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A Review of Root Lesion Nematode: Identification and Plant Resistance

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ABSTRACT

Root lesion nematode, one of the three most devastated plant parasitic nematodes, is widespread in the world. They can invade various food and cash crops, and cause great agriculture loss. Recognition and identification of root lesion nematode are important joint of nematode management, as well as the mechanism research of plant anti-nematode, which is of great benefit to breed resistance varieties. In this review, the recent advances in nematode identification and research of plant anti-nematode are presented, and the importance of non-inaccuracy identification of nematode was emphasized. The mechanism of resistance to root lesion nematode was also discussed.

Keywords: *Pratylenchus*; Isoenzyme; ITS; Resistance Related Protein; Secondary Metabolite

1. Introduction

Root lesion nematode (RLN) belonging to the genus of *Pratylenchus*, range the second plant pathogenic nematode only to root knot nematode (RKN) and cyst nematode (CN), not only because of their wide host ranges but also because of they distribute throughout the world [1]. They feed on a wide range of important crops of primary importance including cereals, legumes, vegetables, fruit (trees), ornamentals, coffee, peanut, ramie, etc. RLNs compromised 97 valid species distributed almost every cool, temperate and tropical environment [2]. RLNs are migratory endoparasites, infecting and developing mainly in the cortical parenchyma which cumbers the absorption of water and nutrients from the soil, thereby causing severe root damage and reduced plant growth. Twelve species of RLN are most common and devastating pests in the temperate regions of the world; they are *Pratylenchus neglectus*, *P. thornei*, *P. coffeae*, *P. penetrans*, *P. scribneri*, *P. brachyurus*, *P. vulnus*, *P. crenatus*, *P. loosi*, *P. goodeyi*, *P. pratensis* and *P. zeae* [1].

Cereals including wheat, rice, barley and maize are main food crops. It is reported that at least 8 species of RLN could infect cereal crops, hereinto *P. thornei* and *P. neglectus* are two quite devastating species. In Australia, Israel, Oregon of American and Mexico, *P. thornei* brings yield loss of wheat 85%, 70%, 50% and 37%, respectively, every year [3]. *P. neglectus* brings yield loss of wheat to Australia and northwest America about 16% - 23% and 8% - 36%, respectively, every year [4,5]. Furthermore, these two RLN species always occur simultaneously. *P. penetrans* also parasitize wheat and barley, and resulted in yield loss of 10% - 19% in Canada [6]. In northwest America, RLN bring economic loss of 51,000,000$ annually [3]. Banana is the primary food and cash crop of many countries in Latin America and Africa; it is also main fruit crops in tropic and subtropical region. Nematode infestation results in banana yield loss of 20% - 30% annually. It is reported that 8 species of RLN could parasitize banana, and *P. coffeae* and *P. goodeyi* are two chiefly RLN constraints of banana production [7].

In light of the economic important of RLN, environment friendship methods for its control are attempt aims of nematologist and phytopathologist. Fast recognition and identification are premise and benefit for making effective control plan. Since utilization of resistance varieties is an ideal method for the control of RLN, knowledge of resistance mechanism and genes of plant hereby are vital to anti-nematode breeding. In this paper, we reviewed the advances of methods for RLN identification, the resistance genes and enzymes and secondary metabolites of host plants. Disadvantages of each identification methods and resistance mechanism were also discussed.

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2. Identification of Rln

2.1. Identification of RLN by Morphological Character

Morphological features (such as body length, max body width, stylet length, spicule length, vulvas-anus distance, etc.) were firstly used to differentiate, identify and classify nematode, and play a basilic role until now [7,8]. After getting the data of morphological features of the targeted nematode, these data were compared with those of model nematode, and the targetted nematode was identified to species level according the nematode taxonomy of Maggenti [9].

Nematode identification by morphological features always needs advanced microscope, abundant knowledge and veteran experiences of nematode classification, so it is not easy to be operated by a freshman. In addition, since there are not distinct differences among some nematodes of RLN, and intra-specific variations often happened; morphological traits are not sufficient to the accurate identification of RLN. Due to the economical importance of RLNs, nematologists and psychopathologists aim to develop a simple and accurate method to identify RLNs, since prior discovery and identification accuracy of nematode are premise of making a control strategy.

2.2. Identification of RLN by Isoenzyme Zymogram

In 1997, Jaumot compared the differences of total protein patterns of five RLNs (*P. coffeae*, *P. thornei*, *P. vulnus*, *P. goodeyi*) and *Radopholus similis* by applying SDS-PAGE electrophoresis and suggested a potential method for differentiating nematode of different species by the relative motility rates of proteins [10]. However, this method could not be used to identify nematode, since there was no criterial protein could be used as reference.

Phenotypes of isoenzymes, such as esterase (EST), Malate dehydrogenase (MDH), glucose oxidase (GOD), superoxide dismutase (SOD) and isocitrate dehydrogenase (IDH) have been to identify and differentiate various nematodes until now [11-13]. Through comparing pattern differences of EST, IDH, phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6PD), Ibrahim and the colleagures categorized *P. crenatus*, *p. neglectus*, *P. penetrans*, *P. thornei*, *P. pinguicaudatus* and *P. fallax* which collected from different regions of UK into two groups. One group contains *P. crenatus*, *p. neglectus* and *P. penetrans*, the other contains *P. thornei*, *P. pinguicaudatus* and *P. fallax*. Nevertheless, the study also showed that phenotype differences of the 5 enzymes were not sufficient to differentiate these RLNs due to the galactic intra-specific similarity (*i.e.* two species in the same group) [14].

Using isoelecro-focusing electrophoresis, Andrés and the colleagues analyzed patterns differences of 6 isoenzymes (EST, MDH, hexoquinase (HK), PGM, IDH and PGI) out of 40 populations of 9 RLNs and *R. similes*. They found only EST, MDH, PGI and PGM could be used to differentiate *P. vulnus*, *P. goodyi*, *P. penetrans*, *P. scribner*i and *R. similis*. Based on the isoenzyme patterns of MDH, PGI and PGM, the greatest intra-specific diversity was found within *P. coffeae*. EST and PGI systems of *R. similis* showed two different patterns indicating intra-specific variation of this nematode [11].

Using isoenzyme patterns to differentiate RLN always needs a mass of nematodes to extract total proteins; additionally, this method could not be competent for analyzing single nematode and thereby not suitable for detecting and quarantining a spot of nematodes in plant.

2.3. Identification of RLN by PCR-Based Molecular Biotechnology

Recently, PCR-based molecular biotechnologies were used widely for differentiation and identification of plant parasitic nematodes. Sequence specific DNA probe combining hybridization was once used to detect and identify nematode [15-17]. However, this method required plenty of nematode to extract DNA and radioactivity examination equipment. It costs time and exists potential radiation to operator.

Analyses and identification methods using nematode genomic DNA as object include RFLP, RAPD, SCAR and rDNA-ITS, etc. Ouri and his colleagues analyzed and differentiated seven *Pratylenchus* species from Japan with PCR-RFLP [18]. Seven *Pratylenchus* species from Brazil were classified by RAPD primers [19]. A method for the identifying *P. thornei* was developed by combing RAPD and SCAR-PCR techniques in 2007 [20].

Ribosomal DNA (rDNA) is abundant in organisms. Its intertranscribed spacer (ITS) sequences are non-coding and similar in different individuals of the same species. Therefore, ITS based PCR is very suitable for identifying species and subspecies of organisms, and is currently widely used to identify nematode. Based on analyzing the ITS sequences of *P. coffeae*, *P. loosi* and *P. penetrans*, specific primers were designed and identification systems were developed for RLNs [21,22]. The ITS sequences character and morphological traits of a *Pratylenchus* species were always combined to the identification of nematode [8,23,24].

A species-specific PCR system was developed for detection and identification of *P. neglectus* and *P. thornei* from soil; moreover, this system could detect single nematode in soil sample [25].

More recently, after comparison and analysis of ITS sequences of eighteen *Pratylenchus* species, Castillo and

his colleagues proposed that identification of RLN by ITS sequence character is feasible. However, their study also showed that some ambiguous regions (high nucleotide variability) in some species' ITS sequences can preclude its use to resolve relationships among all members of the genus [26].

As the fast development of quantitative PCR, this technology was also used for the identification and quantization of RLN. Based on the ITS region character of *P. penetrans*, species-specific primers were designed and a real time quantitative PCR system was developed for *P. penetrans* identification and quantization from both nematode populations and soil sample [27,28]. According to the ITS1 region character of *P. thornei*, Yan's teams designed forward primer (THO-ITS-F2) and reverse primer (THO-ITS-R2) and invented a qRT-PCR based method to detect, identify and quantify *P. thornei* in soil [29].

2.4. RLN Resistance Genes, Enzymes and Secondary Metabolites

2.4.1. Resistance Genes

Compared to that of RKN and CN, less studies of RLN resistance gene was performed. The reported genes were mainly in wheat, barley and banana [30-32]. In Australia, many wheat accessions and varieties were identified to be resistant to *P. thornei* [32-34]. However, only one *P. neglectus*-resistant gene (*Rlnn*1) , which was located in chromosome 7AL and originated from Excalibur wheat, has been identified and validated so far [35].

Studies of descents of W-7984 (resistant) × Opata85 (susceptible), CPI133872 (resistant) × Janz (susceptible), AUS13124 (resistant) × Janz (susceptible), AUS4926 (resistant) × Janz showed many quantitative traits loci (QTL) with resistance to *P. neglectus* and/or *P. thornei*. QTLs with resistance to *P. thornei* inheriting from W-7984 were located on chromosome 2BS, 6DS and 6DL [36]. A major QTL was identified on Chromosome 6DS and was associated with resistance to both *P. thornei* and *P. neglectus*. These two traits were designated as *QRlnt.lrc*-6*D.*1 and *QRlnn.lrc*-6*D.*1 respectively. *QRlnt.lrc*-2*B* and QTL *QRlnt.lrc*-6*D.*2, which located on chromosome 2BS and 6DL respectively, were also associated with resistance to *P. thornei*. These QTLs inherited from CPI133872. QTL *QRlnn.lrc*-4*D.*1 located on chromosome 4DS and *QRlnn.lrc*4*B.*1 located on 4BS were also involved in resistance to *P. neglectus*; the former was inherited from Janz and the latter was inherited from CPI133872 [37,38]. Many other QTLs on chromosome 2B, 3B, 6D and 7A were detected to be involved in resistance to *P. thornei* in the descent derived from cross of AUS13124 × Janz and AUS4926 × Janz, and QTL on chromosome 3B was designated as *QRlnt.cpi*-3*B.*1 [39].

Based on the screened-out resistant material [31], doubled haploid population derived from anthers of cross of two winter barley varieties Igri (resistance) and Franka (susceptible) were studied to search loci for *P. neglectus* resistance. Five major QTLs (*Pne*3*H*-1, *Pne*3*H*-2, *Pne*5*H*, *Pne*6*H* and *Pne*7*H*) mapped on four linkage groups (3H, 5H, 6H and 7H), which derived from Igri, were associated closely with *P. neglectus* resistance [40].

2.4.2. Resistance Enzymes and Secondary Metabolites

After *P. coffeae* infection, the peroxidase (POD) activity increased in both the resistant and susceptible banana genotypes; however, the final enzyme concentrations in resistant accessions were much higher than that of the susceptible ones. Additionally, the resistant accessions recorded higher polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activity than those of the susceptible ones. The higher the PAL activity, the fewer lesion index of roots and corm could be observed [41,42]. Investigations of the relationships of the resistant accessions and some bio-chemical parameters also showed that POD, PAL and POD activity were positively correlated with the resistance [43].

Before the infection of *P. penetrans*, phenylpropanoid pathway mRNA levels in roots of three resistant alfalfa varieties were 1.3 - 1.8 fold higher than that of three susceptible ones. The mRNA encoded many crucial enzymes, such as PAL, chalcone synthase and isoflavone reductase, in the resistance response pathway. Acid β-1, 3-glucanase (GLU) mRNA levels were similar in resistant and susceptible roots of alfalfa. However, it was accumulated more rapidly in resistant than in susceptible ones after nematode infection [44].

Total phenol, tannin and lignin contents in banana roots were also positively correlated with the resistance to RLN in banana, which means the resistance level increases when the above contents accumulate in banana roots [43].

The semi-quantitative RT-PCR analysis revealed that mRNA levels of one enzyme for flavonoid biosynthesis (chalcone synthase) was constitutively higher in roots of the resistant cv. than in the susceptible one [45].

Similar results were also reported in studies of RKN and CN [46,47]. Taken together, protective enzymes (POD, POD, PAL, etc.) and secondary metabolites (tannin, phenol and lignin, etc.) are involved in the process of plant defend RLN infection.

3. Conclusion and Perspectives

Morphological character of RLN is indispensable and supplying base data in nematode identification. Total protein or isoenzymes electrophoresis character could be used to differentiate RLN of various species, but could not be used to identify nematode. PCR based DNA analysis is powerful to detect, distinguish and identify

RLN. It is well-improved and widely applied, especially to the identification of nematode with equivocal body.

RLN, one of the three plant parasitic nematodes, is economical important; but its biological traits, pathogenesis and host resistance mechanisms, especially the plant RLN resistance genes are rarely reported. The resistance gene reported is less than that of the RKN and CN. This is probably due to the economical importance of host and the destruction ponderance the nematode invited. In America and Australia, *P. thornei* and *P. neglectus* infect wheat and resulted in serious yield loss. Since wheat is one of the most important crops, these nematode attracted much attention and studies, and some resistance related QTLs and genes were discovered.

During the past 30 years, much work with regarding to identification, biology and control of RLN, parasitic to wheat, banana, fruit trees and ornamental plants, were done by Chinese researchers. However, most of the studies were mainly on biological and morphological aspects. RLNs did not attracted enough attention probably due to 1) most of the RLNs are foreign species and 2) not resulted in serious disaster of staple crops. However, with the increase of people's various material needs, effective control of RLN plays a vital action to good yield. More work should be carried out in nematode pathogenesis, host resistance and control strategy therefor.

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