Assessment of genetic variability in population of *Ditylenchus destructor* (Thorne 1945) (Tylenchida: Anguinidae) from China

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Accepted for publication 22 November 2009

Summary. *Ditylenchus destructor* is distributed widely in China and causes considerable yield losses of potatoes. Genetic diversity and variation of this species was analysed using Inter-Simple Sequence Repeats (ISSR) markers. Second-stage juveniles from 16 populations were analysed with three primer pairs and 32 fragments were considered for the analysis. Diversity levels within populations were relatively high. Cluster analysis and principle coordinate analysis grouped the majority of the nematode populations into three main clusters. At the regional level, the AMOVA indicated that about 91.4% variations in the data set were from genotypic variations within populations, 5.0% variations due to regional differences, and the remaining 3.6% due to differences among populations within regions. Low levels of genetic variation among populations suggested an extensive gene flow among them. Amphimictic mode in natural populations and passive dispersal of nematodes by anthropogenic activities and natural means would probably be responsible for the results observed. It was shown that the ISSR marker was an efficient method for detecting the genetic structure of *D. destructor* populations at a macro geographical level..

Key words: *Ditylenchus destructor*, genetic diversity, genetic variation, ISSR.

The nematode *Ditylenchus destructor* (Thorne, 1945) is an obligatory endoparasite species, able to grow in over 120 plant species and is the main pathogen affecting production of potato and sweet potato in most regions of China (Thorne, 1945; Yao & Cui, 2001). It belongs to the list of A2 pests regulated as quarantine pests in APPPC (Asia and Pacific Plant Protection Commission), COSAVE (Comite Regional de Sanidad Vegetal Parael Cono Sur) and China (Thorne, 1945; Hooper, 1973; OEPP/EPPO, 1978; Gubina, 1982; Esser, 1985). This species was first recorded in North America and has spread to about 52 countries of America, Europe, Asia, Africa and Oceania (Yao & Cui, 2001). *Ditylenchus destructor* was first detected in China infecting sweet potatoes in North China in 1937 (Ding & Lin, 1982). It was observed on other crops i.e. potato, pea, peanut (Liu et al. 2006), as well as on medicinal materials of the genera *Angelica* L. and *Mentha* L. *etc*. (Chen & Zhen, 1988; Zhang & Zhang, 2007). Now this nematode species is widely distributed in most sweet potato growing areas of China and causes considerable yield losses.

Despite their importance in ecosystems and for agriculture, the genetic structure of *D. destructor* populations is poorly known. Data on gene flow and the genetic structure of natural populations are scarce even for pests of the *Ditylenchus* genus. *Ditylenchus destructor* exhibits particular variability in many aspects. Populations from different hosts have shown different pathogenicity to the same or several other hosts (Ding & Lin, 1982; De Waele, 1989). The isolates of *D. destructor* from *Hyacinthus orientalis* could not infect sweet potato successfully. However, it cannot be classified as a different physiological race as originally was *D. dipsacii*. Variations in susceptibility of different populations to different types of nematicides have also been detected (Ding, 2007). Insecticide resistance level of Funing, Zuozhou and Lulong populations to aldicarb emulsifiable concentrate was twice to four times than that of the Changli population in Hebei province of China. Furthermore, genetic variability has been demonstrated using the D2D3 region of the ribosomal DNA (rDNA-D2D3); there are more than

10 bases that differ among 22 populations (Yu, 2008). High levels of variation were also detected by analysis of the internal transcribed spacer region of the ribosomal DNA (rDNA-ITS) (Wang *et al*., 2007; Wan *et al*., 2008; Zhang & Zhang, 2008). The sequence length of rDNA-ITS region was about 900 bp in A type populations but about 1100 bp in B type. Based on the variation of rDNA-ITS regions, several molecular detection methods were developed to identify two different types of this species (Liu *et al*., 2007, Wan *et al*., 2008).

The degree of genetic differentiation among local populations of a species largely depends on the magnitude of gene flow and other processes such as mutation, genetic drift, and locally differing selection pressure occurring independently in each subpopulation (Lax *et al*., 2007). The use of population genetics to estimate dispersal provides additional information concerning the inbreeding, the genetic structure and the level of gene flow at various scales (Picard *et al.*, 2004). Thorough knowledge of the population structure of plant-parasitic nematodes is essential to develop efficient control strategies (Hyman, 1996). For pest species, measurement of dispersal ability is of crucial importance to formulating control methods to limit the economic losses in agriculture (Lenormand & Raymond, 1998; Carrière *et al*., 2003). The Inter-Simple Sequence Repeats (ISSR) PCR technique has been developed to study genetic diversity in natural populations (Zietkiewicz *et al*., 1994; Metge & Burgermeister, 2006; Lax *et al*., 2007). This technique is rapid as well as sensitive, and capable of differentiating between closely related individuals. ISSRs take advantage of simple sequence repeats (SSR) or microsatellites, which are abundant in all eukaryotic genomes. Unlike SSR markers, the ISSRs do not require any prior knowledge of the genome sequence (Zietkiewicz *et al*., 1994). This method is similar to RAPD-PCR and provides similar genomic information. However, ISSR markers may have certain advantages over RAPDs to assess genetic variation within and among population of the same and closely related species (Wolfe & Liston, 1998; Subbotin *et al*. 1999; Crawford *et al*., 2001; Amiri *et al*. 2003; Ou *et al*., 2008). ISSR primers are longer and have higher annealing temperatures, which results in greater band reproducibility than RAPD markers (Culley & Wolfe, 2001); they also reveal high genetic variability. ISSR markers have great potential for studies of natural populations and have been useful in the study of the population structure of many plant-parasitic nematodes, entomo-pathogenic nematodes and some crops with resistance to nematodes (Zietkiewicz *et al*., 1994; Berner & Schnetter, 2002; Metge & Burgermeister, 2006; Lax *et al*., 2007; Dayteg *et al*., 2008).

The objective of this work was to analyse the levels of genetic variability in Chinese populations of *D. destructor* from different geographic regions and to evaluate the degree of genetic difference among them using ISSR molecular markers.

MATERIAL AND METHODS

Nematode populations. Sixteen populations of *D. destructor* from different geographic regions were considered for this study (Table 1, Fig. 1). In autumn 2007, 10 soil samples of *ca* 3 kg each were random taken from an infested sweet potato field at each site and mixed together in a plastic case separately (except for the locality of KOSE, where 10 garlic samples were taken from the infested garlic intercepted by Shenzhen Entry-Exit Inspection and Quarantine Bureau of China). Second-stage juveniles (J2) were extracted from 200 g of soil by the Baermann's method. All populations were identified as *D. destructor*, according to the description of Thorne (1945) and morphologi-cal/morphometrical characterisations of other populations of the species (Thorne 1945; Ding & Lin, 1982). For nematode multiplication under laboratory conditions, 200 J2 of *D. destructor* were sterilized in 0.05% penicillin and 0.5% chloramphenicol solution for 30 min, and then the populations were cultured for 45 d on a colony of *Fusarium semitectum* Brek. & Rav. at 25°C in the dark. Then nematodes were transferred to another colony of *F. semitectum* to maintain populations until DNA extraction was conducted.

DNA Extraction. For each population, genomic DNA was isolated from individual nematode and fifteen individuals were used in total. Each specimen was crushed with a glass pestle inside the tube after it was immersed in liquid nitrogen for 30 s. Then 32 µl Worm Lysis Buffer (WLB, 500 mM KCl, 10 mM Tris-HCl, 15 mM $MgCl_2$, 1.0 mM DTT, 4.5% Tween20) and 8 µl Proteinase K (20 mg ml^{-1}) were added to each tube. The tubes were incubated at 65°C for 2 h, followed by 10 min at 94°C in a Master cycler 5332 PCR thermal sequencer (Eppendorf). DNA extracted from each nematode was kept at -20°C until use.

ISSR-PCR amplification. Several DNA and primer concentrations used in the reaction mix, number of cycles, annealing temperature and evaluation of multiple 2 µl aliquots from a single juvenile DNA extraction were tested to optimise PCR reaction conditions and guarantee the repeatability of the amplified products obtained. A

total number of 35 ISSR primers were tested (Zietkiewicz *et al*., 1994; Tikunov *et al.*, 2003; Bornet & Branchard, 2001); those that produced clear, reproducible and polymorphic bands were selected for the analysis. Each ISSR reaction was carried out in a total volume of 25 µl containing: 2 µl of DNA, 2.5 µl 10×reaction buffer (500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris-Cl, pH 9.0), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 15 ng of primer and 1 U of Taq DNA Polymerase (TaKaRa Biosciences, China). PCR reactions were programmed for an initial denaturation at 94°C for 5 min, followed by 38 cycles of 30 s at 94°C, 45 s at 52°C, 1 min at 72°C, and a final extension of 10 min at 72°C. Negative controls were added in all assays to discount contamination. A positive control containing DNA at a concentration of 10 ng μl^{-1} of an individual of the plant species *Lycopersicon esculentum* Miller (Solanaceae) was used in each reaction. Its amplification products were known and gave high repeatability for the ISSR primers selected (Tikunov *et al*., 2003). This control allowed us to verify the repeatability of the bands obtained and to check that the amplification experiment developed normally.

The PCR products were separated electrophoretically on 2% agrose gels in 1×TAE buffer. DL2000 DNA Ladder (TaKaRa) was used as the molecular weight marker. Gels were stained with ethidium bromide and photographed with Gel Doc XR image analysis system (Bio-Rad).

Data analysis. Fifteen juveniles from each population were used for the analysis. Only bands that were clear and reproducible were included in the study. Amplified bands were scored as 1/0 (presence/absence) of homologous bands for all samples. The resulting presence/ absence data matrix was analyzed using POPGENE version 1.32 (Yeh *et al*. 1999) to estimate the level of genetic diversity by the percentage of polymorphic bands (PPB), average heterozygosity (H) and Shannon Information Index (I). Isolation by distance was investigated to estimate the correlation between geographical and genetic distance of the populations studied using a Mantel's test and a rank correlation coefficient (Mantel, 1967). In order to investigate the partition of genetic variation within and among populations, ARLEQUIN software (version 1.1) (Schneider *et al*., 1997) was used to carry out analysis of molecular

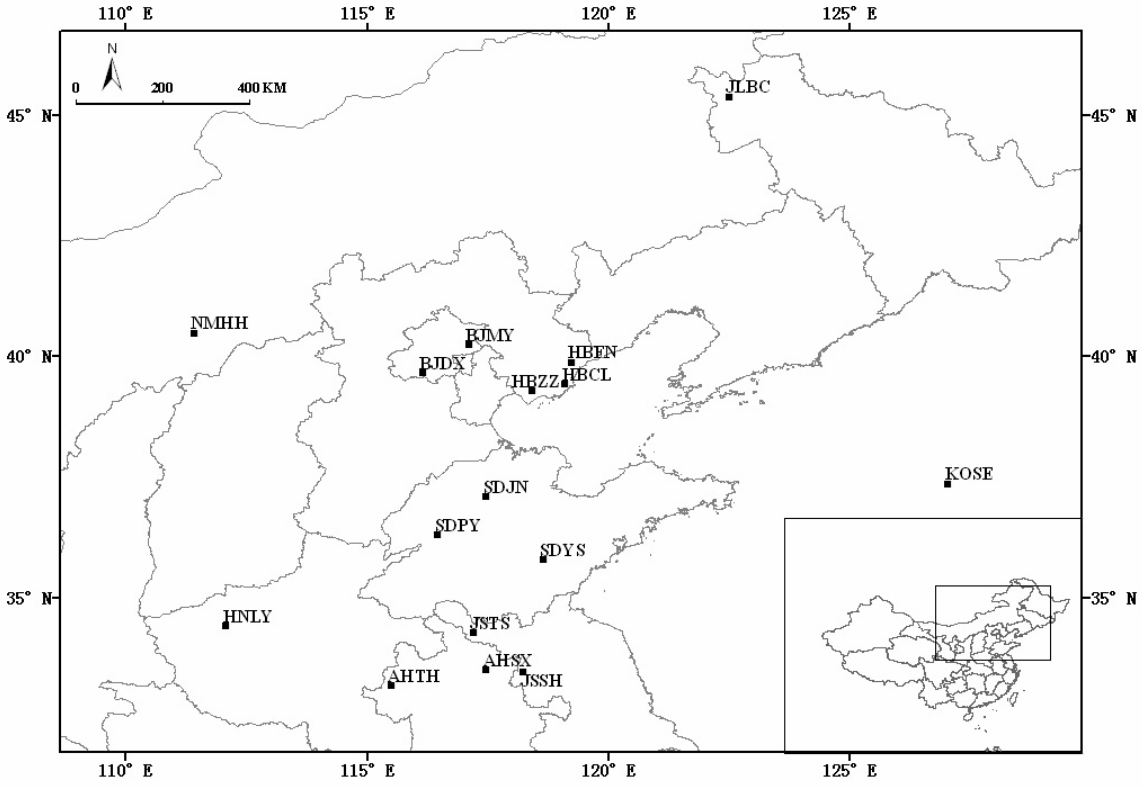

Fig. 1. Geographic location of the 16 *Ditylenchus destructor* populations from China obtained during 2007. For locality code information see Table 1. Altitude, latitude and longitude data of each location were recorded by a GPS locator.

Table 1. Location and genetic diversity of the 16 *Ditylenchus destructor* populations

Code	Province of origin	Locality	Altitude (m)	Longitude (E)	Latitude (N)	PPB (%)	Average heterozygosity (H)	Shannon Information Index (I)
HBZZ	Hebei	Zuozhou	17	118.41	39.28	81.3	0.2603	0.3975
HBCL	Hebei	Changli	32	119.10	39.42	68.8	0.1978	0.3042
HBFN	Hebei	Funing	76	119.22	39.88	68.8	0.2082	0.3130
BJDX	Beijing	Daxing	21	116.15	39.66	71.9	0.2169	0.3380
BJMY	Beijing	Miyun	68	117.10	40.24	75.0	0.2350	0.3620
SDYS	Shandong	Yishui	163	118.64	35.78	78.1	0.2344	0.3601
SDJN	Shandong	Jinan	54	117.46	37.09	75.0	0.2156	0.3384
SDPY	Shandong	Pingyin	212	116.46	36.29	78.1	0.2606	0.3964
JSSH	Jiangshu	Sihong	47	118.23	33.46	75.0	0.2504	0.3823
DDTS	Jiangshu	Tongshan	149	117.2	34.26	78.1	0.2650	0.4019
AHTH	Anhui	Taihe	26	115.49	33.17	78.1	0.2735	0.4123
AHSX	Anhui	Sixian	38	117.46	33.49	78.1	0.2532	0.3865
NMHH	Neimenggu	Huhhot	1075	111.41	40.48	78.1	0.2523	0.3864
JLBC	Jilin	Baicheng	662	122.50	45.38	75.0	0.2335	0.3618
HNLY	Henan	Luoyang	329	112.07	34.40	71.9	0.2227	0.3439
KORE	Korea	Seoul	263	127.03	37.35	71.9	0.2301	0.3512
Average						75.2	0.2381	0.3647
Species						87.5	0.2769	0.4242

variance (AMOVA) with Euclidean distance matrices (φ_{ST}) from ISSR profiles. Pairwise F_{ST} estimates between different populations were also obtained with this program. Unweighted pair group arithmetic average (UPGMA) clustering was performed with the Phylip program, version 3.6, to describe the genetic relationship among the 16 *D. destructor* populations and dendrograms were created (Felsenstein, 2001; Rohlf, 2002).The robustness of the dendrogram was tested by bootstrapping with 1000 permutations. Results of all bands were also pooled into a principle coordinate analysis (PCO) with the Multi-Variate Statistical Package (MVSP) 3.0 (Kovach Computing Services 2005) in the 16 populations. Thus, the genetic variability of sampled populations was summarised into a few major components (e.g. PCO1, PCO2). The PCO on the data was considered as a check of the clusters formed by the cluster analysis.

RESULTS

Genetic diversity. Primers that could not amplify polymorphic bands in all populations or did not amplify polymorphic bands clearly enough were discarded. Sequences of the single non-anchored and two anchored primers selected to amplify ISSR in all individuals are shown in Table 2. High polymorphism and genetic variability was observed using the selected primers (Fig. 2). The number of markers scored per primer ranged between 9 and 13. No population-specific bands were detected. At the species level, the average PPB was 87.5%, heterozygosity (H) was 0.2769 and Shannon information index (I) was 0.4242 (Table 1). Within populations, the PPB varied from 68.8% for population HBCL to 81.3% for population HBZZ, and the mean H was 0.2769, ranging from 0.1978 for population HBCL to 0.2735 for population AHTH. The I value showed similar trends, ranging from 0.3042 for HBCL to 0.4123 for AHTH.

Table 2. Sequence of ISSR primers used for the analysis of *Ditylenchus destructor* populations from China and number of polymorphic bands scored

Primer	Sequence(5'→3')	Number of loci scored
ISSR1	$(AC)_8G$	9
ISSR2	$(GACA)_4$	13
ISSR3	$(AC_{10})AA$	10

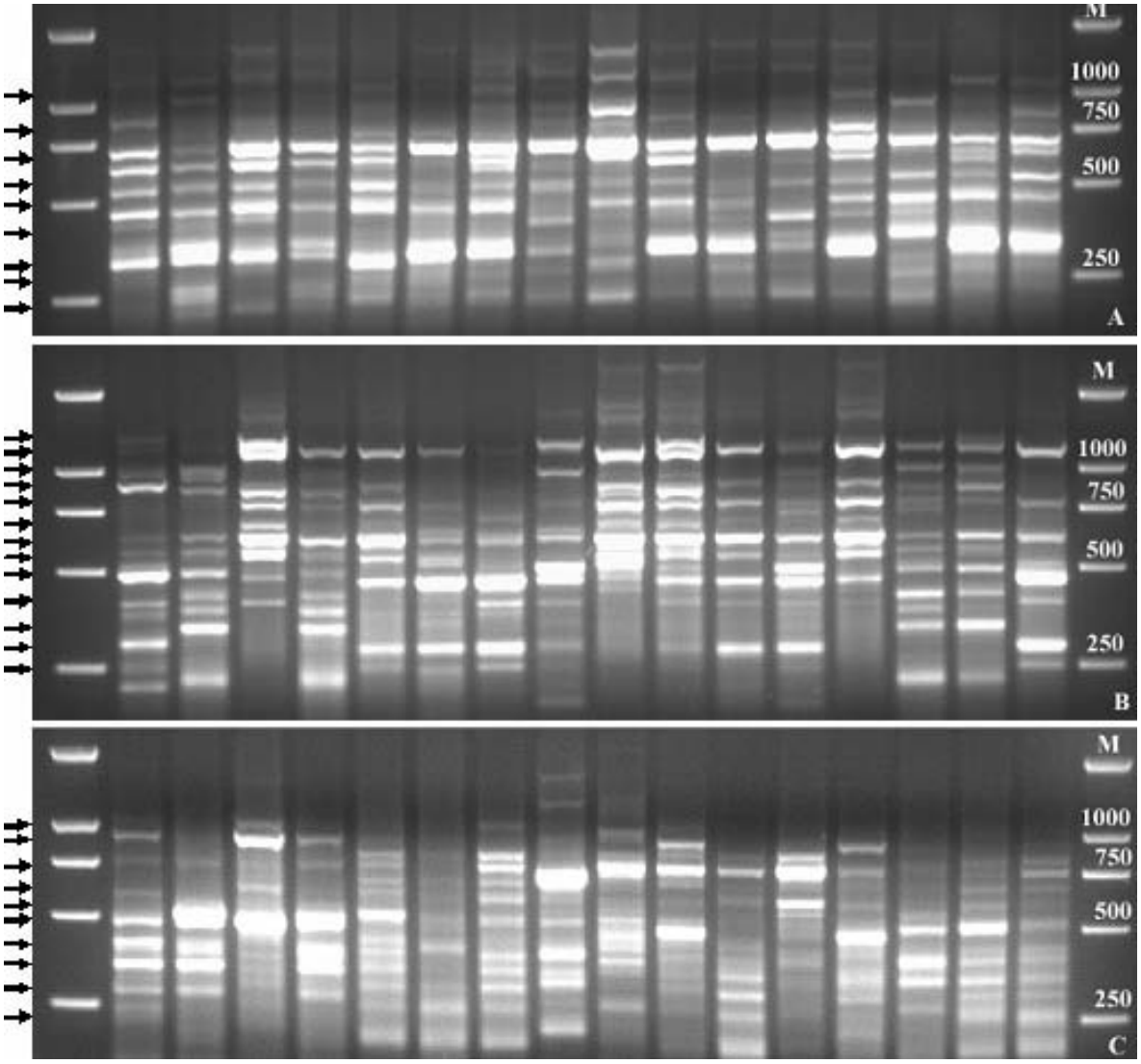

Fig. 2. ISSR bands in individual specimen of *Ditylenchus destructor* of different populations to indicate the selected bands (arrows) for the polymorphism analysis. A: Primer ISSR1; B: Primer ISSR2; C: Primer ISSR3. D: Primer could produce clear polymorphic bands in all populations.

Table 3. Hierarchical analysis of molecular variance (AMOVA) of the 16 *Ditylenchus destructor* populations in China.

Source of variation	d.f.	Sums of squares	Variance Component	Total variance (%)	P-value
Among populations	15	107.69	0.28	8.1	0.001
Within populations	224	703.40	3.14	91.9	0.001
Among regions	8	56.26	0.12	3.6	0.001
Among populations within regions	7	51.43	0.17	5.0	0.001
Within population	224	703.40	3.14	91.4	0.001

Genetic variation. Low levels of genetic variations were observed among *D. destructor* populations of different regions. F_{ST} values comparing pairs of populations ranged between 0.004-0.087, HBCL-NMHH (F_{ST} = 0.087), HBCL-JSTS (F_{ST} = 0.082) being the most different and HBFN-HBCL the most similar (F_{ST} = 0.004). Results of the cluster analysis among populations, based on their pairwise F_{ST} estimates, are shown in Fig. 3. Cluster analysis grouped the majority of the 16 *D. destructor* populations into three main clusters, which corresponded to their geographic distributions. Cluster I comprises the KORE, SDYS, JSSH and JSTS populations, which belong to the B type as classified by the rDNA-ITS region. Populations of A type classified by the rDNA-ITS region were divided to cluster II and cluster III. Cluster II comprises the JLBC, HBCL and HBFN populations, the remaining populations of the A type of rDNA-ITS region are more diverse and belongs to cluster III. However, the NMHH population appeared clearly separated from the rest of the Chinese populations. Principal Coordinate Analysis (PCO) of the data set explained 27.9% and 16.8% of the total phenotypic variance along the first and second axes, respectively, implying a similar relationship among populations (Fig. 4). This confirmed the results obtained with the phenogram and the quantification of intra-population diversity. Mantel test showed a low positive correlation (R^2 = 0.344, P = 0.009, t = 1.577) between pairwise genetic and geographical distances (Fig. 5).

The Hierarchical AMOVA revealed small genetic divergence among populations from different locations. It further showed that the majority of genetic variation (91.9%) existed within populations, whereas small genetic variation (8.1%) occurred among populations. At the regional level, the AMOVA indicated that about 91.4% of the variations were genotypic variations within populations, 3.6% of the variations were due to regional differences, while the remaining 5.0% were due to differences among populations within re

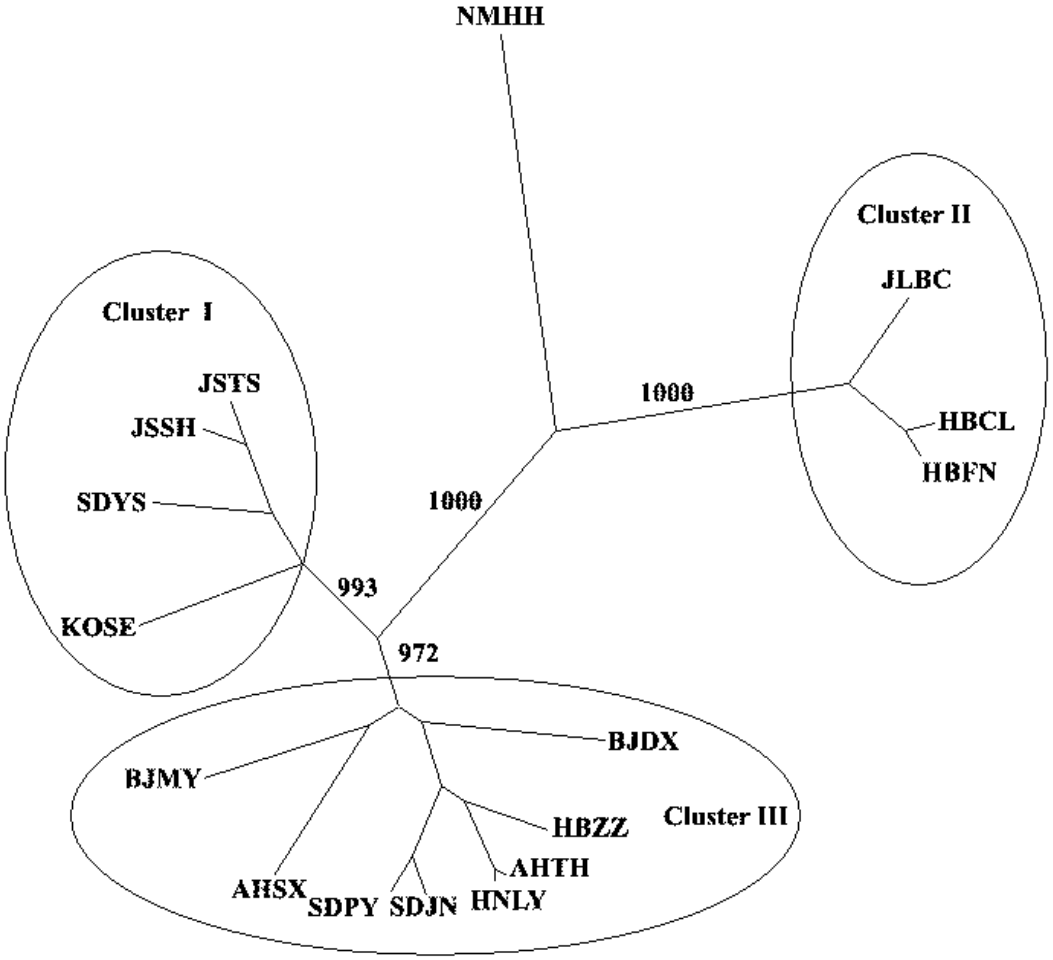

Fig. 3 Relationships among *Ditylenchus destructor* populations based on their pairwise F_{ST} values. UPGMA clustering was constructed using the Phylip program, version 3.6. Only bootstrap values higher than 950 are shown.

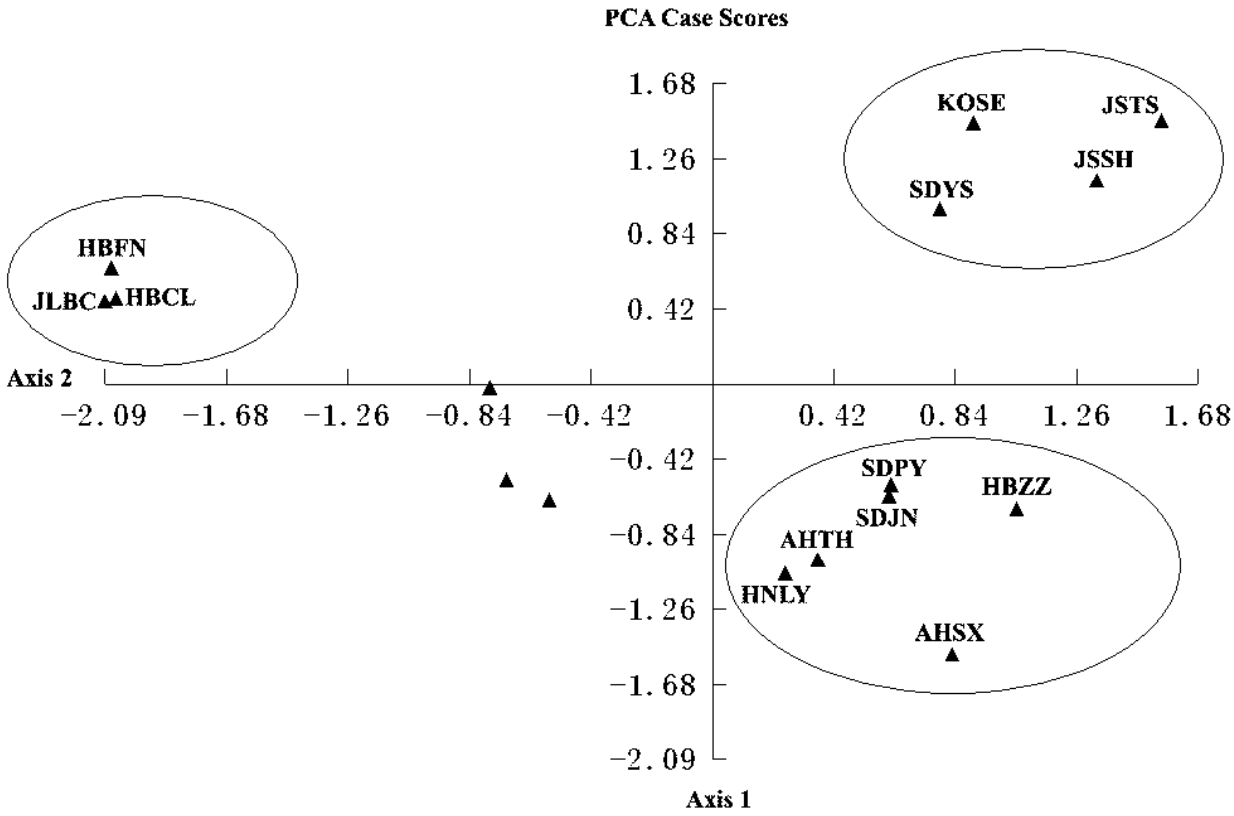

Fig. 4. Principal Coordinate Analysis (PCO) based on Euclidean distance among the 26 *Ditylenchus destructor* populations in China. PCO axis 1 and 2 accounted for 44.7% of the overall variation.

gions (Table 3). Despite the differences in proportion of the total variance, values for all three hierarchical levels were significantly different. This suggests that significant gene flow occurs among populations and even regions.

DISCUSSION

The application of the ISSR technique to detect intra-specific variation in nematodes and the usefulness of this technique in determining population genetic variation of some nematode species has been demonstrated (Berner & Schnetter, 2002; Metge & Burgermeister, 2006; Lax *et al.*, 2007). ISSR markers have been shown to provide useful information for resolving phylogenetic relationships among closely related species and relationships at or below the species level (Mort *et al*., 2003). The present work is the first study of the genetic structure of *D. destructor* populations at a macrogeographical level using ISSR markers. At the species level, genetic variation within population of *D. destructor* is higher than that of *Heterodera glycines* and *Bursaphelenchus xylophilus* using RAPD markers (Zhang *et al*., 1998; Vieira *et al.*, 2007). However, higher genetic variation was detected within fields for *H. schachtii* (Plantard & porter, 2004; Madani *et al*. 2007), *Nacobbus aberrans* (Lax *et al*., 2007) and *Globodera pallida* using different molecular markers.

Genetic similarity index is a reliable index that allows evaluation of genetic variation level among different individuals. In the present study, the Shannon genetic information index (I) was between 0.3042 and 0.4123, meaning that genetic diversity of *D. destructor* was very high. One of the possible sources of the high level of polymorphism found in *D. destructor* populations could be related to the mode of reproduction of the nematode. In the study based on the results obtained for four physiological races of *D. destructor*, Smart & Darling (1963) indicated that amphimixis is the only reproduction mode for this species. Anderson & Darling (1964) hypothesised that *D. destructor* secrete attractants that probably serve to bring nematodes of opposite sexes together prior to mating. They observed that a single male would mate with the same female if contact was made again and occasionally several males

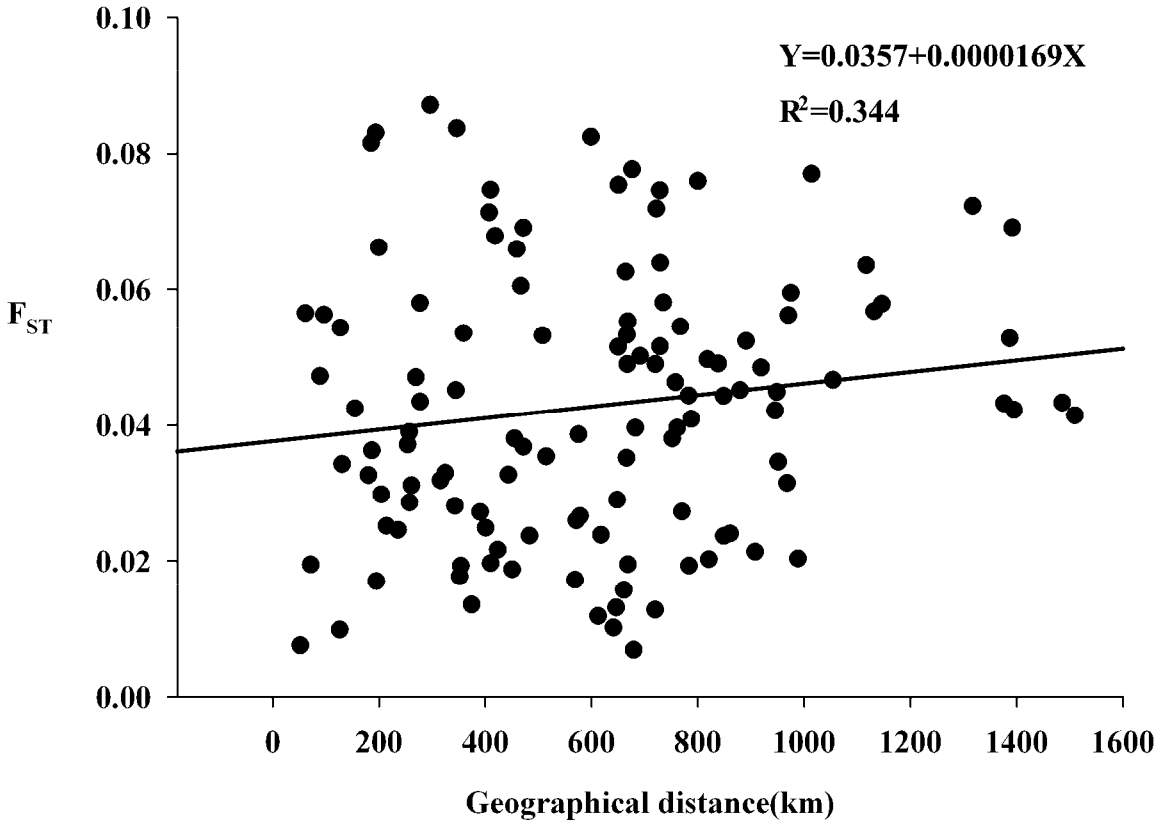

Fig. 5. Plot of pairwise F_{ST} values between populations of *Ditylenchus destructor* against geographical distance

would attempt to mate with the same female at the same time in the laboratory, which might be an indicator of multiple mating of *D. destructor*. In the same field, Wan *et al*. (2008) detected two different types of *D. destructor* rDNA-ITS region. This may be evidence of multiple mating in natural populations of this species. The multiple mating modes would favour genetic diversity among the offspring of *D. destructor*. In addition, the relatively high genetic diversity within populations may have resulted largely from multiple introductions of *D. destructor* from different origins, which might helped this species avoid the founder effect (Da Conceição *et al.*, 2003; Plantard *et al*., 2008).

Ditylenchus destructor exhibits low genetic differentiation among populations and regions despite the great geographic distances (even 1500 km apart between HNLY and JLBC) that separate some of them, suggesting significant gene flow at these spatial scales. Because of their small size, active dispersal is probably limited to a few centimeters or decimeters. The nematodes can move only short distances in the soil and have no natural means of long-range movement. Juveniles from the same egg mass are, respectively, full or half-sibling following fertilisation of the female by one or several males. Although males are slightly larger than J2, their active dispersal is also likely to be limited. This life cycle and behaviour should thus favour mating between siblings and enhances the production of homozygotes (Plantard & Porte, 2004). Even if the migration is very low, gene flow could occur and thus prevents genetic differentiation among populations. For *C. elegans*, which is also a soil nematode but a free-living one feeding on bacteria, Koch *et al.* (2000) suggest long-range dispersal to explain the weak genetic differentiation of this species at the global scale. Moreover, such gene flow could be due to passive transport of nematodes within and among fields by human activities (e.g. transport of soil by farm machinery, sewage farms around sweet tomato factories) or by water (flood, irrigation or drainage) (Plantard & Porte, 2004). In addition, infected root tubers and seedlings are important means of dissemination of this nematode. Third- (J3) and fourth-stage juveniles (J4) and immature females may be found on root tubers and seedlings of sweet potato (Anderson & Darling, 1964; Ding & Lin, 1982). They are sources of inoculum when infected seedlings are planted in sweet potato fields. These dispersal mechanisms would favour the maintenance of high effective

population sizes, which would contribute to increase the genetic diversity of natural populations of this species (Lax *et al*., 2007).

The AMOVA performed with three variance components revealed that 3.6% of the total variation detected in *D. destructor* population using ISSR loci corresponded to significant differences among regions defined on the basis of their geographic origin. At the population level, the present results revealed that genetic variability within populations with ISSR loci (91.4%) in *D. destructor* was higher than that among different populations (5.0%). These results support the fact that there was some degree of genetic similarity among populations from the same region and would confirm the existence of different races or biological entities within the species (Plantard & Porte, 2004; Picard *et al*., 2004). It has been reported that within particular nematode populations, some characteristics, such as host preference and aggressiveness, might be determined by alleles that vary in their frequencies (Triantaphyllou, 1987). Even the gene frequencies could change with time in response to host-induced selection (Kaplan *et al*., 1999). This may have important consequences for the evolution of virulence in *D. destructor*. Indeed, in a classical gene-for-gene relationship with a dominant resistance gene, only homozygous virulent individuals are able to overcome the resistance gene. If a mutation in the virulence-avirulence gene changing the avirulence allele into a virulence allele occurs at a low frequency, mating between siblings will facilitate the association of two virulence alleles in the same individual. Thus, inbreeding induced by such a behaviour should be included in models of virulence evolution in phytoparasitic nematodes (Schouten, 1997; Plantard & Porte, 2004).

In this work, it was possible to define populations of *D. destructor* populations according to their genetic divergence using ISSR markers. Other molecular methods, such as analysis of the rDNA-ITS and rDNA-D2D3 regions, also showed affinities between populations of the species from the same country of origin (with some exception) (Liu *et al*. 2007; Wan *et al.* 2008; Yu, 2008). Sixteen populations of *D. destructor* were grouped to three main clusters using the ISSR marker, which corresponded to their characteristics from analysis of rDNA-ITS regions. The divergence of *D. destructor* also agrees with the results previously obtained by analysing the rDNA-D2D3 regions of 22 populations of *D. destructor* in China (Yu, 2008). These results would confirm the existence of two or three different races or biological entities within the species (Plantard & Porte, 2004; Picard *et al*., 2004). However, the NMHH population appeared clearly separated from the other Chinese populations using ISSR markers. This population is located to the north of Yinshan mountains, the altitude of which is about 1500 m. Because of these geographic characteristics, the nematodes of this population are very unlikely to have a genetic exchange with the other populations. Given the clear genetic differentiation between NMHH and the other populations studies, it would be interesting to conduct a similar study to make a genetic comparison between that population and others.

The ISSR markers revealed high genetic diversity from different localities but low level genetic variation among different regions in *D. destructor* populations of China. It was shown that the ISSR marker was an efficient method for detecting genetic variation among the different geographic populations of this species. Further studies of *D. destructor* using the molecular markers would contribute to elucidate the taxonomic statues and may assist in developing effective control measures for different biological entities of this species.

ACKNOWLEDGEMENT

This work was funded by the National Basic Research and Development Program of China (2009CB119200), National Science Foundation of China (30871627) and National Key Technology Research & Development Program for the Eleventh Five-year Plan in China (2006BAD08A14).

REFERENCES

AMIRI, S., SUBBOTIN, S.A. & MOENS, M. 2003. Comparative morphometrics and RAPD studies of *Heterodera schachtii* and *H. betae* populations. *Russian Journal of Nematology* 11: 87-95.

ANDERSON, R.V. & DARLING, H. 1964. Embryology and reproduction of *Ditylenchus destructor* Thorne, with emphasis on gonad development. *Proceedings of the Helminthological Society of Washington* 31: 240-256.

BERNER, M. & SCHNETTER, W. 2002. Application of ISSR-PCR to characterize entomopathogenic nematodes within a selection program for higher efficiency against white grubs of the cockchafer (*Melolontha* spp.). *Bulletin OILB/SROP* 25: 35-40.

BORNET, B. & BRANCHARD, M. 2001. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter* 19: 209-215.

CARRIÈRE, Y., ELLERS-KIRK, C., SISTERSON, M., ANTILLA, L., WHITLOW, M., DENNEHY, T.J. & TABASHNIK, B.E. 2003. Long-term regional

suppression of pink bollworm by *Bacillus thuringiensis* cotton. *Proceedings of the National Academy of Sciences of the USA* 100: 1519-1523.

CHEN, P.S. & ZHEN, J.W. 1988. [Identification of *Ditylenchus* spp. in Angelica sinensis.] *Plant Protection* 14: 12-14.

CRAWFORD, D., TAGO-NAKAZAWA, M., STUESSY, T.F., ANDERSON, G.J., BERNARDELLO, G. & RUIZ, E. 2001. Intersimple sequence repeat (ISSR) variation in *Lactoris fernandeziana* (Lactoridaceae), a race endemic of the Juan Fernandez Archipelago, Chile. *Molecular Ecology* 16: 185-192.

CULLEY, T.M. & WOLFE, A.D. 2001. Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity* 86: 545-556.

Da CONCEIÇÃO, I., Dos SANTOS, M.C.V., De OLIVEIRA, A.I.M. & De ALMEIDA SANTOS, M.S.N. 2003. Using RAPD markers to analyse genetic diversity in Portuguese potato cyst nematode populations. *Nematology* 5: 137-143.

DAYTEG, C., RASMUSSEN, M., TUVESSON, S., MERKER, A. & JAHOOR, A. 2008. Development of an ISSR-derived PCR marker linked to nematode resistance (*Ha*2) in spring barley. *Plant Breeding* 127: 24-27.

De WAELE, D., JONES, B.L., BOLTON, C. & E. Van den BERG. 1989. *Ditylenchus destructor* in hulls and seeds of peanut. *Journal of Nematology* 21: 10-15.

DING, Z. 2007. [Studies on Differential susceptibility and its mechanisms of different population of *Ditylenchus destructor* to acetylcholinesterase inhibitor.] Ph. D. dissertation. *Hunan Agricultural University, Hunan*.

DING, Z.F. & LIN, M S. 1982. [Identification of *Ditylenchus* sp. on sweet potato, potato and mint.] *Journal of plant protection* 9: 169-172.

ESSER, R P. 1985. Characterization of potato root nematode, *Ditylenchus destructor* Thorne, 1945 (Tylenchidae) for regulatory purpose. Florida department of agriculture and consumer service. *Nematology Circular* 124.

FELSENSTEIN, J. 2001. *PHYLIP: Phylogeny Inference Package, Version 3.6 (alpha2)*. Department of Genetics, University of Washington, Seattle, WA, USA.

GUBINA, V.G. 1982. *Plant and soil nematode genus Ditylenchus*. Izdatelstvo "Nauka", Moscow., p. 69-86.

HOOPER, D.J. 1973. *Ditylenchus destructor. CIH description of plant-parasitic nematodes No. 21*. CAB international, Wallingford, UK, p. 54.

HYMAN, B.C. 1996. Molecular systematics and population biology of phytonematodes: some unifying principle. *Fundamental and applied nematology* 19: 309-313.

KAPLAN, M., CASWELL-CHEN, E.P. & WILLIAMSON, V.M. 1999. Assessment of host-induced selection on three geographic isolates of *Heterodera schachtii* using RAPD and AFLP markers. *Phytopathology* 89: 68-73.

KOCH, R., van LUENEN, H.G.A. M., VAN DER HORST, M., THIJSSEN, K. & PLASTERK, R.H.A. 2000. Single nucleotide polymorphisms in wild isolates of *Caenorhabditis elegans*. *Genome Research* 10: 1690-1696.

LAX, P., RONDAN DUENAS, J.C., GARDENAL, C.N. & DOUCET, M.E. 2007. Assessment of genetic variability in populations of *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Pratylenchidae) from Argentina. *Nematology* 9: 261-270.

LENORMAND, T. & RAYMOND, M. 1998. Resistance management: the stable zone strategy. *Proceedings of the Royal Society of London Series B: Biology Sciences*. 265: 1985-1990.

LIU, B., MEI, Y.Y. & ZHENG, J.W. 2007. [Species-specific detection of inter-populations of *Ditylenchus destructor*.] *Journal of Zhejiang University (Agric. & Life Sci.)* 33: 490-496.

LIU, X.B., GE, J.J., TAN, Z.Q. & CAO, A.X. 2006. [The first report of *Ditylenchus destructor* infecting potatoes in China.] *Plant Protection* 32: 157-158.

MADANI, M., KYNDT, T., COLPAERT, N., SUBBOTIN, S.A., GHEYSEN, G. & MOENS, M. 2007. Plolymorphism among sugar beet cyst nematode *Heterodera schachtii* populations as inferred from AFLP and ITS rRNA gene analyses. *Russian Journal of Nematology* 15: 117-128.

MANTEL, N.A. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

METGE, K. & BURGERMEISTER, W. 2006. Intraspecific variation in isolates of *Bursaphelenchus xylophilus* (Nematoda : Aphelenchoididae) revealed by ISSR and RAPD fingerprints. *Journal of Plant Diseases & Protection* 113: 275-282.

MORT, M.E., CRAWFORD, D.J., SANTOS-GUERRA, A., FRANCISCO-VERA, I., ESSELMAN, E. J. & WOLFE, A. D. 2003. Relationships among the Macronesian members of *Tolpis* (Asterraceae: Lactuceae) based upon analyses of inter simple sequence repeat (ISSR) markers. *Taxon* 52: 511-518.

OEPP/EPPO. 1978. Data sheets on quarantine organisms No. 123, Ditylenchus destructor. *Bulletin OEPP/EPPO Bulletin* 8: 1-5.

OU, S., PENG, D., LI, Y. & MOENS, M. 2008. Identification of *Heterodera glycines* using PCR with sequence characterized amplified region (SCAR) primers. *Nematology* 10: 397- 403.

PICARD, D., PLANTARD, O., SCURRAH, M. & MUGNIERY, D. 2004. Inbreeding and population structure of the potato cyst nematode(*Globodera pallida*) in its native area(Peru). *Molecular ecology* 13: 2899-2908.

PLANTARD, O., PICARD, D., VALETTE, S., SCURRAH, M., GRENIER, E. & MUGNIERY, D. 2008. Origin and genetic diversity of Western European populations of the potato cyst nematode (*Globodera pallida*) inferred from mitochondrial sequences and microsatellite loci. *Molecular Ecology* 17: 2208-2218.

PLANTARD, O. & PORTE, C. 2004. Population genetic structure of the sugar beet cyst nematode *Heterodera schachtii*: a genochorist and amphimictic species with highly inbred but weakly differentiated population. *Molecular ecology* 13: 33-41.

ROHLF, F.J. 2002. *NTSYSpc: Numerical Taxonomy System, ver.2.1*. Exeter Publishing, Ltd. Setauket, NY, USA.

SCHNEIDER, S., EXCOFFIER, L., KUEFFER, J.M. & ROESSLI, D. 1997. *ARLEQUIN, Version 1.1: software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

SCHOUTEN, H.J. 1997. Modeling the effect of random genetic drift on the virulence of potato cyst nematodes. *Nematologica* 43: 173-184.

SMART, G.C.Jr. & DARLING, H.M. 1963. Pathogenic variation and nutritional requirement of *Ditylenchus destructor*. *Phytopathology* 53: 374-381.

SUBBOTIN, S.A., HALFORD, P.D. & PERRY, R.N. 1999. Identification of populations of potato cyst nematodes from Russia using protein electrophoresis, rDNA-RFLPs and RAPDs. *Russian Journal of Nematology* 7: 57-63.

THORNE, G. 1945. *Ditylenchus destructor* sp., the potato rot nematode and *Ditylenchus dipsaci* (Kuhn-1857) Filipjev-1936, the teasel nematode (Nematode: Tylenchidae). *Proceedings of the Helminthological Society of Washington.* 12: 27-33.

TIKUNOV, Y.M., KHRUSTALEVA, L.I. & KARLOV, G.I. 2003. Application of ISSR markers in the genus *Lycopersicon*. *Euphytica* 131: 71-80.

TRIANTAPHYLLOU, A.C. 1987. Genetics of nematode parasitism on plants: Veech, J. A. & Dickson D. W. (Dds). *Vistas on nematology*. Society of Nematologists, Hyattsville, MD, USA, 354-363.

VIEIRA, P., BURGERMEISTER, W., MOTA, M., METGE, K. & SILVA, G. 2007. Lack of genetic variation of *Bursaphelenchus xylophilus* in Portugal revealed by RAPD-PCR analyses. *Journal of Nematology* 39: 2, 118-126.

WAN, F., PENG, D.L., YANG, Y.W. & HE, Y.Q. 2008. [Species specific molecular diagnosis of *Ditylenchus destructor* populations occurring in China.] *Acta Phytopathologica Sinica* 38: 263-270.

WANG, J.C., JI, L., HUANG, G.M., YANG, X.L. & LIN, M.S. 2007. [Alignments of rDNA- ITS sequences and phylogeny of different geo-populations of *Ditylenchus destructor* in China.] *Journal of Agricultural University of Heibei* 30: 79-83.

WOLFE, A.D. & LISTON, A. 1998. Contribution of PCR-based methods to plant systematics and evolutionary biology: Soltis DE, Soltis PS, Doyle JJ, editors. *Molecular Systematics of Plants II: DNA Sequencing*, New York: Kluwer 43-86.

YAO, W.G. & CUI, M.L. 2001. [*Pests of potato and quarantine measures*.] China Agricultural Press, Beijing, 300-307.

YEH, F.C., YANG, R. & BOYLE, T. 1999. Popgene, version 1.32. Microsoft window-based freeware for population genetic analysis. University of Alberta, Edmonton. Available at: http:// www. ualberta.ca/fyeh/index.htm.

YU, H.Y. 2008. [*Molecular cloning and sequences analysis of 28S rDNA-D2/D3 regions of Ditylenchus destructor on sweet potato in China*.] M. S. dissertation. Yunan Agricultural University, Yunnan.

ZHANG, G.Z. & ZHANG, H.W. 2007. First report of root rot of American ginseng (*Panax quinquefolium*) caused by *Ditylenchus destructor* in China. *Plant Disease* 91: 459.

ZHANG, L., DEAN, R.A., KNAP, H.T. & LEWIS, S.A. 1998. Diversity among a *Heterodera glycines* field isolate and derived inbreds based on RAPD analysis and reproduction on soybean genotypes. *Journal of Nematology* 30: 477-484.

ZHANG, S.L. & ZHANG, S.S. 2008. [PCR and sequence analyse of rDNA-ITSl region of sweet potato stem nematode.] *Acta phytopathologica Sinica* 38: 132-135.

ZIETKIEWICZ, E., RAFALSKI, A. & LABUDA, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

Wen-Kun Huang, De-Liang Peng, Dong-Sheng Zhang, Hong-Yun Jiang, Zhong Ding, Huan Peng, Hai-Bo Long. Оценка генетического разнообразия в популяциях *Ditylenchus destructor* (Thorne 1945) (Tylenchida: Anguinidae) в Китае.

Резюме. Нематоды *Ditylenchus destructor* широко распространены в Китае и вызывают значительные потери урожая картофеля. Генетическое разнообразие и изменчивость этого вида исследовали с использованием метода ISSR-маркеров (Inter-Simple Sequence Repeats). Личинки 2-й стадии из 16 популяций исследовали с использованием трех пар праймеров, что позволяло проводить анализ 32 фрагментов ДНК. Уровень генетического разнообразия был довольно высоким. Кластерный анализ и метод основных координат подразделяли изученные популяции на три основные группы. На региональном уровне метод AMOVA показал, что около 91,4% наблюдаемой изменчивости связано с различиями генотипов в пределах популяций, 5,0% вариабельности определяется региональными различиями и остальные 3,6% определяются различиями между популяциями в пределах каждого из регионов. Невысокий уровень генетической изменчивости между популяциями предполагает значительный обмен генами между ними. Амфимиктическое размножение в природных популяциях и пассивный разнос за счет деятельности человека, а также естественные причины, вероятно, определяют наблюдаемую картину. Показано, что ISSR-маркеры представляют собой эффективный метод выявления генетической структуры популяций *D. destructor* на уровне географически удаленных популяций.