3 μm) and body width (range 11 to 18 μm ; mean: 15.0 μm) were observed. The average a, b and c values were significantly ($P < 0.05$) higher in the population from Batangas compared with the other four populations.

MOLECULAR CHARACTERISATION

The 386-bp nuclear rDNA region (ITS) of the *M. graminicola* populations did not show variation between populations. Based on a BLAST search, all populations have 100% identity (E. value 0.0) with *M. graminicola* population VN6 (GenBank accession no. KF250479). Similarly, no significant variation was found in the mitochondrial genome of the *M. graminicola* populations from the Philippines. The mitochondrial DNA sequences of mtMg-1 and mtMg-3 regions were 100% identical (E. value 0.0) to the *M. graminicola* mitochondrial sequence (GenBank accession no. NC_024275.1; Besnard *et al.*, 2014). However, the analysis on the mtMg-2 region that includes the 3' end of *NAD4* gene, the complete tRNA-His gene and a non-coding region at the 5' extremity of the 111R repeat region revealed heteroplasmy. In this 1891-bp sequence, six nucleotide sites presented heteroplasmy (transition or transversion) but no indel was detected (Table 4). For five loci, polymorphism was between nucleotides A and T (transversion) and for the sixth locus we observed a transition between C and T (therefore, respectively, encoding for W

Table 3. Morphometrics of the second-stage juveniles (J2) of five *Meloidogyne graminicola* populations collected from different rice-growing areas in the Philippines (n = 18 for each population), and populations from USA and Nepal. Values are in μm and in the form: mean $\pm$ standard deviation (range).

<i>M. graminicola</i> population	Body length	Pharynx length	Tail length	Stylet length	Body width	a	b	c
Batangas	446.6 $\pm$ 34.3 a (391-503)	109.8 $\pm$ 11.5 b (84-135)	76.9 $\pm$ 9.2 a (59-95)	14.2 $\pm$ 0.8 a (13-15)	14.2 $\pm$ 1.4 a (11-17)	31.6 $\pm$ 3.6 a (25.7-38.8)	4.1 $\pm$ 0.6 a (3.2-5.7)	5.9 $\pm$ 0.7 a (5.0-7.6)
Bulacan	412.6 $\pm$ 34.7 b (354-494)	122.8 $\pm$ 17.8 a (95-158)	82.0 $\pm$ 10.6 a (57-95)	13.0 $\pm$ 1.3 a (10-15)	15.5 $\pm$ 1.9 a (13-18)	26.9 $\pm$ 3.4 b (20.8-31.8)	3.4 $\pm$ 0.5 b (2.7-4.5)	5.1 $\pm$ 0.6 b (4.2-6.2)
Cotabato	386.7 $\pm$ 35.2 b (346-480)	117.7 $\pm$ 10.5 ab (99-129)	82.8 $\pm$ 8.8 a (68-97)	13.1 $\pm$ 3.1 a (11-15)	15.4 $\pm$ 1.4 a (14-18)	25.3 $\pm$ 3.4 b (20.5-32.4)	3.3 $\pm$ 0.4 b (2.9-4.7)	4.7 $\pm$ 0.5 b (4.0-5.9)
Laguna	399.7 $\pm$ 34.5 b (340-465)	112.5 $\pm$ 13.6 ab (93-135)	80.8 $\pm$ 10.6 a (63-97)	13.0 $\pm$ 1.4 a (9-15)	14.8 $\pm$ 1.3 a (11-17)	27.4 $\pm$ 4.5 b (21.6-40.4)	3.6 $\pm$ 0.4 b (2.9-4.1)	5.1 $\pm$ 1.0 b (3.8-7.0)
Tarlac	395.3 $\pm$ 24.2 b (346-440)	118.0 $\pm$ 12.8 ab (95-139)	80.1 $\pm$ 10.3 a (66-94)	13.0 $\pm$ 1.2 a (11-15)	15.3 $\pm$ 1.6 a (12-18)	26.2 $\pm$ 3.0 b (22.6-31.7)	3.4 $\pm$ 0.5 b (2.9-4.6)	5.0 $\pm$ 0.6 b (3.8-6.1)
USA ¹	441.0 (415-484)	126.0 (112-140)	70.9 (67-76)	11.4 (11-12)	17.8 (17-19)	24.8 (22.3-27.3)	3.2 (2.9-4.0)	6.2 (5.5-6.7)
Nepal ²	450.9 (425-477)			11.4 (10-16)		25.8 (22.3-30.5)	3.8 (3.5-4.2)	6.4 (5.2-8.1)

Means in the same column followed by the same letter do not differ significantly between the five *M. graminicola* populations according to Tukey's HSD test ($P < 0.05$).

a - body length/maximum body width.

b - body length/pharynx length.

c - body length/tail length.

¹Golden & Birchfield (1965); Mulk (1976).

²Pokharel *et al.* (2007).

Table 4. Mitochondrial heteroplasmic single-nucleotide polymorphisms.

Position on the reference genome (NC_024275)	Gene	Ref Base	Var Base	IUPAC nucleotide code	Nematode population with heteroplasmy
577	<i>NAD4</i>	C	T	Y	Batangas, Cotabato
1332	N.C	A	T	W	Batangas, Cotabato, Laguna
1524	N.C	A	T	W	Batangas, Cotabato, Tarlac
1534	N.C	A	T	W	Batangas, Cotabato, Laguna
1536	N.C	A	T	W	Batangas, Cotabato, Laguna
1559	N.C	A	T	W	Batangas, Cotabato, Laguna

Ref Base = Reference Allele; Var Base = Variant Allele; N.C = No Coding sequence.

and Y according to the IUPAC nucleotide code). Further analysis of those polymorphism sites revealed that five were in a non-coding region and one was in the *NAD4* gene generating a silent mutation. The populations from Batangas and Cotabato presented a higher degree of heteroplasmy with the presence of six heteroplasmic positions while the population from Tarlac had only two heteroplasmic positions and thus in the non-coding region. None of the individuals we surveyed was homoplasmic.

PATHOGENICITY STUDY

Nematode reproduction

No significant differences in reproduction among the five populations on the resistant varieties were observed (Table 5). The highest average number of J2 (g root)⁻¹ occurred with the Batangas population on 'RAM131', while the lowest average number of J2 (g root)⁻¹ was with the Bulacan population on 'TOG5674' (1,709 vs 154 J2 (g root)⁻¹, respectively). The highest average number of J2 (root system)⁻¹ was with the Laguna population on 'CG14' while the lowest average number of J2 (root system)⁻¹ was with the Bulacan popula-

tion on 'TOG5674'. The lowest MFs were observed on 'TOG5674' and 'TOG5675' (MF with one exception > 1). The MF on 'CG14' and 'RAM131' were somewhat higher (Table 5).

No significant differences in reproduction among the five populations on the susceptible varieties were observed. The highest average number of J2 (g root)⁻¹ occurred with the Tarlac population on 'IR64', while the lowest average number of J2 (g root)⁻¹ occurred also with the Tarlac population on 'UPLRi-5'. The highest average number of J2 (root system)⁻¹ was with the Laguna population on 'UPLRi-5', while the lowest average number of J2 (root system)⁻¹ was with the Batangas population on 'IR64'. The MF of 'IR64' and 'UPLRi-5' ranged on average from 12.4 to 15 and from 12.4 to 19.8, respectively.

Host response

The five *M. graminicola* populations examined in this study induced a comparable severity of root galling (in general < 3) on the four resistant *O. glaberrima* varieties. The Laguna population significantly ($P < 0.05$) induced root galls on 'TOG5675' and 'RAM131'. No significant differences in severity of root galling among the five populations on the susceptible varieties were observed, ranging from 4.4 to 5.0.

All five populations reduced the fresh root weights of the resistant varieties compared with the non-inoculated control plants but only significantly ($P < 0.05$) on 'TOG5674' and 'CG14' (Table 6). Significant differences in percentage reduction in fresh root weights among the five *M. graminicola* populations were not observed. All five populations also reduced the fresh shoot weights of the resistant varieties compared with the non-inoculated control plants but only significantly ($P < 0.05$) on 'TOG5674' with the Tarlac population. No significant percentage reduction in plant height of the resistant varieties was caused by any of the five *M. graminicola* populations. With a few exceptions, the percentage reduction in plant height was < 5%. Compared with the non-inoculated control plants, the Batangas population significantly ($P < 0.05$) reduced the fresh root weight of 'IR64', while the Batangas, Tarlac, Bulacan and Laguna populations reduced the fresh root weight of 'UPLRi-5'. Compared with the non-inoculated control plants, all populations, except the Tarlac population, significantly ($P < 0.05$) reduced the fresh shoot weight of 'IR64' while the Batangas population significantly ($P < 0.05$) reduced the fresh shoot weight of 'UPLRi-5'. Compared

Table 5. Reproduction of five populations of *Meloidogyne graminicola* from the Philippines on resistant (^R) and susceptible (^S) rice varieties, and severity of root galling at maturity.

Rice variety	Nematode population	No. of J2 (g root) ⁻¹	No. of J2 (root system) ⁻¹	Multiplication factor	Root galling index
TOG5674 ^R	Batangas	277 ± 304	3905 ± 3320	0.7 ± 0.5	2.6 ± 1.3
	Bulacan	154 ± 111	2316 ± 2003	0.4 ± 0.3	1.6 ± 0.7
	Cotabato	227 ± 137	2889 ± 1754	0.5 ± 0.3	1.6 ± 0.9
	Laguna	357 ± 236	4395 ± 2887	0.7 ± 0.5	3.0 ± 1.1
	Tarlac	343 ± 385	3406 ± 3150	0.6 ± 0.5	1.8 ± 0.9
TOG5675 ^R	Batangas	559 ± 544	4155 ± 3436	0.7 ± 0.8	2.5 ± 0.8 ab
	Bulacan	279 ± 269	3813 ± 3603	0.6 ± 0.6	1.5 ± 0.8 a
	Cotabato	660 ± 662	6848 ± 7337	1.1 ± 1.2	2.7 ± 0.8 ab
	Laguna	567 ± 639	5475 ± 6420	0.9 ± 1.1	3.3 ± 1.0 b
	Tarlac	379 ± 365	4636 ± 4233	0.8 ± 0.7	2.3 ± 1.5 ab
CG14 ^R	Batangas	850 ± 761 ab	13 845 ± 15 768	2.3 ± 2.6	2.3 ± 0.5
	Bulacan	356 ± 358 a	5418 ± 6771	0.9 ± 1.1	1.4 ± 0.5
	Cotabato	489 ± 385 ab	6656 ± 4661	1.1 ± 0.8	1.8 ± 0.7
	Laguna	901 ± 706 b	14 530 ± 9546	2.2 ± 1.7	2.1 ± 0.8
	Tarlac	545 ± 427 ab	9659 ± 9739	1.6 ± 1.6	1.8 ± 1.0
RAM131 ^R	Batangas	1709 ± 1497 b	12 064 ± 6336 b	2.0 ± 1.1 b	1.6 ± 0.7 ab
	Bulacan	263 ± 326 a	2578 ± 2546 a	0.4 ± 0.4 a	0.9 ± 0.3 a
	Cotabato	481 ± 443 ab	3842 ± 3096 ab	0.6 ± 0.5 ab	1.4 ± 0.7 a
	Laguna	942 ± 1036 ab	9099 ± 10 040 ab	1.5 ± 1.7 ab	2.9 ± 1.6 b
	Tarlac	446 ± 519 ab	4815 ± 5598 ab	0.8 ± 1.0 ab	1.6 ± 0.9 ab
IR64 ^S	Batangas	5572 ± 4076	74 139 ± 57 322	12.4 ± 9.6	4.5 ± 0.5
	Bulacan	5439 ± 3560	88 744 ± 51 224	14.8 ± 8.5	4.5 ± 0.5
	Cotabato	5321 ± 5043	90 069 ± 59 596	15.0 ± 9.9	4.9 ± 0.3
	Laguna	4775 ± 2410	84 794 ± 43 672	14.1 ± 7.3	4.6 ± 0.6
	Tarlac	5611 ± 4550	89 086 ± 64 544	14.8 ± 10.8	4.6 ± 0.5
UPLRi-5 ^S	Batangas	3483 ± 2186	90 413 ± 61 119	15.1 ± 0.5	4.8 ± 0.5
	Bulacan	3444 ± 3385	102 867 ± 87 062	17.1 ± 0.3	4.4 ± 1.8
	Cotabato	3166 ± 2269	109 758 ± 72 598	18.3 ± 0.3	4.5 ± 0.5
	Laguna	3583 ± 2847	118 909 ± 79 150	19.8 ± 0.5	4.6 ± 0.5
	Tarlac	2399 ± 1217	74 374 ± 38 085	12.4 ± 0.5	5.0 ± 0.0

Number of second-stage juveniles (J2) inoculated: 6000 plant⁻¹. Data are means ± standard deviation (n = 8). Means of the same rice variety followed by the same letter are not significantly different according to Tukey's HSD test ($P < 0.05$). Multiplication Factor = number of J2 per root system/6000 J2.

with the non-inoculated control plants, all populations significantly ($P < 0.05$) reduced the plant height of 'UPLRi-5'.

No significant differences were observed on the yield-contributing traits and yield of the plants inoculated with each of the five *M. graminicola* populations (Tables 7-9). All five populations reduced the number of tillers per plant, number of panicles per plant, and number of spikelets per panicle of the resistant varieties compared with the non-inoculated control plants but only the Batangas, Cotabato, Laguna and Tarlac populations caused a significant ($P < 0.05$) reduction in the number of tillers per plant on 'TOG5674' and the Tarlac population in

the number of panicles per plant. Although the five *M. graminicola* populations caused reductions in number of spikelets per panicle on the susceptible varieties compared with the non-inoculated control plants, these percentage reductions were only significant ($P < 0.05$) for the Cotabato population on 'IR64' and the Batangas population on 'UPLRi-5'.

Although all five *M. graminicola* populations mostly reduced the percentage of filled grains per panicle, filled grain weight per plant and weight of 100 grains per plant compared with the non-inoculated control plants of both the resistant and the susceptible varieties, no significant reductions were observed, with the exception of

Table 6. Fresh root and shoot weight, and plant height at maturity of resistant (^R) and susceptible (^S) rice varieties inoculated with five different populations of *Meloidogyne graminicola* from the Philippines, and percentage reduction when compared with non-inoculated control plants.

Rice variety	Nematode population	Fresh root weight		Fresh shoot weight		Plant height at maturity	
		g	Reduction (%)	g	Reduction (%)	cm	Reduction (%)
TOG5674 ^R	Batangas	16.4 Aa	−24.1	123.9 Aa	−20.8	129.8	0.7
	Bulacan	14.5 Aa	−32.9	148.1 Aa	−5.3	125.0	−3.0
	Cotabato	13.6 Aa	−37.2	124.5 Aa	−20.4	126.2	−2.1
	Laguna	12.9 Aa	−40.4	113.9 Aa	−27.2	129.4	0.4
	Tarlac	12.1 Aa	−44.1	110.0 Ab	−29.7	128.5	−0.3
	Non-inoculated	21.6 b	0.0	156.4 a	0.0	128.9	0.0
TOG5675 ^R	Batangas	12.4	−21.7	100.1	−27.0	114.0	−6.1
	Bulacan	13.3	−15.9	105.6	−23.0	124.4	2.4
	Cotabato	12.0	−24.1	107.7	−21.5	123.7	1.9
	Laguna	11.6	−26.8	103.0	−24.9	119.8	−1.4
	Tarlac	13.2	−16.9	123.2	−10.2	123.5	1.6
	Non-inoculated	15.9	0.0	137.2	0.0	121.5	0.0
CG14 ^R	Batangas	16.0 Aa	−22.6	122.1	−6.2	135.0	1.8
	Bulacan	14.8 Aa	−28.2	93.4	−28.2	126.6	−4.5
	Cotabato	14.9 Aa	−27.6	110.1	−15.4	135.9	2.5
	Laguna	17.2 Aa	−16.6	113.4	−12.8	130.9	−1.3
	Tarlac	14.5 Aa	−29.6	112.9	−13.2	137.4	3.6
	Non-inoculated	20.6 b	0.0	130.1	0.0	132.6	0.0
RAM131 ^R	Batangas	7.6	−31.5	93.5	−3.9	102.3	−2.8
	Bulacan	9.3	−16.2	88.9	−9.0	103.9	−1.2
	Cotabato	9.2	−17.1	79.7	−18.4	87.4	−16.9
	Laguna	8.1	−27.0	74.1	−24.2	107.4	2.1
	Tarlac	10.6	−4.5	85.9	−12.1	82.7	−21.4
	Non-inoculated	11.1	0.0	97.7	0.0	105.2	0.0
IR64 ^S	Batangas	13.3 Aa	−48.0	85.1 Aa	−41.8	70.5	−11.6
	Bulacan	18.6 Ab	−27.2	100.8 Aa	−31.1	68.8	−13.8
	Cotabato	20.5 Ab	−19.7	100.7 Aa	−31.2	75.2	−5.8
	Laguna	18.9 Ab	−26.0	90.1 Aa	−38.4	64.4	−19.3
	Tarlac	18.1 Ab	−29.0	105.3 b	−28.0	68.1	−14.6
	Non-inoculated	25.5 b	0.0	146.3 b	0.0	79.7	0.0
UPLRi-5 ^S	Batangas	26.5 Aa	−40.5	156.4 Aa	−25.6	114.8 Aa	−7.9
	Bulacan	34.6 Aa	−22.3	187.3 Ab	−10.9	106.7 Aa	−14.5
	Cotabato	37.4 Ab	−16.0	190.1 Ab	−9.6	109.0 Aa	−12.6
	Laguna	36.0 Aa	−19.1	172.8 Ab	−17.8	102.4 Aa	−17.9
	Tarlac	33.2 Aa	−25.4	160.1 Ab	−23.9	105.8 Aa	−15.1
	Non-inoculated	44.5 b	0.0	210.3 b	0.0	124.7 b	0.0

Means of the same rice varieties followed by the same uppercase letter are not significantly different among the five *M. graminicola* populations according to Tukey's HSD test ($P < 0.05$). (n = 8).

Means of the same rice varieties followed by the same lowercase letter are not significantly different from the non-inoculated control plants according to Dunnett's test ($P < 0.05$). (n = 8).

the Batangas population in 'TOG5675' and 'RAM131'. A high reduction in filled grain weight per plant of 'IR64' was caused by the Batangas, Cotabato and Laguna populations, while all five populations caused a

high reduction in filled grain weight per plant on 'UPLRi-5'.

The percentage yield reduction was positively correlated with the number of J2 per root system of the rice

Table 7. Yield component parameters of resistant (^R) and susceptible (^S) rice varieties inoculated with five different populations of *Meloidogyne graminicola* from the Philippines, and percentage reduction when compared with non-inoculated control plants.

Rice variety	Nematode population	Number of tillers per plant		Number of panicles per plant		Number of spikelets per panicle	
		n	Reduction (%)	n	Reduction (%)	n	Reduction (%)
TOG5674 ^R	Batangas	9.9 Aa	−36.9	19.6 Ab	−13.3	80.8	−2.7
	Bulacan	13.6 Aa	−13.4	19.9 Aa	−11.9	78.1	−5.9
	Cotabato	11.0 Aa	−29.9	18.4 Ab	−18.6	77.1	−7.1
	Laguna	10.7 Aa	−31.8	16.0 Ab	−29.1	78.0	−6.0
	Tarlac	9.7 Aa	−38.2	15.5 Aa	−31.3	78.3	−5.7
	Non-inoculated	15.7 b	0.0	22.6 b	0.0	83.0	0.0
TOG5675 ^R	Batangas	8.7	−22.3	16.0	−28.4	59.4	−14.5
	Bulacan	8.9	−20.5	17.4	−22.2	66.8	−3.9
	Cotabato	8.4	−25.0	20.9	−6.6	61.8	−11.0
	Laguna	9.9	−11.6	20.8	−7.1	60.6	−12.7
	Tarlac	9.4	−16.1	18.9	−15.5	69.7	0.3
	Non-inoculated	11.2	0.0	22.3	0.0	69.4	0.0
CG14 ^R	Batangas	8.5	−25.4	19.4	−0.6	98.1	−3.1
	Bulacan	7.9	−30.7	14.3	−26.9	96.0	−5.2
	Cotabato	8.4	−26.3	16.9	−13.5	102.9	1.7
	Laguna	8.1	−28.9	17.0	−12.8	99.3	−1.9
	Tarlac	6.4	−43.9	17.6	−9.6	90.9	−10.2
	Non-inoculated	11.4	0.0	19.5	0.0	101.3	0.0
RAM131 ^R	Batangas	8.7	−29.3	7.3	4.3	55.8	−5.6
	Bulacan	12.0	−2.4	7.7	10.0	53.6	−9.4
	Cotabato	12.7	3.3	5.7	−18.6	41.2	−30.3
	Laguna	9.5	−22.8	8.8	25.7	43.6	−26.3
	Tarlac	10.0	−18.7	5.3	−25.0	35.6	−39.9
	Non-inoculated	12.3	0.0	7.0	0.0	59.1	0.0
IR64 ^S	Batangas	11.9	−16.8	13.0	−34.6	74.4 Ab	−30.8
	Bulacan	9.5	−33.6	16.3	−18.0	75.4 Ab	−29.8
	Cotabato	11.4	−20.3	14.9	−25.0	66.9 Aa	−37.8
	Laguna	10.8	−24.5	12.9	−35.2	71.8 Ab	−33.2
	Tarlac	12.8	−10.5	16.3	−18.2	83.1 Ab	−22.6
	Non-inoculated	14.3	0.0	19.9	0.0	107.4 b	0.0
UPLRi-5 ^S	Batangas	8.0	−20.8	10.5	−30.6	109.7 Aa	−31.4
	Bulacan	9.8	−3.0	13.5	−10.7	133.9 Ab	−16.2
	Cotabato	9.5	−5.9	13.6	−9.9	137.9 Ab	−13.7
	Laguna	8.1	−19.8	13.6	−9.9	121.4 Ab	−24.1
	Tarlac	9.4	−6.9	13.5	−10.7	119.6 Ab	−25.1
	Non-inoculated	10.1	0.0	15.1	0.0	159.8 b	0.0

Means of the same rice varieties followed by the same uppercase letter are not significantly different among the five *M. graminicola* populations according to Tukey's HSD test ($P < 0.05$). (n = 8).

Means of the same rice varieties followed by the same lowercase letter are not significantly different from the non-inoculated control plants according to Dunnett's test ($P < 0.05$). (n = 8).

genotypes ($P < 0.05$, $r = 0.80$). Figure 3 shows the percentage yield reduction in *M. graminicola*-infected resistant and susceptible rice varieties when compared with non-inoculated control plants. On average, the four resis-

tant rice varieties suffered a yield reduction of 18.4%. Among the resistant varieties, the highest average percentage yield reduction was always caused by the Batangas population. The highest percentage yield reduction

Table 8. Yield-contributing traits data of resistant (^R) and susceptible (^S) rice varieties inoculated with five different populations of *Meloidogyne graminicola* populations (6000 second-stage juveniles plant⁻¹) from the Philippines, and percentage change when compared with no-inoculated control plants.

Rice genotype	Nematode population	Percentage filled grains per panicle		Weight of 100 grains per plant		Filled grain weight per plant	
		(%)	% change	(g)	% change	(g)	% change
TOG5674 ^R	Batangas	70.9	-8.3	2.3	15.6	9.4	-22.5
	Bulacan	73.1	-5.4	2.2	9.1	12.1	0.4
	Cotabato	77.4	0.1	2.2	13.4	10.7	-11.5
	Laguna	74.7	-3.4	2.0	-0.4	9.9	-17.8
	Tarlac	75.5	-2.3	2.0	2.9	10.6	-12.2
	Non-inoculated	77.3	0.0	2.0	0.0	12.1	0.0
TOG5675 ^R	Batangas	61.5	-15.2	1.9	-11.6	7.2 Aa	-35.8
	Bulacan	64.2	-11.5	2.0	-7.7	9.7 Ab	-13.7
	Cotabato	79.4	9.4	2.2	3.0	13.8 Ab	23.4
	Laguna	75.7	4.3	2.3	4.0	8.9 Ab	-20.3
	Tarlac	67.8	-6.6	2.2	-0.6	11.0 Ab	-1.6
	Non-inoculated	72.6	0.0	2.2	0.0	11.2 b	0.0
CG14 ^R	Batangas	65.3	-4.7	2.0	-1.7	11.2	-13.7
	Bulacan	60.7	-11.4	2.0	-0.9	11.5	-11.7
	Cotabato	72.9	6.5	2.2	7.6	14.1	8.2
	Laguna	66.5	-2.9	1.9	-7.0	12.0	-7.5
	Tarlac	71.5	4.4	2.1	4.5	11.7	-9.8
	Non-inoculated	68.5	0.0	2.0	0.0	13.0	0.0
RAM131 ^R	Batangas	31.5	-35.6	1.5	-2.7	2.2 Aa	-58.9
	Bulacan	38.6	-21.1	1.4	-10.7	3.8 Ab	-30.9
	Cotabato	24.8	-49.3	1.6	3.1	4.3 Ab	-21.2
	Laguna	27.5	-43.8	1.4	-7.4	5.5 Ab	1.5
	Tarlac	35.4	-27.6	1.6	1.1	5.1 Ab	-5.5
	Non-inoculated	48.9	0.0	1.6	0.0	5.4 b	0.0
IR64 ^S	Batangas	65.0	-10.7	1.9	-3.3	4.9 Aa	-66.7
	Bulacan	62.4	-14.2	2.0	1.7	7.7 Ab	-47.0
	Cotabato	62.6	-14.0	1.9	-2.2	5.7 Aa	-61.0
	Laguna	62.7	-13.8	1.9	-2.0	4.5 Aa	-69.2
	Tarlac	68.4	-6.0	2.0	-0.3	7.7 Ab	-47.0
	Non-inoculated	72.8	0.0	2.0	0.0	14.6 b	0.0
UPLRi-5 ^S	Batangas	62.5	-10.8	2.1	-6.8	10.1 Aa	-49.3
	Bulacan	56.7	-19.1	2.1	-5.5	9.8 Aa	-50.8
	Cotabato	59.5	-15.1	2.0	-8.2	11.7 Aa	-41.3
	Laguna	57.4	-18.1	2.1	-5.1	11.3 Aa	-43.1
	Tarlac	59.2	-15.5	2.1	-5.7	12.7 Aa	-36.3
	Non-inoculated	70.1	0.0	2.2	0.0	19.9 b	0.0

Means of the same rice varieties followed by the same uppercase letter are not significantly different among the five *M. graminicola* populations according to Tukey's HSD test ($P < 0.05$). (n = 8).

Means of the same rice varieties followed by the same lowercase letter are not significantly different from the non-inoculated control plants according to Dunnett's test ($P < 0.05$). (n = 8).

by this population was observed on 'RAM131' (58.9%; $P < 0.05$) and on 'TOG5675' (35.8%; $P < 0.05$). Among the resistant rice varieties, the lowest average per-

centage yield reductions were caused by each of the other four *M. graminicola* populations. On average, the two susceptible rice varieties suffered a yield reduction of 51.2%.

Table 9. Average fresh root weight and average percentage change in plant growth data and yield-contributing traits of resistant (^R) and susceptible (^S) rice varieties inoculated with five *Meloidogyne graminicola* populations (6000 second-stage juveniles plant⁻¹) from the Philippines compared with non-inoculated control plants for each rice variety, and for the resistant and susceptible varieties combined.

Rice genotype	Average fresh root weight (g)	Fresh root weight	Fresh shoot weight	Plant height at maturity	Number of tillers per plant	Number of panicles per plant	Number of spikelets per panicle	Percentage filled grains per panicle	Weight of 100 grains per plant	Filled grain weight per plant
TOG5674 ^R	26.1	-35.8	-20.7	-1.8	-30.1	-20.9	-5.5	-4.9	-0.4	-16.0
TOG5675 ^R	15.9	-21.1	-21.3	-3.8	-19.1	-15.9	-10.5	-11.1	-6.7	-17.8
CG14 ^R	20.6	-24.9	-15.2	-2.9	-31.1	-12.7	-5.1	-6.3	-6.7	-10.7
RAM131 ^R	11.1	-19.3	-13.5	-10.6	-18.3	-21.8	-22.3	-35.5	-6.9	-29.1
IR64 ^S	25.5	-30.0	-34.1	-13.0	-21.1	-26.2	-30.8	-11.7	-2.0	-58.2
UPLRi-5 ^S	44.5	-24.7	-17.6	-13.6	-11.3	-14.4	-22.1	-15.7	-6.3	-44.2
Resistant	18.4	-25.3	-17.7	-4.8	-24.6	-17.8	-10.9	-14.4	-5.1	-18.4
Susceptible	35.0	-27.3	-25.8	-13.3	-16.2	-20.3	-26.5	-13.7	-4.1	-51.2

For both susceptible varieties, the percentage yield reductions were significantly ($P < 0.05$) different compared with the non-inoculated control plants. The percentage yield reduction was on average higher on 'IR64' (58.2%) compared with 'UPLRi-5' (44.2%). On 'IR64', the highest percentage yield reduction was caused by the Laguna population (69.2%) closely followed by the Batangas population (66.7%), and the lowest percentage yield reduction was by the Bulacan and Tarlac populations (both 47%). On 'UPLRi-5', the highest percentage yield reduction was caused by the Bulacan population (50.8%) closely followed by the Batangas population (49.3%), and the lowest by the Tarlac population (36.3%).

For both the resistant and susceptible rice varieties, on average, fresh root and shoot weight were more affected among the plant growth traits compared with plant height (Table 9). Among the resistant varieties, on average, the number of tillers per plant was the most affected yield component (24.6%) while the weight of 100 grains per plant was the least affected yield component (5.1%). Between the two susceptible varieties, on average, filled grain weight per plant was the most affected yield component (51.2%) while the weight of 100 grains per plant weight was the least affected yield component (4.1%).

Discussion

The five *M. graminicola* populations collected from rice-growing areas in the Philippines varying in agro-

ecological conditions showed comparable morphology, morphometrics and molecular characteristics. The morphology of the J2 of the five *M. graminicola* populations examined was similar to the descriptions by Golden & Birchfield (1965), Mulk (1976) and Pokharel *et al.* (2007). The J2 measurements were within the range of the measurements reported so far for this nematode species (Mulk, 1976) but with interspecific variations among the five populations based on body length and stylet length, and among different *M. graminicola* populations in the USA (Golden & Birchfield, 1965), Nepal (Pokharel *et al.*, 2007) and Vietnam (Bellafiore *et al.*, 2015). The perineal pattern of the *M. graminicola* females were, with some minor variability, similar to the published perineal patterns of this nematode species (Mulk, 1976; Pokharel *et al.*, 2007). Phenotypic similarity was also observed among the J2 and females of 33 *M. graminicola* populations from Nepal (Pokharel *et al.*, 2007).

Molecular analysis of the *M. graminicola* populations from the Philippines revealed that molecular traits determined by the nuclear ribosomal DNA-ITS region were stable across all isolates. The same amplicon size was found in all *M. graminicola* isolates and there were no discernible differences in the nucleotide sequence. Based on the analysis of molecular diversity using this nuclear marker, these populations presented less inter-population diversity than the ones observed in Vietnam (Bellafiore *et al.*, 2015) and in Nepal (Pokharel *et al.*, 2007). We could not conclude that all nuclear genetic traits were conserved across all isolates and, in order

Fig. 3. Effect of the five *Meloidogyne graminicola* populations from the Philippines on the number of juveniles per root system and the yield reduction of four resistant (^R) and two susceptible (^S) rice genotypes grown in a sandy loam soil in the glasshouse. Number of nematodes initially inoculated (P_i): 6000 second-stage juveniles (J2) plant⁻¹. Median boxplot with 25-75% (boxes) and minimum-maximum (error bars) values.

to address this question, other markers like microsatellites or single-nucleotide polymorphism (SNP) have to be developed. In the absence of information on the *M. graminicola* nuclear genome, we took advantage of the published mitochondrial genome to develop mitochondrial markers, as the polymorphism of mitochondrial DNA markers are known to be higher than that with nuclear markers from the genus *Meloidogyne* (Humphreys-Pereira & Elling, 2013). From the three mtDNA markers used and covering up to 5000-bp (corresponding to 25% of the total mtDNA genome) few heteroplasmic

sites were found. Only one of them, mtMg-2, which includes the 5' extremity of *NAD4* and a non-coding region, presented six heteroplasmic sites. mtMg-1 (3' *COX1*, a non-coding region and 5' *ND1*) and mtMg-3 (tRNA^{S2}, *ATP6* and 5' *ND5*) were conserved between isolates and did not present heteroplasmic sites. This is in agreement with a previous study done on 15 populations from southern Europe on the dagger nematodes *Xiphinema* spp., where the molecular intraspecific diversity was studied using the *COX1* gene. For example, a maximum of two heteroplasmic sites were found for *X. pachtaicum*

in a 443-bp mitochondrial *COX1* amplicon (Gutiérrez-Gutiérrez *et al.*, 2011). However, we did not find any heteroplasmic sites in the *COX1* gene, and we could only speculate that the molecular diversity at the intrapopulation level in *M. graminicola* in the Philippines was even lower than that of *X. pachtaicum* in southern Europe. Moreover, the molecular intraspecific diversity observed was very low compared to that observed in other *Meloidogyne* spp. (Humphreys-Pereira & Elling, 2013). Hence, we could hypothesise that this genetic homogeneity in the *M. graminicola* populations in the Philippines reflects their predominant parthenogenetic reproduction mode and/or that these populations have experienced a similar colonisation history with probably a common ancestor.

The low variability in the morphometrics of J2 of the populations examined in this study can thus be accounted for by differences among individuals and not differences among populations. The molecular analysis of this study shows few differences in gene sequence of J2 belonging to the same *M. graminicola* population. Reproduction of the *M. graminicola* populations in resistant and susceptible varieties observed in this study indicates that the five *M. graminicola* populations have similar aggressiveness without any clear resistance-breaking populations.

The severity of root galling is a good indicator of virulence of a root-knot nematode species (Zhou *et al.*, 2000; Anwar & McKenry, 2007) and that is also the case for *M. graminicola* infection of rice. Root galls can limit the uptake of water and nutrients by the roots and their transportation inside the vascular cylinder of the roots. A great severity of root galling can thus cause considerable damage affecting plant growth and reducing yield. The study of De Waele *et al.* (2013) showed a significant correlation between the severity of root galling and yield of rice. On the two susceptible *O. sativa* varieties, all *M. graminicola* populations examined induced greater severity of root galling than *O. glaberrima* varieties. As a result, the yield of *O. glaberrima* varieties was much less reduced compared with the yield of *O. sativa* varieties, thus confirming the previous reports.

In only a minority of the instances (4 out of 36), infection with either of the five *M. graminicola* populations resulted in a significant percentage change in all nine plant growth and yield contributing traits measured of the inoculated *O. glaberrima* plants compared with the non-inoculated control plants. However, in seven out of the 18

instances, infection with either of the five *M. graminicola* populations resulted in a significant percentage change in all nine plant growth and yield-contributing traits measured of the inoculated *O. sativa* plants compared with the non-inoculated control plants. Based on the low number of instances and the inconsistency of the effects of *M. graminicola* on the inoculated plants compared with the non-inoculated plants, one might be tempted to suggest that the five *M. graminicola* populations from the Philippines examined have no major differences in their effect on plant growth and grain production of rice varieties. However, this suggestion is not supported by a closer examination of the data of the effect of the different populations on filled grain weight per plant (yield). In two out of the four resistant *O. glaberrima* varieties included in this study, the Batangas population caused a significantly higher percentage yield reduction compared with the other *M. graminicola* populations. However, on *O. sativa* 'IR64', the Batangas population caused a significantly higher percentage yield reduction compared with two of the other *M. graminicola* populations while, in contrast, on the other *O. sativa* variety, 'UPLRi-5', the Batangas population caused a similar percentage yield reduction compared with all other *M. graminicola* populations. This observation may indicate that there are differences in virulence among the *M. graminicola* populations examined but we predict that these differences will be relatively minor. Nevertheless, this aspect should be further investigated and clarified.

Based on the results of this study, some of the plant traits of the *O. sativa* varieties measured are more reduced by *M. graminicola* infection than other ones. This is, for example, the case for fresh root weight, number of spikelets per panicle, fresh shoot weight and number of panicles per plant. Much reduction in these plant traits as a result of *M. graminicola* infection was also observed in the study of De Waele *et al.* (2013) in *O. sativa* 'IR64' and 'UPLRi-5' vs *O. glaberrima* 'CG14' and 'TOG5674'. Susceptible *O. sativa* varieties grown in raised beds in their study were reduced by 27.5% in fresh root weight, 31.4% in number of spikelets per panicle and 26.2% in number of panicles per plant when infected with *M. graminicola*. This indicates that *M. graminicola* can cause more damage to *O. sativa* genotypes than *O. glaberrima* varieties.

Based on the results of this study, some of the plant traits measured are less reduced by *M. graminicola* infection in the *O. glaberrima* varieties compared with the *O. sativa* varieties. The results of this study seems to

confirm the observation by De Waele *et al.* (2013) carried out in raised beds that *O. glaberrima* varieties are not only resistant or less susceptible to *M. graminicola* but also less sensitive compared with *O. sativa* varieties. However, because the pathogen pressure per root unit (1 g) in this study was 3-5 times lower in the *O. glaberrima* varieties compared with the *O. sativa* varieties, it may also be possible that this difference in nematode susceptibility is the cause of the lower percentage yield reduction in the *O. glaberrima* varieties.

In this study, the number of tillers per plant was, on average, already much more reduced in the *O. glaberrima* varieties at 30 days after planting compared with the *O. sativa* varieties. This seems to contradict the previous suggestion that *O. glaberrima* varieties are less sensitive to *M. graminicola* infection than *O. sativa* varieties, but *O. sativa* varieties (such as 'IR64' and 'UPLRi-5') are known to have moderate to high tillering ability during the vegetative stage (Chang & Loresto, 1986; Mahadevappa *et al.*, 1991). As a result, infection with *M. graminicola* apparently does not reduce the number of tillers per plant in *O. sativa* as much as in *O. glaberrima* varieties. However, tillers of the *O. sativa* varieties were thinner compared with the *O. glaberrima* varieties, which had a higher tiller girth (data not shown) but which are known to be more prone to lodging (Futakuchi & Sié, 2009).

All five *M. graminicola* populations from the Philippines examined in this study show a comparable and low virulence to the selected resistant *O. glaberrima* varieties and a high virulence to the selected susceptible *O. sativa* varieties. A high virulence on the susceptible rice genotypes may be due to an inherent compatibility of the *M. graminicola* populations with these varieties that, in turn, generates a highly virulent interaction, leading to a high nematode reproduction, plant damage and yield loss. Inherent incompatibility of the *M. graminicola* populations with the resistant rice varieties leads to both a low nematode reproduction and less damage and yield loss.

The assessment of variability in reproduction and pathogenicity of plant-parasitic nematode populations is important for the development of management strategies and decisions. Although only five *M. graminicola* populations were included in this study and one should be careful in generalising the observations of this study, the absence of variability in reproduction among the *M. graminicola* populations collected from five rice-producing regions differing in agro-ecology suggests that, at least in the

Philippines, the opportunity to use improved rice varieties with resistance to *M. graminicola* might be useful on a large scale (on the condition that this resistance can be introgressed from *O. glaberrima* into *O. sativa* varieties), but this may not be the case in other rice-growing regions. Resistance to nematodes in improved agricultural crop varieties may be durable if the target nematode species has a low level of genetic variability (Kaloshian *et al.*, 1996) and thus also a low phenotypic variability. However, in Nepal, Pokharel *et al.* (2007) found a high and similar variability in aggressiveness among 33 *M. graminicola* populations measured on the basis of their severity of root galling and nematode reproduction on both a resistant ('Labelle') and susceptible Asian rice genotype ('LA 110'). The study of Pokharel *et al.* (2007) showed that 'Labelle', a previously known resistant rice variety (Yik & Birchfield, 1979), was as susceptible to *M. graminicola* infection as 'LA 110'. So far, all indications are that the resistance to *M. graminicola* infection found in *O. glaberrima* varieties is constant, showing less variability (Soriano *et al.*, 1999; De Waele pers. comm.). This (preliminary) observation may indicate that the resistance genes in *O. glaberrima* may be more durable and more useful in more rice-growing regions than the resistance genes in *O. sativa*. This is an observation that will need further examination to validate.

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