

## Present status and future directions for management of root lesion nematode (*Pratylenchus thornei*) in chickpea

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### ABSTRACT

Chickpea is a popular legume crop in Asia and Africa's semi-arid regions. Crop production gains, on the other hand, have been modest, owing to biotic and abiotic stressors. Among the different biotic stresses, nematodes portray serious threat to chickpea production and colossal losses have been reported due to stress-free infection by other pathogens on infection with root lesion nematode (RLN). The worldwide distribution of two major species of RLN namely *P. thornei* and *P. neglectus* made them a focus research area especially on management aspect. In dryland farming areas of southeastern Australia, the *P. thornei* alone can cause yield losses of up to 40% in cereals and legumes. Despite the fact that chickpea breeders have been working persistently to generate superior chickpea varieties with increased resilience or tolerance to biotic and abiotic challenges, contemporary biotechnology technologies can help to speed up this process. To incorporate these tools and/or accelerate breeding programmes, identification of RLN resistant source with its genetic factor is first step in developing improved cultivars. However, study of resistance screening methods for chickpea against RLN is limited and standardization of different factors for development of a stable screening methodology distinguishing various levels of resistance is the need of the hour in RLN research.

### Introduction

One of the most commercially important food legume crops is chickpea (*Cicer arietinum* L.). It is grown on an estimated 14.56 million hectares in more than 55 countries, producing 14.78 million tonnes (FAOSTAT, 2017). India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, the United States, and Canada are the top chickpea producers (FAOSTAT, 2019). It is planted on 10.22 mha in India, with a production of

9.53 mt and a productivity of 967 kg ha<sup>-1</sup>. Madhya Pradesh, Chhattisgarh, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh, and Karnataka collectively produce 95.71 per cent of the country's chickpea production and 90 per cent of the country's area (Anon, 2013-14).

Chickpea is a nutrient-rich cool-season pulse crop that plays a critical role in ensuring global food security as a source of dietary protein and key

amino acids. Fixing atmospheric nitrogen, contributing to soil fertility, acting as a disease break, and suppressing weeds are all essential roles it performs in farming systems. The global average yield of chickpea is less than 1 t/ha, significantly less than the 6 t/ha potential output under favourable and irrigated circumstances (Varshney *et al.*, 2017). This huge gap between actual and predicted chickpea yields is attributable to biotic challenges including insects, bacteria, fungi, nematodes, and viruses, as well as abiotic factors like drought, nutritional shortages, salinity, and cold (Roorkiwal *et al.*, 2016; Kumar *et al.*, 2021).

Since 1961, chickpea productivity has consistently increased, but its vulnerability to biotic and abiotic stressors has also increased, presumably due to the restricted number of germplasm accessions/donor parents used and reused (Muehlbauer and Sarker, 2017). Chickpea is attacked by air borne, seed borne, and soil borne pathogens (Chattopadhyay *et al.*, 2001). More than 75 pathogens have been reported to infect chickpea (Nene *et al.*, 1984). Globally, the loss of chickpea output due to plant parasite nematodes is estimated to be 14 per cent among the various biotic stressors (Sasser and Freckman, 1987). Accurate nematode species determination necessitates a thorough understanding of nematode taxonomy and/or the use of molecular diagnostic methods. Root-knot nematodes (*Meloidogyne artiella*, *M. incognita*, and *M. javanica*), chickpea cyst nematode (*Heterodera ciceri*), and root-lesion nematode (*Pratylenchus thornei*) are the most common worms that infect chickpea.

The root lesion nematodes, *Pratylenchus* spp. [*Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida, Pratylenchidae)], is among one of the most important constraints to legume production and have a wide distribution in many regions in Turkey (82% of chickpea fields) and affect many agricultural crops around the world (Tanha *et al.*, 2009; Behmand *et al.*, 2019). *Pratylenchus thornei*, *P. neglectus*, *Pratylenchus penetrans* Cobb, 1917 and *Pratylenchus crenatus* Loof, 1960 (Tylenchida: Pratylenchidae), are the most important root lesion nematodes in the world (Vanstone *et al.*, 1998). Among the root lesion nematodes, *P. thornei* and *P. neglectus* are globally distributed and they enter the root tissue of host plant for feeding and

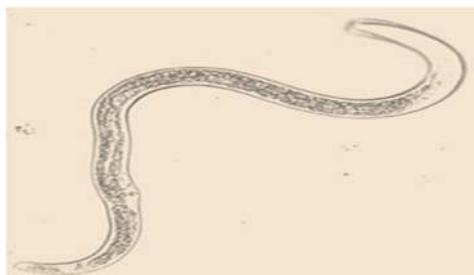
reproduction (Nicol *et al.*, 2004). Also, some studies indicated that in terms of damage caused by these nematodes is of second importance as a nematode problem in the world after root-knot nematodes (Barker & Noe, 1987; Jatala & Bridge, 1990). *P. thornei* is one of the most dominating species of plant parasitic nematodes that can cause yield losses of up to 40% in cereals and legumes in dryland cropping areas of southeastern Australia (Thompson *et al.*, 1995; Vanstone *et al.*, 1998), chickpea in India with particular reference to Madhya Pradesh (Tiwari *et al.*, 1992). Chickpea, infested with RLNs showed symptoms of stunted growth and leaf chlorosis and causes yield losses greater than 50% in chickpeas (Castilo *et al.*, 1998; Castilo & Vovlas, 2007). Conventional breeding technologies are being continuously employed for development of elite chickpea varieties. However, success is limited and time consuming for incorporation of resistance factor against different biotic and abiotic factors. The modern biotechnological tools have significantly facilitated generation of huge amount of genomic resources development to accelerate such activities. To incorporate these tools and/or accelerate breeding programmes, identification of RLN resistant source with its genetic factor is first step in developing improved cultivars. However, study of resistance screening methods for chickpea against RLN is limited and standardization of different factors for development of a stable screening methodology distinguishing various levels of resistance is the need of the hour in RLN research. Recent advancement in generation of genomic resources in chickpea (Hiremath *et al.*, 2011, 2012; Gujaria *et al.*, 2011) will certainly lead to identify of QTL conferring resistance to RLN in chickpea which can be utilized further for incorporation in molecular breeding platform.

### Root Lesion Nematode

The Root Lesion Nematode (RLN), *Pratylenchus thornei* Sher and Allen, is a migratory endoparasite (Figure 1) that causes large yield losses and is regarded one of the most important plant-parasitic nematodes (Tiwari *et al.*, 1992). Inside the root cortex, RLNs penetrate, feed, and migrate, causing necrotic lesions and root cavities (Figure 2). Under ideal conditions, the nematode reproduces mitotically and parthenogenetically (Fortuner, 1977), producing eggs in the cortex and completing its life cycle in 6 weeks. More than 60 species of

**Table 1: Successful management of nematodes in crops using eco-friendly approaches.**

Management approach	Crop	Reference
<b>Soil amendment by different plant bi-products</b>		
Oil cakes of neem, castor bean, groundnut, linseed, sunflower and soybean	Chickpea	Tiyagi and Shamim, 2004
Powdered (seed kernel, seed coat, and Ahook at 20 per cent w/w) neem formulations	Chickpea	Mojumder, 1999
Liquid (Neemark and Nimbecidine @5 per cent v/w) neem formulations	Chickpea	Mojumder, 1999
Mustard and Linseed cakes	Groundnut	Sebastian and Gupta, 1995
Jatropha cake @ 2 t/ha	Tomato	Patel and Patel, 2007
Mustard, castor and Jatropha cakes @ 30 g/plant	Bottle guard	Verma and Nandal, 2007
<b>Bioagents</b>		
<i>Trichoderma harzianum</i>	Chickpea	Pant & Pandey, 2002
	Maize	Windham <i>et al.</i> , 1989
	Tomato	Rao <i>et al.</i> 1997
<i>T. koningii</i>	Maize	Windham <i>et al.</i> , 1989
<i>T. harzianum</i> with neem cake	Tomato	Reddy <i>et al.</i> , 1998
<i>Trichoderma</i> and <i>Gliocladium</i>	Sunflower	Shankaranarayanan <i>et al.</i> , 1999
<i>Trichoderma viride</i>	Chickpea	Pandey <i>et al.</i> , 2003, Dwivedi <i>et al.</i> , 2008
<i>Pseudomonas fluorescens</i>	Chickpea	Dwivedi <i>et al.</i> , 2008
<i>Paecilomyces lilacinus</i>	Chickpea	Zaki and Maqbool, 1992; Vyas <i>et al.</i> , 1997
	Mashbean	Shahzad <i>et al.</i> , 1996
	Groundnut	Vyas <i>et al.</i> , 1997
	Tomato	Lin <i>et al.</i> , 1993; Ekanayake and Jayasundara, 1994; Parveen and Gaffar, 1998; Khan and Saxena, 1996
	Medicinal herbs	Park <i>et al.</i> , 1993
	betelvine	Jonathan <i>et al.</i> , 1995; Nakat <i>et al.</i> , 1998; Hazarika <i>et al.</i> , 1998; Pathak and Saikia, 1999
	Mung bean, Okra	Shahzad and Gaffar, 1987, 1989; Esteshamul-Haque <i>et al.</i> , 1995
<i>Pochonia chlamydosporia</i>	Vegetables	Kerry and Diaz, 2004
	Okra	Dhawan <i>et al.</i> , 2007
	Pistachio	Ebadi <i>et al.</i> , 2018
	Monocot and dicot hosts	Tolba <i>et al.</i> , 2021

**Figure 1: *Pratylenchus thornei*, a root lesion nematode of chickpea.****Figure 2: Symptoms due to *Pratylenchus thornei*, a root lesion nematode on chickpea roots as lesions.**

*Pratylenchus* exist (Loof, 1991), all of which can be distinguished only by small morphological and morphometric changes. In North Africa (Di Vito *et al.*, 1994a), Turkey (Di Vito *et al.*, 1994b), and Spain (Castillo *et al.*, 1996), RLNs are the most common plant-parasitic nematode detected in chickpea crops.

The root-lesion nematode *Pratylenchus thornei* is the most common species that causes damage to chickpea crops around the world. *P. thornei* is found in major chickpea-growing countries such as Australia (Thompson *et al.*, 2000), India, and Pakistan, India (Sharma *et al.*, 1992), North Africa (Di Vito *et al.*, 1994a), Turkey (Di Vito *et al.*, 1994b), and Spain (Castillo *et al.*, 1996). *P. thornei* is emerging as a serious threat to chickpea production in India, which is the world's largest producer and consumer of gram. High populations of *P. thornei* have been reported in Madhya Pradesh (Baghel and Singh, 2013), Rajasthan (Ali and Sharma, 2003), Maharashtra (Varaprasad *et al.*, 1997), and Uttar Pradesh (Sebastian and Gupta, 1995). During chickpea crop surveys in North Africa and the Mediterranean region, Brazil, and North America, numerous more *Pratylenchus* species have been identified and reported.

Root-lesion nematodes are migratory endoparasites that cause severe necrosis of epidermal, cortical, and endodermal cells in chickpea roots by feeding in the cortical parenchyma. A combination of stylet thrusting and enzymatic weakening of the host cell walls facilitates both root penetration and migration inside root tissues (Castillo & Vovlas, 2007). Wheat, in addition to chickpea, has been identified as a possible RLN host (Di vito *et al.*, 1987).

#### **Chickpea-RLN interactions**

Taylor *et al.* (2000) established parasitic behavior of *Pratylenchus thornei* in chickpea and further specified that it's life cycle can be from 45 to 65 days depending upon different environmental features and host availability. However, under artificial conditions using carrot disk culture, *P. thornei* can take 25 to 35 days to complete its life cycle under the temperature incubation at 20 to 25°C (Castillo *et al.*, 1995). Therefore, many generations of *P. thornei* can happen in one crop season (Sikora *et al.*, 2018). Bridge and Starr (2007) affirmed that all the motile stages of *Pratylenchus* are parasitic. They further specified

the behavior of migratory endoparasites and their feeding was confined inside the cortex, deposited eggs singly in the cavities formed by migration of nematode inside the paranchymatic cells of plant roots. De Waele and Elsen (2007) reported that reproduction in the females of *Pratylenchus thornei* is by mitotic parthenogenesis and males are rare. Pudasaini *et al.* (2008) confirmed that females of *P. thornei* deposit eggs in the soil. The eggs and nematodes of *P. thornei* can withstand in the soil under the conditions when host plants are not available. Under the circumstances of slow drying of soil, a high proportion of these nematodes can withstand and survive under these prevailing dry vicinity (Thompson *et al.*, 2017, 2018).

Depending upon the population of *P. thornei*, damage may vary and as a consequence of its infection huge segments of cortex are degraded, and subsequently absorption capacity of roots is also significantly reduced (Jaques and Schwass, 1956). The population of *P. thornei* remained inside the cortical tissues of chickpea roots, according to Tiwari *et al.* (1992). *P. thornei* infection results in dark brown to black lesions on chickpea roots. Root-lesion nematode damage is often less noticeable than root-knot or cyst nematode damage (Sharma *et al.*, 1992), and signs of *P. thornei* root damage do not necessarily manifest themselves on above-ground plant parts. In addition to these symptoms, sometimes plant weight reduction, reduced per cent pollen fertility, lesser number of pods, lower water absorption capacity and lesser chlorophyll content of leaves could also be witnessed due to RLN when compared with healthy chickpea plant (Tiyagi and Parveen, 1992). The above ground symptoms imitate nutrients and water deficiencies symptoms (Taylor *et al.*, 1999). The lesions generally first appear on roots as water-soaked area after initiation of penetration of root epidermis by *P. thornei*. The elliptical shaped water-soaked lesions of 1 to 2 mm in length are formed over time and change in colour takes place to olive green and finally to radish brown colour (De Waele and Elson, 2007).

The roots of chickpea are significantly damaged upon infection by *P. thornei*. However, chickpea plant inoculation with different numbers of lesion nematodes (10, 100, 1000, and 10000 RLN/plant) identified 100 nematode/kg soil as threshold value for significant growth character reductions. At the lowest inoculum level, nematode multiplication

**Table 2: Identification of resistance sources for *P. thornei* in chickpea.**

Total No. of lines screened	No. of lines		Source of germplasm	Reference
	Resistant	Moderately resistant		
215	35	68	Indian Institute of Pulse Research (IIPR), Kanpur; JNKVV, Jabalpur, India	Tiwari <i>et al.</i> , 1992
600	0	17	International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Telangana, India and IIPR, Kanpur, India	Ali and Ahmad, 2000
453	1	14	International Center for Agricultural Research in the Dry Areas (ICARDA), ICRISAT, Australian cultivars and breeding lines	Thompson <i>et al.</i> , 2011
147	21	18	Collection of primary, secondary and tertiary gene pool (Primary: <i>C. echinospermum</i> , <i>C. reticulatum</i> ; Secondary: <i>C. bijugum</i> , <i>C. judaicum</i> , <i>C. pinnatifidum</i> , <i>C. chorassanicum</i> ; Tertiary: <i>C. cuneatum</i> , <i>C. yamashitae</i> )	Di Vito <i>et al.</i> , 1995
96	2	0	ICRISAT accessions	Jatav and Tiwari, 2019
174	13	40	Collection of primary, gene pool ( <i>C. echinospermum</i> , <i>C. reticulatum</i> )	Reen <i>et al.</i> , 2019

was at its peak (Walia and Seshadri, 1985). Threshold damage level of RLN in chickpea has been identified as 2 nematodes/g of soil (Bhatt, 1994). The nematode's reproduction index decreases with increase of inoculum densities greater than 5000 nematodes per plant (Castillo *et al.*, 1995). The temperature ranging from 10 - 25°C, favours the egg hatching in *P. thornei* with steady increase in root penetration in the first 11 days after inoculation. All the migratory stages of *P. thornei* are proficient enough for root penetration and give rise to symptom expression (Castillo *et al.*, 1996). In total, 47% to 84% of the population of RLN is could be identified in the vertical layer of soil from the top 0–10 cm, and 64–94% of the RLN population could be recovered from the top 20 cm soil zone (Taylor and Evans, 1998). However, it may be present upto 30 cm (Smiley *et al.*, 2008; Jatav, 2019)

#### Management strategies for nematode population

Nematicides are nematode-killing chemicals which include two primary categories of synthetic nematicides, fumigants and nonfumigants. Fumigants are often sold as liquids that react with soil water to release gases that kill a variety of organisms (including plants). They're biocides that can be used in a variety of situations. When the soil

temperatures are acceptable, fumigants should be used in the fall or spring. In soil water, nonfumigant nematicides do not volatilize. In some cases, they can be used before, during, or even after planting. The range of these substances is frequently not as extensive as that of fumigants. They not only control nematodes, but also reduce the population of beneficial nematodes. Besides using nematicides, eco-friendly management strategies like use of different plant bi-products and bioagents are eco-friendly approaches for management of nematodes. Plant-parasitic nematode management has traditionally relied on organic amendments. When disintegrating materials emit poisonous substances, nematode population levels may drop quickly, but longer-term impacts could include an increase in nematode antagonists. Plant-parasitic nematodes tolerance may be enhanced due to improved crop nutrition and plant development as a result of amendment application. The success in nematode control may be governed by several factors including the type of used material, processing/composting of material, rate of application, test arena, crop rotation practice adoption and different agronomic techniques, soil type, climate conditions, and other environmental factors. The suppressive effects of different plant bi-products as organic amendments have been reported to exhibit not only detrimental effects on

nematode populations but also in improvement of structure of soil and water holding capacity (WHC) ultimately resulting in enhanced growth and yield. Chickpea root-knot nematodes can be effectively suppressed adopting pre-sowing seed treatment practice using different biopesticides, insecticides, and bioagents (Mishra *et al.*, 2003).

Apart from soil amendments, different biagents have also been identified in the capacity of controlling nematode population in soil. Seed or soil application of bioagents not only increases the egg parasitization but also significantly enhances plant growth parameters. Successful application of these plant bi-products and bioagents in chickpea and other crops is listed in table 1.

### Resistance sources for RLN

When it comes to screening germplasm for sources of resistance, precise and reliable phenotyping is critical. For generating the phenotypic data of different lines/varieties, precise phenotyping is required under controlled environmental conditions using a known initial population of nematodes and/or eggs. The RLNs need to be extracted and quantified (in terms of nematode population) either from both roots and soil or any one of them before and after experimentation in case of migratory root-lesion nematodes. *P. thornei* resistance levels have been reported in connection to the reproduction factor (final nematode population/initial nematode population) by researchers (Tiwari *et al.*, 1992; Di Vito *et al.*, 1995), or as number of nematodes (population count) per unit of root and/or soil (Thompson *et al.*, 2011; Reen *et al.*, 2019). Visual lesions observed on infected roots should not be counted and measured for consideration of nematode population or indicator of resistant reaction because these lesions are just symptoms and not a direct indicator of population of nematodes (Ali and Ahmad, 2000).

In India (Tiwari *et al.*, 1992; Ali and Ahmad, 2000, Gautam, 2021) and Australia (Thompson *et al.*, 2011), sources of *P. thornei* resistance and moderate resistance have been recognized in the *C. arietinum* cultigen, in breeding lines and in accessions in the ICRISAT genebank. Tiwari *et al.*, 1992 reported 35 resistant and 68 moderately resistant varieties out of 215 screened varieties of chickpea for the resistance against *P. thornei* and

found. Di Vito *et al.*, 1995 screened 141 varieties taken from primary and secondary gene pool and observed seventeen different varieties in each category of resistant and moderately resistant. A comprehensive list of chickpea varieties screened against *P. thornei* is provided in table 2. In general, it has been reported that wild chickpea exhibited higher level of resistance in comparison to of *C. arietinum* cultivars (Reen *et al.*, 2019; Zwart *et al.*, 2019).

### Conclusion

Human population expansion coupled with stunning changes in global food consumption patterns under unpropitious climatic alterations is posing formidable obstacles towards attaining sustainable global food security. Chickpeas are recognised as cost-effective sources of plant-based protein for human consumption, as well as being good to the environment due to their intrinsic nitrogen-fixing capacity. Chickpea development is critical in the rapidly transitioning global landscape where booming anthropogenic activities are causing irreversible natural resource depletion. Nematodes are important pests of agricultural crops, causing annual global economic losses of more than 100 billion dollars. Furthermore, chickpea damage from nematodes can make it more susceptible to disease and other pressures. Three essential aspects for the integrated control of plant-parasitic nematodes in chickpea are accurate diagnosis, effective crop rotations or fallow times, and identification, development of tolerant/resistant crop cultivars. To accomplish these elements in nematode management, extensive expertise of nematode taxonomy and/or application of molecular diagnostic tools are essential. Due to the extensive host range of nematodes, crop rotation options are limited in nematode-infested farms, and nematicides are avoided for environmental and economic reasons. The employment of bioagents with resistant cultivars is the most successful and long-term technique for overcoming restrictions to chickpea production induced by plant-parasitic nematodes. Growing resistant cultivars offers the benefit of avoiding nematode reproduction and lowering existing crop output losses. Furthermore, when resistant cultivars are grown, nematode populations remaining in the soil to damage later crops are reduced, benefiting the entire farming system.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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