Genetic diversity of the root-knot nematode Meloidogyne graminicola in rice (Oryzasativa L.) based on mitochondrial COI gene

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Abstract

The root-knot nematode Meloidogyne graminicola is an important parasitic nematode that causes huge economic losses to rice production in China. In the present study, 54 M. graminicola populations were collected from the major rice-growing areas of ten provinces in China. The cytochrome oxidase subunit I (COI) gene sequence of M. graminicola populations in the studied locations were PCR-amplified, sequenced, and evaluated for genetic diversity analysis. The total number of mutations, haplotypes (Hap) numbers, the average number of nucleotide differences (k), haplotype diversity (Hd), and nucleotide diversity (π) of mtCOI gene were 39, 15, 5.37, 0.646, and 0.00682, respectively. The significant differences in Fst value (0.593) and a low level of gene flow (0.333) were detected between the 54 M. graminicola populations. High genetic diversity was observed within each population and a small genetic distance was found between them. The phylogenetic analyses showed that 54 M. graminicola populations were divided into three large groups corresponding to the Central Region (CR), Southern China (SC) and the Yangtze Valley (YV). Hap8 was the most widely distributed and was considered to be an origin of haplotype, revealing a separate evolutionary origin in China. The high genetic differentiation of M. graminicola populations was result of variation within each of the defined geographical groups, according to an analysis of molecular variance. No significant relationship was observed between the genetic distance and geographical distance in M. graminicola populations. To the best of our knowledge, this is the first study conductedon the genetic diversity of M. graminicola infesting rice in China and provides a theoretical basis for the future management of the M. graminicola population and potential approach for increasing production of rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of most important food crops in China, with an annual cultivated area of 30 million hectares and a yield of 206 million tons (Deng et al., 2019). Due to the popularization and application of direct-seeding cultivation technology, the harm of rice root-knot nematode was becoming more and more serious (Jabbar et al., 2020). Among all the root-knot nematodes (RKNs) that harm rice,*Meloidogyne graminicola* is considered to be one of the most harmful plant parasitic nematodes in rice cultivation system (Jabbar et al., 2020; Onkendi et al., 2013; Pokharel et al., 2007; Mantelin et al., 2017). *M. graminicola* can infiltrate the roots, cause root galling, inhibit plant defense systems, manipulate the plant's metabolic system, and establish giant cells for its nutrition(Jabbar et al., 2020; Luo et al., 2020). Therefore, over time, the plant loses its vitality, which eventually leads to a significant yield loss (Bridge et al., 2005; Jabbar et al., 2020). *M. graminicola* is widely distributed in the tropical and subtropical regions of China, India, Bangladesh, Thailand, the United States, and other countries (Singh 2010). In China, it was recorded for the first time on *Alliumtistulosum* in Hainan province (Zhao et al., 2001). In recent years, the distribution

of this nematode has expanded from Hainan Province to Guangdong, Guangxi, Fujian, Yunnan, Hunan, Hubei, Anhui, Sichuan, Jiangxi, Henan and other provinces. A total of about 1 million hectares of rice were found to be infected, with a high incidence(Du 2003; Liu et al., 2011; Luo et al., 2020).Several studies have shown that *M. graminicola* infections usually result in a 10-20% reduction in rice yield, and in severe cases, a 50-72% reduction in rice yield (Khan and Ahamad 2009; Jabbar et al., 2020; Luo et al., 2020).Thus, *M. graminicola* has becomea major threat to rice production(Huang et al., 2018; Khan 2019; Jabbar et al., 2020).

Mitochondrial DNA (mtDNA) has become an important genetic marker for the study of nematode molecular phylogeography due to its matrilineal inheritance, fast evolutionary rate, and lack of recombination (Derycke et al., 2005; Saccone et al., 2000).Recent studies in nematology have employed mitochondrial cytochrome oxidase subunit I (COI) as a molecular marker to analyze the intra specific genetic structure of closely related *Xiphinema* species (Gutierrez-Gutierrez et al., 2011).Sun et al. (2005) and Deng et al. (2016) used barcoding techniques of mitochondrial COII-LrRNA gene fragments to analyze the differences between *Meloidogyne* spp. and *Rotylenchulusreniformis*populations,and found that *M. incongnita* , *M. javanica* ,*M. arenaria* and *R. reniformis* could be identified by mtDNA-PCR. Rashidifard (2019) studied the molecular characteristics of 37 *Meloidogyne* populations from four provinces in South Africa and showed that *COII-16S* could accurately identify different*M* . *enterolobii* populations. Additionally, the characterization of *COII* and *16S* rRNA has been proved to be useful for the identification of different *Meloidogyne* species from different geographical regions of the world (OnkendiandMoleleki 2013). Janssen et al. (2016) proved that analysis based on mitochondrial haplotypes can reveal the evolution and genetic variation of root nematodes, and pointed out that the barcode region Nad5 can reliably identify the major lineages of tropical root-knot nematodes.Based on the mtCOI gene, four main plant-parasitic *Aphelenchoides* species were successfully diagnosed, and the multiple origins of the parasitic genus were proved (Sánchez-Monge et al. 2017).Ye et al. (2007) constructed 19*Bursaphelenchus* spp. phylogenetic trees using sequences including COI genes and analysed the phylogenetic relationships among species in this genus.Genetic diversity of the root-knot nematode *M. enterolobii* in mulberry has been analyzed by Shao et al. (2020) and observed that the high level of gene flow, a high genetic variation and a small genetic distance among *M. enterolobii* populations. A comprehensive phylogenetic analysis of several hundred COI and ITS rRNA gene sequences from the *Heterodera avenae* group showed that COI haplotypes corresponded to certain pathotypes of cereal cyst nematodes. Therefore, compared to other molecular markers,mitochondrial DNA markers emerged as a valuable tool in the study of genetic diversity, population differentiation, and evolutionary relationship between closely related nematode species.

To date, there are many studies focused on the genetic diversity of RKN populations on different crops, but no work has been done on the genetic diversity of *M. graminicola* in China. Therefore, the genetic diversity, genetic differentiation and origin of *M. graminicola*collected from10provinces of China based on the *COI* gene were analysed. It will provide a theoretical basis to reveal the historical dynamics of *M. graminicola* populations and develop efficient management strategies in China.

MATERIALS AND METHODS

Nematode collection: The nematode samples used in this study were randomly collected from the main rice areas in Guangdong, Guangxi, Fujian, Hainan, Anhui, Jiangsu, Henan, Sichuan, Jiangxi, and Hunan Provinces, China (Table 1). One sample was collected in each site and 4-9 different geographical samples were collected in each province. Single nematode at the second stage (J2) was picked separately from single root-knot after hatching from eggs. Each site was replicated 3 times. All three J2s in each site were defined as a population.

DNA extraction, PCR amplification, and sequencing: DNA extraction was described as Liao et al. (2001). The species identities were determined by ribosomal DNA sequencing of the ITS region. The universal primers for root knot nematode identification were 26S (TTTCACTCGCCGTTACTAAGG) and V5367(TTGATTACGTCCCTGCCTTT) as described in Vrain et al. (1992). Single J2 of *M. graminicola* was cut into two pieces with a scalpel under a dissecting microscope and placed into 8μL of worm lysis

buffer (WLB) solution containing1μL protein K (20 mg/mL) in a PCR tube (Zhuo et al., 2008). The PCR tube was incubated for 30 min at 65, then 15 min at 95. The final suspension was used as a DNA template for PCR amplification (Zhang et al. 2001).

The primer pairs COI-F (5'-ATCAGGAGTGAGATCTATTTCTAG-3') and COI-R (5'-CGAGGTTGCCCTTGTCCAAA-3') which designed using Primer 5 based on the 1-1500bp sequence of the accession number KJ139963 were used for the amplification of the *mtCOI* gene region. The 25μL PCR mixture contained 12.5 μL 2× PCR buffer for KOD FX (Toyobo Life Science Co., Ltd.), 5 μL 2 mM dNTPs, 1 μL of each primer (10 μM), 2μL (20ng) DNA, and 4μL distilled water. The PCR amplification was carried out in a lab cycler (Applied Biosystems) as described in Shao et al. (2020).

All PCR products were separated by electrophoresis on a 1% TBE agarose gel and purified by Tiangen Gel Extraction Kit (Tiangen Biotech Co., Ltd.), then cloned into the $p^{MD19\text{-}T}$ Vector (Takara Bio Inc.), and transformed into DH5 alpha Competent Cells. The amplified products were sequenced (BGI Genomics, BGI-Shenzhen) and the haplotypes were calculated using DNASP 5.0 (Librado and Rozas, 2009). After sequencing, the sequences obtained were submitted to GenBank and get its accession numbers.

Genetic diversity analysis: The original sequence was retrieved and the flanking sequences at both ends were deleted for further data analysis. Haplotypes were analysed using Alignment Transformation EnviRonment (http://sing.ei.uvigo.es/ALTER/). The percentage of variant loci in the sequence, parsimony informative loci, nucleotide diversity index(π), haplotype diversity (Hd), population genetic differentiation index, Fst values, Gst values, and the average number of nucleotide changes (K) of the 54 *M. graminicola*populations were calculated by Tajima (1989) and Fu (1997). Gene flow (Nm) between populations was calculated based on the mitochondrial-specific gene formula Fst=1/(1+2Nm) (Takahata and Palumbi 1985). The gene fragments were tested for neutrality using Tajima's D and Fu's Fs neutrality tests at the population and group levels. Phylogenetic trees were constructed using PhyloSuite software based on a Bayesian approach (GTR model) (Zhang et al., 2019). To study the genetic relationship between haplotypes, Network v.4.6.1 software (www.Fluxusengineering.com) was used to draw the mediation network between haplotypes (Bandelt et al., 1999). The correlation between genetic distance and geographic distance was calculated using SPSS (22.0) based on the mantel test method. Molecular analysis of variation (AMOVA) was performed using Arlequin 3.1 software (Excoffer et al., 1992) to estimate genetic variation among and within populations.

RESULTS

Sequence characteristics and variation study of the mtCOI gene fragment in M. graminicola populations: The COI gene fragments of 54 *M. graminicola* populations isolated in 10 provinces of China were PCR-amplified and sequenced. The sequence fragment lengths of 787 bp were obtained by sequence splicing and multiple sequence alignment. The accession numbers of the generated sequences are shown in Table 1. Thirty-nine polymorphic loci were found (4.9% of the total number of bases examine), with 12 S-singleton sites and 27 parsimony-informative sites, accounting for 30.8% and 69.2% of the total polymorphisms, respectively. The S-singleton sites on the *mtCOI* gene fragment were found at positions of 205, 260, 285, 301, 305, 312, 315, 379, 418, 498, 546and 582, whereas the parsimony-informative sites were found at positions of 51, 114, 182, 188, 195, 266, 289, 330, 333, 353, 381, 414, 441,446, 447, 468, 519, 523, 565, 577, 586, 609, 628, 634, 642, 651 and 681, respectively. The contents of a, t, c, and g were 28.1%, 46.3%, 7.4%, and 18.2%, respectively, and the content of a+t was 74.4%, indicating a significant a/t bias. The conversion/transversion rate R was 0.6.

Phylogenetic analysis of M. graminicola populations based on COI genes: Phylogenetic suite software was used to perform Bayesian interference analysis and construct a phylogenetic tree of COI genes in*M. graminicola* populations (Fig.1). *M.enterolobii* strains from NCBI with the same *mtCOI* gene were selected as out groups for the phylogenetic tree. Fifty-four populations of *M. graminicola* were divided into three clades; the population from Henan province, Jiangsu and Anhui Province in Yangtze valley (YP) were grouped in Cluster 1; Cluster 2 comprises the populations from central region of China (CR) (Hunan, Sichuan, Jiangxi provinces) and eight French strains of *M. graminicola* selected from NCBI, Cluster 3 includes all populations in southern China (SC) (Guangdong, Guangxi, Fujian and Hainan provinces).

Nucleotide and haplotype diversity analysis of M. graminicola populations: The haplotype diversity (Hd) and nucleotide diversity (π) of *mtCOI* genes in *M. graminicola* populations (Table 2) showed that the Hd was 0.646, indicating that the total population was higher in haplotypes. The nucleotide diversity (π) and nucleotide mean difference number (k) were 0.00682 and 5.370, respectively. The Tajima's D (-1.252) and Fu's Fs values (-3.06764) of the total population were less than zero (0), which indicated that the entire population conformedto the law of neutrality.

Among the *mtCOI* gene sequences of the three groups, the highest point of variation (31) was observed in the YP group (Table 2), and the lowest mutation sites were observed in the CR group. The haplotype diversity (Hd) of the three groups ranged from 0.143 to 0.772, while the lowest (0.143) and the highest (0.804) Hd value was in CR group and YP group, respectively. The π values of the three groups ranged from 0.00018-0.01127, the smallest π value was detected in the CR groups (0.00018) and the largest π value was detected in the YP group (0.01127). The highest mean number of nucleotide differences (k) among groups was found in the YP group whilethe lowest was found in the CR group. The neutral test results for the three groups were shown to be less than zero (0) for Tajima's D and Fu's Fs values and were not significantly different.

Haplotype frequency analysis of M. graminicola: There are 15 different haplotypes (Hap 1-15) that have been discovered in 54*M. graminicola* populations (Table 3). Hap8 appeared significantly more frequent among all individuals tested, accounting for 59.3% (32/54). Hap10 was detected in four populations, accounting for 7.4% (4/54). Hap1, Hap6, Hap7, Hap12, and Hap15 accounted for 3.7% (2/54) of the populations examined. Among these 15 haplotypes identified, 8 haplotypes occurred only once and were found to be endemic in *M. graminicola* populations. The haplotype results (Fig. 2) of the clusters showed that only one haplotype (Hap8) was shared among the three clusters. Nine haplotypes species were in the YP group, of which were identified as endemic haplotypes and the highest haplotype frequency was identified (60%). There were five haplotypes in the CR group, with a haplotype frequency of 33.3%. Four haplotype species was found in SC group with the lowest haplotype frequency of 26.7%.

Haplotype mediation network map of M. graminicola populations: The *M. graminicola* populations' *mtCOI* genes were then used to construct the haplotype-mediated network map (Fig.3). The haplotype-mediated network formed a circular topological distribution pattern, indicating that the *M. graminicola*populations have historically undergone expansion. Hap8 occurred most frequently and had the largest area distribution. The haplotype of the three clusters in common atHap8. Therefore, Hap8 may be the original haplotype of *M. graminicola* . However, the haplotypes in Henan province were grouped separately. Hap5 (HENXX) was a transitional haplotype linking the Henan haplotype to other haplotypes. Hence, this network may clarify the evolutionary relationships between each haplotype and the geographical distribution of each group, bolstering the phylogenetic tree.

Genetic differentiation and gene flow analysis of the M. graminicola populations: The genetic differentiation and gene flow of the 54 populations of *M. graminicola* were analyzed based on*mtCOI* gene sequence data (Table 4). The overall genetic differentiation coefficient Gst value, fixation coefficient Fst value, and gene flow Nm value among the populations of *M. graminicola*were 0.431, 0.593, and 0.33, respectively. These suggested that there was a large genetic differentiation (Fst$>$ 0.25) and a low gene exchange (Nm $<$ 1) among the studied populations. The minimum Fst value observed between the population of Hainan (HAN) and Fujian (FUJ)was 0.047, while the highest gene flow was 10.125, which indicated that the gene exchange between these two populations was frequent with low genetic differences. The gene flow between the Guangdong (GUD) population and the Jiangsu (JIS) population was higher (1.75), indicating that there was some gene exchange between them.Similarly, the gene flow between populations of GUD, JIX, ANH, HAN, and JIS were all 1,suggesting that there was low gene exchange between these populations.

Genetic and geographic distance analysis of M. graminicola populations: Using the Kimura2-Parameter model, the genetic distances between different *M. Graminicola* populations were calculated based on *mtCOI* gene sequences (Table 5). Results showed that the genetic distances between different populations varied from0 to0.013. Among them, the lowest genetic distances (0.000) was observed between the populations of

HAI and SIC, FUJ; GUD and JIX, ANH, JIS; JIX and ANH, JIS; ANH and JIS; SIC and FUJ, while the highest genetic distance (0.013) was observed between Henan, GUX, and Hunan.

Based on *mtCOI* gene, the relationship between genetic distance and geographic distance of different *M. graminicola* populations were examined using SPSS software (Fig.4). The findings revealed that among the collected samples, there was no significant correlation between genetic distance and the natural logarithm (LN km) matrix of geographical distance (Table 5) ($R<0.2$), $P>0.05$), suggesting that geographic distance was not the major factor contributing to *M. graminicola* populations differentiation.

Analysis of molecular variance (AMOVA) of the *M.graminicola* groups: This analysis results of AMOVA based on different *M. graminicola* populations were shown in Table 6. The genetic differentiation (F_{ST}) among different populations was low (F_{ST}= 0.17847 $P < 0.0001$), 96.3% of total variation was mainly occurred within populations whereas only 3.7% of the total variation was within groups. These findings suggested that the *M. graminicola* populations' genetic variation was basically from within the population.

CONCLUSION AND DISCUSSION

In this study, we examined the genetic diversity, population structure, and relationship between geographic distribution and genetic distance of the *M. graminicola* population using the *mtDNA* gene. The genetic diversity of the *M. graminicola* population was first studied in this study using mitochondrial genes in China. High level of genetic variation (Fst = 0.593) and little gene exchange (Nm = 0.333) were observed in *M. graminicola* populations. In addition, the overall genetic variation in *M. graminicola* may primarily due to variation within geographic groups,and no substantial relationship between genetic distance and geographic distribution was observed. This study further enriched the phylogenetic information of *M. graminicola* and provided the basic evidence for the inherent genetic factors of damage from *M. graminicola* .

Genetic diversity is not only the basis of biodiversity, but also the driving force of species evolution. The reduction or loss of genetic diversity poses a huge threat to populations or species living in a constantly changing environment (Hedrén 2004). Haplotype polymorphisms (Hd) and nucleotide polymorphisms (π) are commonly used to measure the genetic diversity of species or populations (Hao et al., 2014). The 54 nematode populations used in this study had a total of 15 haplotypes with a Hd of 0.646, indicating high haplotype diversity in *M. graminicola* populations in China. However, the total nucleotide diversity was very low (π=0.00682). The high haplotype diversity and low nucleotide diversity implied that *M. graminicola* populations had a bottleneck, followed by rapid population growth. The high haplotype diversity observed among these populations, with substantial nucleotide similarity might have resulted from the accumulation of mutations (Shao et al., 2020). As many invertebrates with large maternal active populations and robust reproductive capacities are known for having high haplotype diversity and low nucleotide diversity (Grantand Bowen 1998; Lavery et al., 2008). According to Tajima's D and Fu's FS analyses, all*M. graminicola* populations in the present research might have experienced population expansion during evolution.

The genetic differentiation index (Fst) and gene flow (Nm) are two important indicators that reflect genetic differences and gene exchange among populations (Rousset 1997). The coefficient of genetic differentiation (Fst) is usually used to measure the degree of differentiation between different populations. In the range of 0 to 1, the larger the Fst value, the higher the degree of differentiation between populations (Wright, 1978). Based on the present research (Table 3), the Fst values (0.593) among all *M. graminicola* populations were greater than 0.25, which showed that there was a high genetic differentiation among all populations. The gene flow values (0.33) were all less than 1, and gene communication between populations was blocked, which may be because these groups are relatively stable in space and time, resulting in less gene exchange among them. On the other hand, over time, the accumulation of mutations may lead to high levels of genetic differentiation among populations, which may be related to the isolation of geographical distance and the weaker migration ability and slower migration speed of nematode populations.

Deng et al. (2016) analyzed the genetic diversity of *R.reniformis*in China based on the sequence of *COII* gene and found a high variation among different *R. reniformis* populations based on the*COII-LrRNA* sequence, which helps them to adapt to changes in the environment. The result is consistent with the results of our

study. Wang (2015) investigated the genetic structure of *H. glycines* in China using *COI* genes. The Fst value and Nm value of the*H.glycines* population was 0.27442 and 1.322, respectively. The results indicate some genetic differentiation between populations in general, but also a high level of gene exchange. This finding differs from our observation in this study, and it might be due to the different nematodes species. The Fst value (0.0169) and a high level of gene flow (7.02) were detected among the 19 *M. enterolobii* populations, and high genetic variation within each population and a small genetic distance among populations were observedbased on the diversity analysis of mitochondrial COI gene.These results are totally inconsistent with our study, probably because the time of *M. enterolobii* and*M. graminicola* in China is different, and the mode of transmission may be different, though both are root knot nematodes.

The phylogenetic results revealed that the *M. graminicola*populations in China were divided into three groups: Cluster1 SC, Cluster2 YP and Cluster3 CR (Fig. 1). The *M. graminicola* strains of French selected from NCBI were not clustered separately, but with Cluster 2 and Cluster 3 of *M. graminicola* from China, respectively. It showed that there were relatively few genetic differences between the *M. graminicola* populations in France and the Chinese populations, and there were no genetic differences due to geographic differences. It was also possible that the Chinese *M. graminicola* populations had the same origin as the *M. graminicola* populations from French.Additionally, the haplotype analysis indicated that the Hap8 was shared with all *M. Graminicola* groups in China and that the other haplotypes were evolved from Hap8. Furthermore, the high genetic variation and low gene exchange among the populations as well as the absence of a relationship between haplotype and geographic region, further supported the hypothesis that the different *M. graminicola* populations isolated from China originated from different places (Yu 2009). The Hap8 was splited into three major groups, with no clustering among haplotypes from the same geographical group. These findings added to the evidence that there was litter genetic flow among *M. graminicola* populations, resulting in high genetic diversity. The haplotypes of Henan Province were separated from other haplotypes by a significant genetic distance. The genetic distance between them varied significantly, which might be related to the rotation of rice with wheat.*M. graminicola* was firstly spread from the main rice-producing areas to the wheat and rice rotation areas in 2020 (Liu et al., 2021) and wheat was often planted in Henan Province. The population in Henan Province is the northernmost population in China and the different climate, planting mode and host may affect the infection and development of *M. graminicola* , and then contribute different genetic variation in this population.

In the present research, no significant correlation ship was observed between genetic distance and geographic distance (Fig 4). This might be a result of the effects of natural irrigation and long-range seed transporting. Based on the Mitochondrial *COI* gene, Shao et al. (2020) analysed the genetic diversity of the *M. enterolobii*populations in China. The findings also reveal that the genetic distance of *M. enterolobii* populations does not match their geographical distance.Similarly, the genetic differentiation of *H. schachtii*was found to be less influenced by geographical distance when studying the population genetic structure of the sugar beet cyst nematode in French (Plantard and Porte , 2003).Therefore, we hypothesize that the J2 of *M. graminicola* would passively be transported to a long distance as a result of human agricultural operations or natural factors such as wind, rain, and water. Then it would affect the genetic structure of *M. graminicola* populations in China, resulting in a weak correlation between genetic distance and geographic distance.

The genetic diversity of the mitochondrial genes of *M. graminicola* populations was reported for the first time in China. The genetic diversity of *M. graminicola* populations in China were found to be small and gene exchange were hindered among them. The present study provided a theoretical basis for the management of the*M. graminicola* and wouldbe helpful in increasing the production of rice in the future.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The assembled gene sequences of *Meloidogyne graminicola* are available in NCBI (MZ522726- MZ522779). The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

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Figure legends

Fig. 1 The phylogenetic tree of *M. graminicola* population in China based on the COI gene. The *mtCOI* sequences were imported into MAFFT for multiple sequence matching, and then the compared sequences were imported into Gblock of PhyloSuite software for trimming, and the optimized data were used for optimal model selection by ModelFinder software. Bayesian inference with GTR+F+G4 model for mtCOIsequence was conducted in MrBayes 3.2.6 plugin in PhyloSuite.

Fig.2 Histogram of haplotype frequencies in each *M. graminicola*groups.

Fig. 3Median-Joining haplotype network of 54 *M. graminicola*populations based on *mtCOI* gene using NETWORK software 5.0. Each numbered circle (Hap1-Hap15) represents an unique haplotype, and the size of the circle is proportional to the overall frequency of each haplotype in the entire sample of the species. Each line connecting the haplotypes refers to a mutational step. Marks on the lines indicate the number of steps. Colors correspond to different sampling locations and names are given in abbreviations by initials of the group.

Fig 4 Correlation between genetic distance and geographical distance in the *M. graminicola* populations based on *mtCOII*gene.Genetic distance and geographical distance of the populations are listed in Appendix 1 and Appendix 2, respectively.

Table 1*Meloidogyne graminicola* samples obtained from*Oryzasativa* growing areas of China

Group	Province	Location	Population	Longitude	Latitude	GenBank accession number
YP	Anhui	Bengfu	ANHBF	E117°20’41”	N32°58’13”	MZ522753
		Datong	ANHDT	E117°3’12”	N32°37’54”	MZ522754
		Fengyang	ANHFY	E117°31’54”	N32°52’29”	MZ522752
		Huainan	ANHHN	E117°02’54”	N32°64’59”	MZ522750
		Huaiyuan	ANHHY	E117°20’50”	N32°97’07”	MZ522751
		Wuhu	ANHWH	E118°37’64”	N31°32’63”	MZ522749
SC	Fujian	Datian	FUJDT	E117°84’71”	N25°69’26”	MZ522762
		Fuzhou	FUJFZ	E119°30’62”	N26°07’53”	MZ522760
		Longyan	FUJLY	E117°02’78”	N25°09’16”	MZ522764
		Jiangle	FUJJL	E117°23’56”	N26°30’54”	MZ522761
		Xiamen	FUJXM	E118°11’02”	N24°49’04”	MZ522765

Group	Province	Location	Population	Longitude	Latitude	GenBank accession number
		Zhangzhou	FUJZZ	E117°66'18"	N24°51'08"	MZ522763
SC	Guangdong	Maoming	GUDMM	E110°91'92"	N21°65'97"	MZ522744
		Qingyuan	GUDQY	E113°03'67"	N23°70'41"	MZ522743
		Shaoguang	GUDSG	E113°59'15"	N24°80'13"	MZ522741
		Yangjiang	GUDYJ	E111°97'51"	N21°85'92"	MZ522742
SC	Guangxi	Guigan	GUXGG	E109°60'21"	N23°11'58"	MZ522772
		Nanning	GUXNL	E108°32'04"	N22°82'40"	MZ522770
		Qinzhou	GUXQZ	E108°62'41"	N21°96'71"	MZ522773
		Yulin	GUXYL	E110°15'43"	N22°63'13"	MZ522771
		Wuzhou	GUXWZ	E11°31'62"	N23°47'23"	MZ522774
SC	Hainan	Haikou	HANHK	E110°38'39"	N20°02'45"	MZ522736
		Ledong	HANLD	E109°17'	N 18°73'	MZ522739
		Qionghai	HANQH	E110°46'67"	N19°24'60"	MZ522738
		Sanya	HANSY	E109°50'82"	N18°24'78"	MZ522740
		Wenchang	HANWC	E110°75'4"	N19°61'23"	MZ522735
		Zhangzhou	HANZZ	E109°57'67"	N19°51'74"	MZ522737
YP	Henan	Dengzhou	HENDZ	E112°08'96"	N32°68'57"	MZ522734
		Jiaozuo	HENJZ	E113°23'82"	N35°23'90"	MZ522733
		Kaifeng	HENKF	E114°34'14"	N34°79'70"	MZ522732
		Nanyang	HENNY	E112°54'09"	N32°99'90"	MZ522727
		Pingdingshan	HENPD	E113°30'77"	N33°73'52"	MZ522731
		Puyang	HENPY	E115°04'12"	N35°76'82"	MZ522729
		Xinxiang	HENXX	E113°88'39"	N35°30'26"	MZ522730
		Xinyang	HENXY	E114°07'50"	N32°12'32"	MZ522726
		Zhuaadian	HENZM	E114°02'47"	N32°98'11"	MZ522728
CR	Hunan	Changde	HUNCD	E111°69'13"	N29°04'22"	MZ522775
		Loudi	HUNLD	E112°00'84"	N27°72'81"	MZ522779
		Shaoyang	HUNSY	E111°46'92"	N27°23'78"	MZ522777
		Xiangtan	HUNXT	E112°92'50"	N27°84'67"	MZ522778
		Yiyang	HUNYY	E112°35'50"	N28°57'66"	MZ522776
YP	Jiangsu	Donghai	JISDH	E118°23'23"	N34°11'45"	MZ522768
		Nanjing	JISNJ	E118°52'18"	N32°2'21"	MZ522766
		Xinhua	JISXH	E119°85'23"	N32°90'94"	MZ522769
		Xuzhou	JISXZ	E117°16'58"	N34°15'49"	MZ522767
CR	Jiangxi	Nanchang	JIXNC	E115°94'39"	N28°54'54"	MZ522747
		Ningdu	JIXND	E115°40'18"	N26°05'48"	MZ522746
		Shangrao	JIXSR	E117°97'11"	N28°44'42"	MZ522745
		WanNian	JIXWN	E117°06'88"	N28°69'53"	MZ522748
CR	Sichuan	Chendu	SICCD	E 104°06'	N 30°67'	MZ522754
		Dazhou	SICDZ	E107°50'22"	N31°20'94"	MZ522759
		Leshan	SICLS	E103°76'12"	N29°58'20"	MZ522758
		Yibing	SICYB	E104°63'08"	N28°76'01"	MZ522757
		Nanchong	SICNC	E106°08'29"	N30°79'52"	MZ522756

Note: The name of each provincial is indicated in three abbreviated capital letters. Anhui: ANH; Fujian: FUG; Guangdong: GUD; Guangxi: GUX; Hainan: HAN; Henan: HEN; Hunan: HUN; Jiangsu: JIS; Jiangxi: JIX; Sichuan: SIC;

Table 2 Genetic diversity and neutrality test of mtDNA COI gene among*Meloidogyne graminicola* groups

Group	Number of mutations	Haplotype, diversity (Hd)	Nucleotide, diversity (π)	Average number of nucleotide differences(K)	Tajima's, D	Fu's, Fs
SC	7	0.705	0.00150	1.181	-1.274	-2.828
YP	31	0.772	0.01127	9.813	-0.42264	-3.105
CR	1	0.143	0.00018	0.143	-1.1552	-0.595

Table 3 Haplotypes identified in *Meloidogyne graminicola*populations based on *mtCOI* gene

Haplotype	N	Geographic distribution
Hap1	2	HENNY, HENXY
Hap2	1	HENZM
Hap3	1	HENPY
Hap4	1	HENXX
Hap5	1	HENPD
Hap6	2	ANHFY, HENKF
Hap7	2	HENDZ, HENJZ
Hap8	32	ANHDT, ANHHN, ANHHY, ANHWH, HANZZ, GDMM, GDSG, GUDQY, GUDYJ, FUJDT, FUJFZ, F
Hap9	1	HUNYY
Hap10	4	GUXGG, GUXNL, GUXQZ, GUXYL
Hap11	1	GUXWZ
Hap12	2	FUJJL, FUJLY
Hap13	1	HUNSY
Hap14	1	HANQH
Hap15	2	HUNLD, HANYY

Table 4 Fst and Nm among populations of *Meloidogyne graminicola*based on COI gene

	HEN	HAN	GUD	JIX	ANH	SIC	FUJ	JIS	GUX	HUN
HEN		0.329	0.491	0.518	0.518	0.506	0.529	0.511	0.527	0.527
HAN	1.021		0.173	0.352	0.352	0.000	0.047	0.332	0.428	0.430
GUD	0.519	2.395		0.333	0.333	0.511	0.778	0.222	0.546	0.541
JIX	0.465	0.920	1.000		0.000	0.800	1.000	0.000	0.625	0.611
ANH	0.465	0.920	1.000	0.000		0.800	1.00	0.000	0.625	0.611
SIC	0.487	0.000	0.478	0.125	0.125		0.000	0.711	0.722	0.711
FUJ	0.446	10.125	0.143	0.000	0.000	0.000		0.889	0.833	0.816
JIS	0.479	1.006	1.750	0.000	0.000	0.203	0.062		0.541	0.537
GUX	0.449	0.669	0.400	0.300	0.300	0.192	0.100	0.425		0.618
HUN	0.449	0.662	0.408	0.318	0.318	0.204	0.113	0.432	0.310	

*The upper triangle is the Fst value and the lower triangle is the value of Nm. Fst was calculated using DNAsp5.0. Nm was calculated by Fst=1/(1+2Nm)

Table5 Natural logarithm of the geographic distances and genetic distance between different *Meloidogyne graminicola* populations based on *mtCOI* gene

	HEN	HAN	GUD	JIX	ANH	SIC	FUJ	JIS	GUX	HUN
HEN		0.007	0.011	0.012	0.012	0.012	0.012	0.012	0.013	0.013
HAN	7.447		0.001	0.002	0.002	0.000	0.000	0.002	0.003	0.003
GUD	7.198	7.174		0.000	0.000	0.001	0.001	0.000	0.001	0.002
JIX	6.567	7.035	6.509		0.000	0.002	0.002	0.000	0.001	0.001
ANH	6.032	7.318	6.984	5.956		0.002	0.002	0.000	0.001	0.001
SIC	6.917	7.180	7.110	7.047	7.138		0.000	0.002	0.003	0.003
FUJ	6.986	7.059	6.546	6.101	6.497	7.459		0.002	0.004	0.004
JIS	6.319	7.374	7.023	6.150	4.987	7.245	6.461		0.010	0.010
GUX	7.270	5.900	6.219	6.885	7.265	6.871	7.066	7.321		0.003
HUN	6.623	6.861	6.365	5.601	6.371	6.818	6.505	6.504	6.630	

*The lower triangle is natural logarithm of the geographic distances, and the upper triangle is genetic distance.

Table 6 Analysis of molecular variance (AMOVA) within and among groups of *M. graminicola* based on *mtCOI* gene

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Between groups	2	32.782	0.75671	3.700
Within group	52	203.462	3.48326	96.300
Total variation	54	236.244	4.23997	100.000

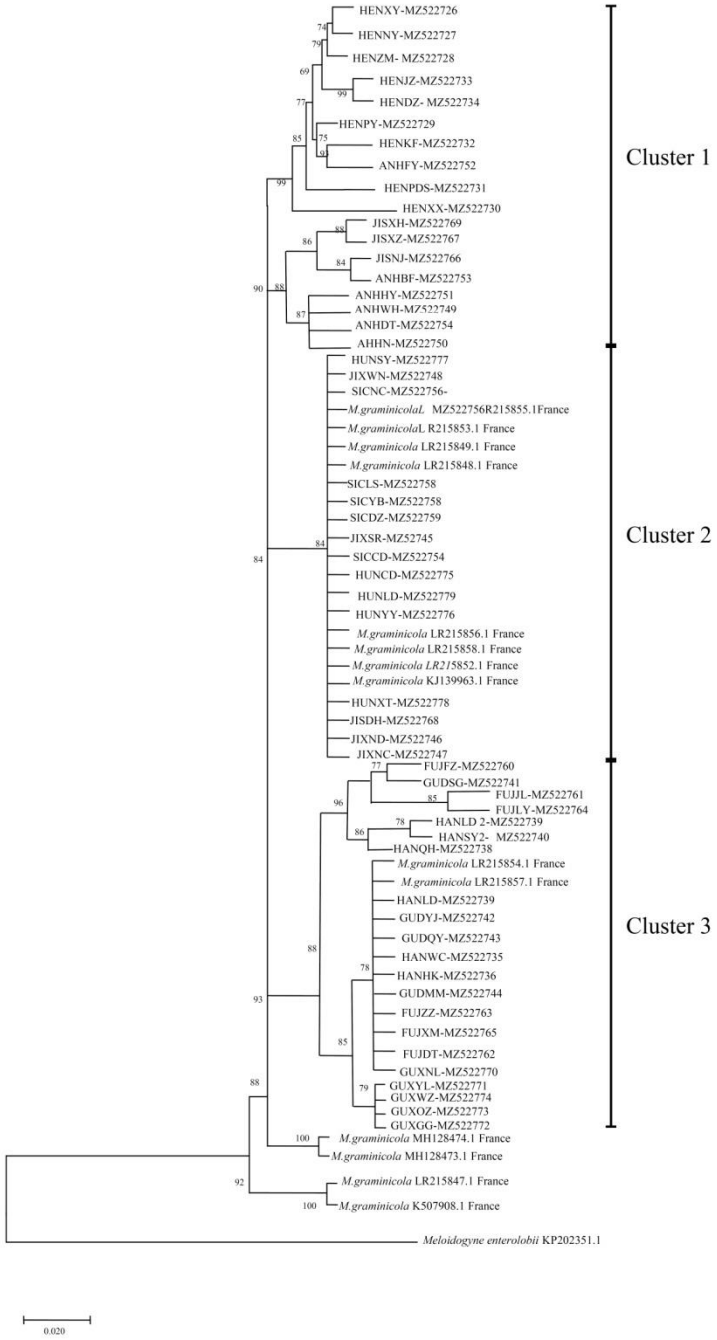

Fig.1 The phylogenetic tree of *M. Graminicola* population from China and France of NCBI based on the COI gene constructed. The *mtCOI* sequences were imported into MAFFT for multiple sequence matching, and then the compared sequences were imported into Gblock of PhyloSuite software for trimming, and the optimized data were used for optimal model selection by ModelFinder software Bayesian. Inference with GTR+F+G4 model for mtCOI sequence was conducted in MrBayes 3.2.6 plugin in PhyloSuite. The scale is 0.020.

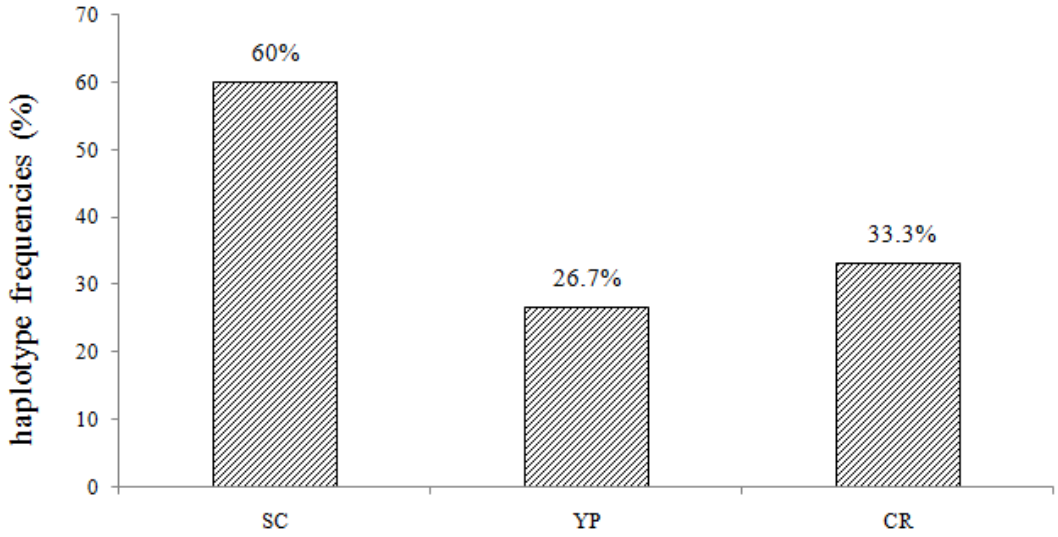

Fig.2 Histogram of haplotype frequencies in each *M. Graminicola* groups

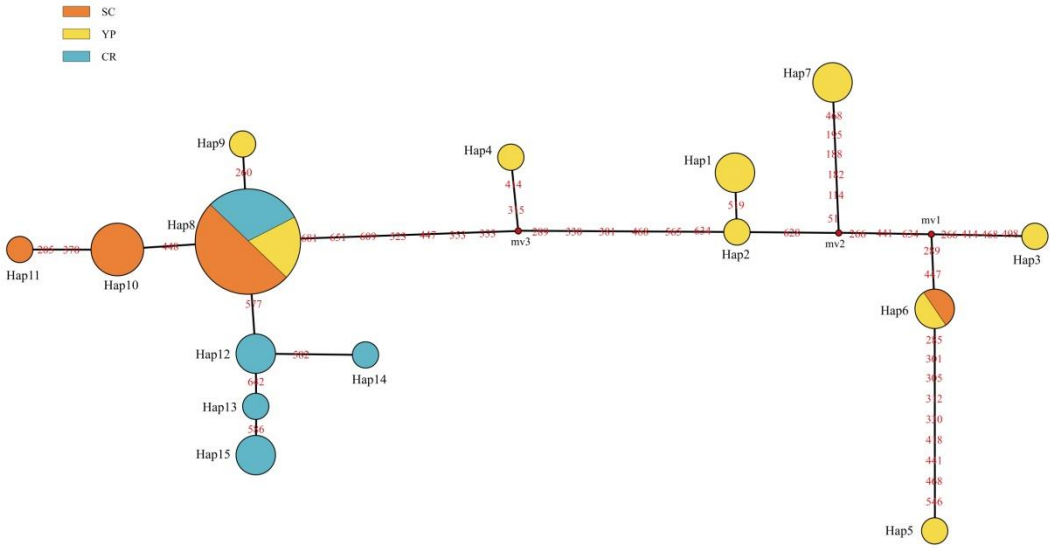

Fig.3 Median-Joining haplotype network of 54*M. graminicola* populations based on *mtCOI* gene using NETWORK 5.0 soft. Each numbered circle (Hap1-Hap15) represents a unique haplotype, and the size of the circle is proportional to the overall frequency of each haplotype in the entire sample of the species. Each line connecting the haplotypes refers to a mutational step. Marks on the lines indicate the number of steps. Colors correspond to different sampling locations and names are given in abbreviationsby initials of the group.

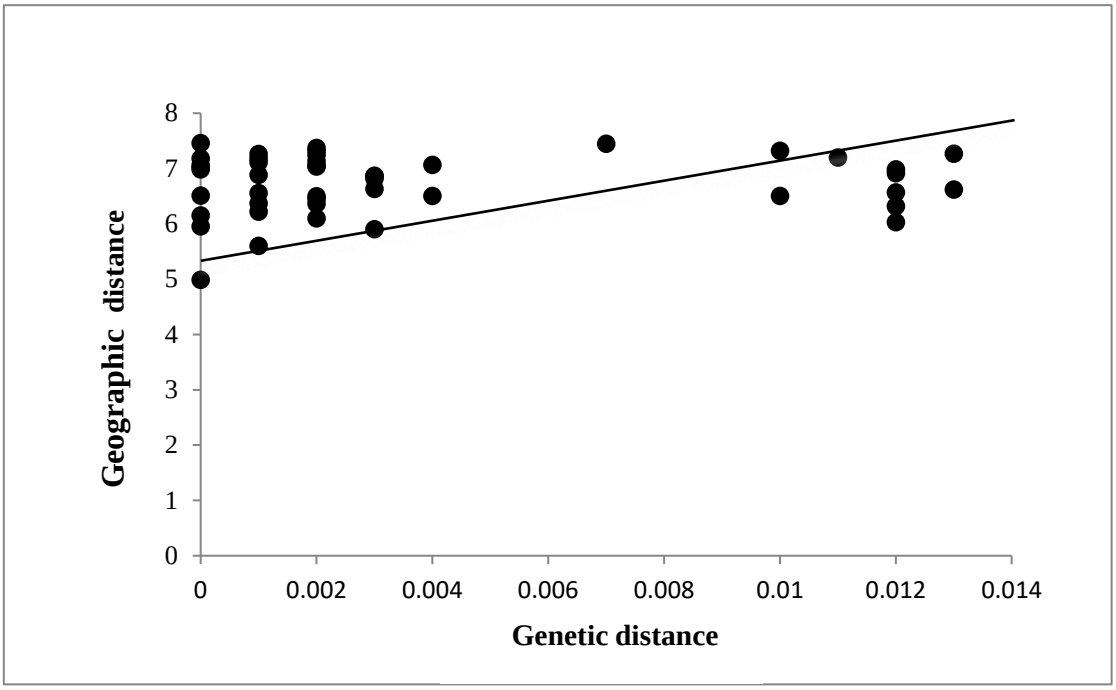

Fig.4 Correlation between genetic distanceand geographical distance in the *M. graminicola* populations based on *mtCOII* gene using SPSS soft. Genetic distance and geographical distance of the populations are in Appendix 1 and Appendix 2, respectively.