

RESEARCH NOTE/NOTA DE INVESTIGACIÓN

RESISTANCE OF MOROCCAN WHEAT LINES AGAINST THE ROOT- LESION NEMATODE *PRATYLENCHUS THORNEI*

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ABSTRACT

Laasli, S.-E., F. Mokrini, M. Ferrahi, I. Driss, S. Udupa, R. Lahlali, and A. A. Dababat. 2024. Resistance of Moroccan wheat lines against the root-lesion nematode *Pratylenchus thornei*. *Nematropica* 54:22-28.

The root lesion nematode, *Pratylenchus thornei*, causes high yield losses in rainfed wheat fields in Morocco, as well as worldwide. Growing resistant varieties is one of the most effective methods for controlling nematodes. Therefore, a collection of 69 wheat lines (*Triticum aestivum* and *T. durum*), provided by the National Institute of Agricultural Research (INRA-Meknes, Morocco) and the International Center for Agricultural Research in the Dry Areas (ICARDA-Rabat, Morocco), were screened for resistance to *P. thornei* in tubes (15 × 20 × 120 mm³) under greenhouse conditions. The resistance level was evaluated based on the number of nematodes extracted from roots and soil 9 weeks after infestation. Three lines, L3 (DW-37), L14 (DW-37), and L54 (USG3535), were found to be moderately resistant (Reproduction factor <1) to *P. thornei*.

Key words: Nematode, *Pratylenchus thornei*, resistance, *Triticum aestivum*, wheat

RESUMEN

Laasli, S.-E., F. Mokrini, M. Ferrahi, I. Driss, S. Udupa, R. Lahlali, and A. A. Dababat. 2024. Resistencia de las líneas de trigo marroquíes contra nematodos lesionadores de la raíz (*Pratylenchus thornei*). *Nematropica* 54:22-28.

Los nematodos lesionadores de la raíz *Pratylenchus thornei* causan grandes pérdidas de rendimiento en los campos de trigo de secano en Marruecos, así como en todo el mundo. El cultivo de variedades resistentes es uno de los métodos más efectivos para controlar los nematodos. Por tanto, se ha recopilado una colección de 69 líneas de trigo (*Triticum aestivum* y *T. durum*), proporcionada por el Instituto Nacional de Investigación Agrícola (INRA-Meknes, Marruecos) y el Centro Internacional de Investigación Agrícola en las Zonas Áridas (ICARDA-Rabat, Marruecos), fueron examinados para detectar resistencia a *P. thornei* en tubos (15 × 20 × 120 mm³) en condiciones de invernadero. El nivel de resistencia se evaluó con base en el número de nematodos extraídos de las raíces y el suelo nueve semanas después de la infestación. Tres

líneas L3 (DW-37), L14 (DW-37) y L54 (USG3535) se encontraron como moderadamente resistentes (factor de reproducción <1) a *P. thornei*.

Palabras clave: Nematodo, resistencia, *Pratylenchus thornei*, *Triticum aestivum*, trigo

Among the cereal crops, wheat (*Triticum aestivum* and *T. durum*) occupies a decent position regarding production, nutrition source, and acreage patterns, especially in developing countries (Nicol *et al.*, 2011). In 2020-21, global wheat production was estimated to exceed 768 million metric tons (USDA, 2020). Wheat has the ability to adapt various geo-climatic conditions as well as dietary traditions as it grows under both irrigated and non-irrigated conditions (Dababat *et al.*, 2015). Around 12.6% of the global annual wheat yield loss is attributed to plant-parasitic nematode damage, which represents an annual monetary loss of \$216 billion (Nyaku *et al.*, 2017). In cereals, plant-parasitic nematodes mostly belong to two groups, root lesion nematodes (RLN, *Pratylenchus* spp.) and cereal cyst nematodes (CCN, *Heterodera* spp.). Root lesion nematodes are widespread and considered one of the most important groups of plant-parasitic nematodes in the world (Castillo and Vovlas, 2007). In Morocco, *Pratylenchus* spp. constitute the most important group of plant-parasitic nematodes in different wheat-growing areas (Mokrini *et al.*, 2016). Two species of RLN, *Pratylenchus thornei* and *Pratylenchus penetrans* were identified in different wheat-producing regions of Morocco (Mokrini *et al.*, 2016).

Many strategies have been developed to manage RLN occurrence, including chemical control, cultural practices, and the use of resistant wheat lines (Dababat *et al.*, 2019; Mokrini *et al.*, 2019). The use of resistant and tolerant wheat cultivars is considered one of the most eco-environmental and promising methods for managing RLN in different cropping systems (Nicol *et al.*, 2011; Mokrini *et al.*, 2019). The objective of this study was to investigate the resistance of wheat lines to *P. thornei*.

Sixty-nine wheat germplasm collections provided by the National Institute of Agricultural Research (INRA) and the International Center for Agricultural Research in the Dry Areas (ICARDA) were screened for resistance against *P. thornei* (Table 1). The germplasm represented a collection

of 40 lines of durum wheat (INRA-Meknes, Morocco) and 29 lines of both durum and winter wheat (ICARDA-Rabat, Morocco). Seeds were surface sterilized with 3% sodium hypochlorite and rinsed several times in sterilized distilled water, then placed in sterilized Petri dishes with moist blotting paper and left to germinate for 3 days at 23°C. A single seedling with 3, 1-2 cm long seminal roots was transplanted in a plastic tube (15 × 20 × 120 mm) containing a potting mixture of sterilized sand and field soil (70:30 v/v). Sand and field soils were sterilized at 110°C for 2 hr and organic fertilizer was sterilized at 70°C for 5 hr. Four replicates of each entry were arranged in a completely randomized design in a greenhouse with temperatures between 22°C and 24°C. Plants were sprayed daily with water using an atomizer. Two standard durum wheat lines, CROC_1/AE.SQUARROSA (224//OPATA and Ourgh, were chosen as check lines for their recognized resistance (CLr) and susceptibility (CLs) to *P. thornei*, respectively (Mokrini *et al.*, 2018).

Experiments were carried out using one population of *P. thornei* collected from the Zaers region of Morocco. This population was extracted from wheat roots and soil collected in 2020. The population was maintained *in vitro* on carrot-disc cultures according to Moody *et al.* (1973) after their morphological and morphometrical identification. The nematodes were extracted from carrot disks using the modified Baermann method (Hooper, 1986). To obtain a uniform population of *P. thornei*, the inoculum of this population was further processed using a 20-µm sieve to separate eggs from juveniles. Nematode suspensions, containing all vermiform stages of *P. thornei*, were prepared using tap water. One week after planting, each seedling was inoculated with a suspension containing 400 individuals of *P. thornei* (Toktay *et al.*, 2012). The inoculum suspension was transferred into 3, 2-cm deep holes 0.5 cm distance from the seedling. The plants were kept in a growth chamber for 9 weeks.

Table 1. Durum and winter wheat lines evaluated against *Pratylenchus thornei* plus the two check lines with their resistance reaction.

Code	Line	Type of wheat ^v	Origin ^w	Reproduction Factor (RF)	RR ^x
L1	21 DW-01	DW	INRA-Meknes	2.29 ± 0.3 ^y	MS
L2	21 DW-02	DW	INRA-Meknes	4.69 ± 0.37	HS
L3	21 DW-03	DW	INRA-Meknes	0.5 ± 0.2	R
L4	21 DW-04	DW	INRA-Meknes	3.01 ± 0.6	S
L5	21 DW-05	DW	INRA-Meknes	2.28 ± 0.2	MS
L6	21 DW-06	DW	INRA-Meknes	3.27 ± 0.4	S
L7	21 DW-07	DW	INRA-Meknes	3.98 ± 0.2	S
L8	21 DW-08	DW	INRA-Meknes	3.11 ± 0.5	S
L9	21 DW-09	DW	INRA-Meknes	2.67 ± 0.4	MS
L10	21 DW-10	DW	INRA-Meknes	5.34 ± 0.4	HS
L11	21 DW-11	DW	INRA-Meknes	2.33 ± 0.3	MS
L12	21 DW-12	DW	INRA-Meknes	7.14 ± 0.4	HS
L13	21 DW-13	DW	INRA-Meknes	3.3 ± 0.4	HS
L14	21 DW-14	DW	INRA-Meknes	0.54 ± 0.1	R
L15	21 DW-15	DW	INRA-Meknes	5.19 ± 0.3	HS
L16	21 DW-16	DW	INRA-Meknes	2.71 ± 0.3	MS
L17	21 DW-17	DW	INRA-Meknes	5.91 ± 0.3	HS
L18	21 DW-18	DW	INRA-Meknes	2.77 ± 0.3	MS
L19	21 DW-19	DW	INRA-Meknes	2.03 ± 0.2	MS
L20	21 DW-20	DW	INRA-Meknes	3.58 ± 0.1	S
L21	21 DW-21	DW	INRA-Meknes	2.05 ± 0.3	MS
L22	21 DW-22	DW	INRA-Meknes	5.21 ± 0.2	HS
L23	21 DW-23	DW	INRA-Meknes	7.29 ± 0.5	HS
L24	21 DW-24	DW	INRA-Meknes	2.37 ± 0.3	MS
L25	21 DW-25	DW	INRA-Meknes	3.52 ± 0.1	S
L26	21 DW-26	DW	INRA-Meknes	3.78 ± 0.4	S
L27	21 DW-27	DW	INRA-Meknes	2.22 ± 0.2	MS
L28	21 DW-28	DW	INRA-Meknes	4.57 ± 0.2	HS
L29	21 DW-29	DW	INRA-Meknes	5.45 ± 0.3	HS
L30	21 DW-30	DW	INRA-Meknes	3.52 ± 0.1	S
L31	21 DW-31	DW	INRA-Meknes	4.58 ± 0.1	HS
L32	21 DW-32	DW	INRA-Meknes	3.11 ± 0.2	S
L33	21 DW-33	DW	INRA-Meknes	4.22 ± 0.1	HS
L34	21 DW-34	DW	INRA-Meknes	1.7 ± 0.2	MR
L35	21 DW-35	DW	INRA-Meknes	4.04 ± 0.2	HS
L36	21 DW-36	DW	INRA-Meknes	2.84 ± 0.3	MS
L37	21 DW-37	DW	INRA-Meknes	2.99 ± 0.5	MS
L38	21 DW-38	DW	INRA-Meknes	4.49 ± 0.4	HS
L39	21 DW-39	DW	INRA-Meknes	2.04 ± 0.3	MS
L40	21 DW-40	DW	INRA-Meknes	3.35 ± 0.3	S
L41	1265	WW	ICARDA-Rabat	5.04 ± 0.3	HS
L42	1256	WW	ICARDA-Rabat	4.56 ± 0.3	HS
L43	Icamor	DW	ICARDA-Rabat	2.31 ± 0.1	MS
L44	Dha Nass	DW	ICARDA-Rabat	2.53 ± 0.5	MS
L45	BT7	DW	ICARDA-Rabat	3.03 ± 0.2	S
L46	BD5	DW	ICARDA-Rabat	1.67 ± 0.2	MR
L47	Florence	DW	ICARDA-Rabat	4.16 ± 0.5	HS
L48	Kharoba	DW	ICARDA-Rabat	3.08 ± 0.1	S
L49	BT33	DW	ICARDA-Rabat	4.38 ± 0.3	HS

Table 1. Continued.

Code	Line	Type of wheat ^v	Origin ^w	Reproduction Factor (RF)	RR ^x
L50	1257	WW	ICARDA-Rabat	3.81 ± 0.3	S
L51	1255	WW	ICARDA-Rabat	3.27 ± 0.1	S
L52	BD3	DW	ICARDA-Rabat	6.69 ± 1.3	HS
L53	Parula	DW	ICARDA-Rabat	3.09 ± 0.2	S
L54	USG3535	DW	ICARDA-Rabat	0.79 ± 0.1	R
L55	1179	WW	ICARDA-Rabat	3.98 ± 0.1	S
L56	1258Bidri	DW	ICARDA-Rabat	4.91 ± 0.4	HS
L57	DW5004	DW	ICARDA-Rabat	5.24 ± 0.3	HS
L58	Sumia3	DW	ICARDA-Rabat	4.95 ± 0.3	HS
L59	1267	WW	ICARDA-Rabat	7.45 ± 0.4	HS
L60	1266	WW	ICARDA-Rabat	4.96 ± 0.3	HS
L61	Aguilal	DW	ICARDA-Rabat	3.68 ± 0.2	S
L62	Nax2-BW5907	DW	ICARDA-Rabat	6.34 ± 0.5	HS
L63	BT8	DW	ICARDA-Rabat	4.63 ± 0.3	HS
L64	Nax1-BW5020	DW	ICARDA-Rabat	6.07 ± 0.3	HS
L65	MGB61272	DW	ICARDA-Rabat	3.7 ± 0.1	S
L66	SF 74	DW	ICARDA-Rabat	2.19 ± 0.1	MS
L67	MGB61195	DW	ICARDA-Rabat	2.87 ± 0.3	MS
	CROC_1/AE.SQU				
CLr ^z	ARROSA (224)//OP	DW	CYMMIT	0.3 ± 0.08	R
CLs ^z	Ourgh	DW	Morocco	4.09 ± 0.2	HS

^vDW = Durum Wheat, WW = Winter Wheat

^wNational Institute of Agricultural Research (INRA-Meknes, Morocco) and the International Center for Agricultural Research in the Dry Areas (ICARDA-Rabat, Morocco)

^xabbreviations in this column : RR = Resistance Reaction, R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible, HS = Highly Susceptible.

^yValues are the mean ± standard error (n = 4)

^zCLr = Resistant check line, CLs = Susceptible check line.

Plants were harvested 9 weeks after inoculation and aboveground plant parts were removed. The soil was then removed from the roots by gently shaking the plants. Nematodes were extracted from soil and roots using a modified Baermann funnel method (Hooper, 1986). The roots were washed separately for every plant. Nematodes were released from the roots by cutting the root system into 2-cm pieces and macerating them in water for 1 min at high speed in a commercial blender prior to placement on funnels. The reproduction factor (RF) of *P. thornei* was determined by dividing the final nematode population (Pf) by the initial nematode population (Pi). The Pf of each line was determined from the total number of vermiform stages of *P. thornei* extracted from both the soil and roots.

The resistant reaction was assessed according to five distinctive groups which were: Resistant (R)

= RF ≤ 1; Moderately Resistant (MR) = RF between 1-2, slightly more nematodes than in a resistant check; Moderately Susceptible (MS) = RF between 2-3, significantly more nematodes than in a resistant check, but not as many as in the susceptible check; Susceptible (S) = RF between 3-4, same nematode densities as of the susceptible check; and Highly Susceptible (HS) = RF more than 4, more nematodes than in the susceptible check (Dababat *et al.*, 2016).

Data were processed using analysis of variance (ANOVA) after being assessed for normalization assumptions by the Anderson–Darling normality test (Stephens, 1974). The Protective Least Significant Difference (LSD) test was adopted to detect significant differences between lines at $P < 0.001$ using SPSS software V 17.0 (SPSS Inc., Chicago, IL, USA). The population structure of the lines was distinguished

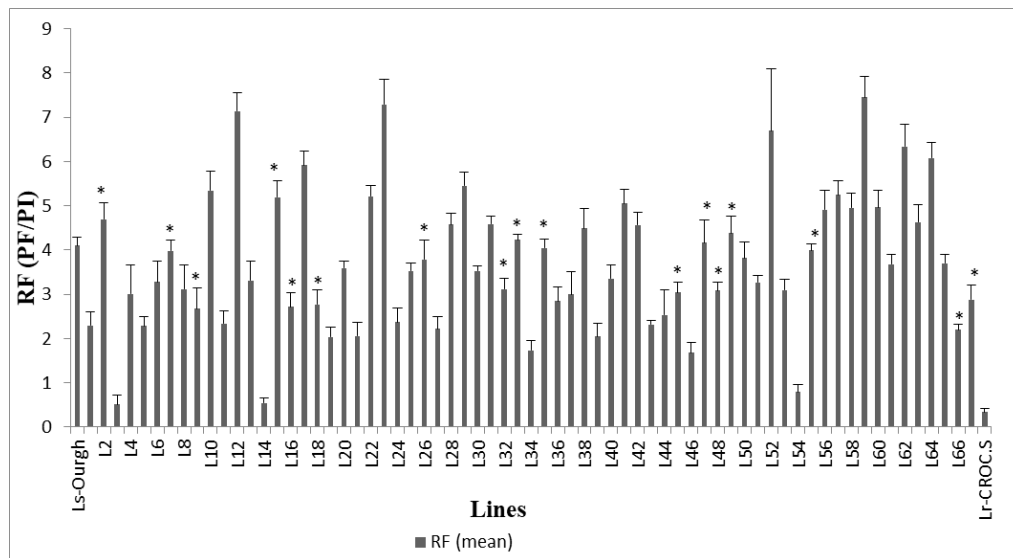


Figure 1. *Pratylenchus thornei* reproduction factor [RF = final population density (PF)/initial population density (PI)] on wheat lines maintained in a growth chamber. Stars represent homogenous groups based on the LSD test for each line at $P < 0.001$. Error lines represent the standard error (SE) ($n = 4$).

by Linear Discriminant Analysis (LDA) to determine putative groups of wheat lines based on their resistance and susceptibility to *P. thornei*. Nine weeks after inoculation, the RF of *P. thornei* in the 67 lines of wheat ranged from 0.5 to 7.4 (Fig. 1; Table 1). The RF values of both check lines ranged from 0.33 (Lr) to 4.1 (Ls). Lines L3, L14, and L54 were the most resistant, with RF values of 0.5, 0.54, and 0.79, respectively (Table 1). In addition, L34 and L46 were moderately resistant, with RF values of 1.7 and 1.6, respectively.

Population structure based on LDA displayed five distinct groups, depicting the resistance reaction against *P. thornei* among the 67 lines evaluated (excluding both check lines) (Fig. 2). The first two groups consisted of three resistant (R) lines, including L3, L14, and L54, followed by four moderately resistant (MR) lines, including L34 and L46. The third group was comprised of 15 moderately susceptible (MS) lines, including (L1, L5, L9, L11, L16, L18, L19, L21, L24, L27, L36, L39, L43, L44, L66, and L67). Eighteen susceptible lines (S) were determined, followed by 27 highly susceptible lines. Several studies have evaluated RLN resistance in wheat lines through growth chambers, glasshouses, and field experiments. For instance, Laasli *et al.* (2022)

found that out of 150 spring wheat lines from the 18 KASIB-CORE nursery, 48 were resistant to *P. thornei*. Similarly, Dababat *et al.* (2019) discovered that out of 484 CIMMYT spring wheat varieties, 56 were resistant to *P. thornei* under controlled growth room conditions. In Türkiye, Duman *et al.* (2021) tested 19 spring wheat cultivars against *P. thornei* and found that nine accessions had moderate resistance. Imren *et al.* (2015) also evaluated 82 durum wheat lines grown in Türkiye and found that, while none had total resistance to *P. thornei*, 29 were moderately resistant. Kranti and Kanwar (2012) also screened various *T. durum* and *T. aestivum* against *P. thornei* and found that nine lines were resistant to a *P. thornei* population from India. Additionally, many other CIMMYT wheat lines have been reported to be partially or totally resistant to *P. thornei*.

In conclusion, three lines of wheat, L3 (DW-37), L14 (DW-37), and L54 (USG3535) were resistant to *P. thornei*. This study provides baseline insights for selecting genuine Moroccan wheat lines for future breeding and disease management programs involving resistant attributes. However, the field performance of these lines against *P. thornei* parasitism should be evaluated before they are released to farmers to confirm resistance.

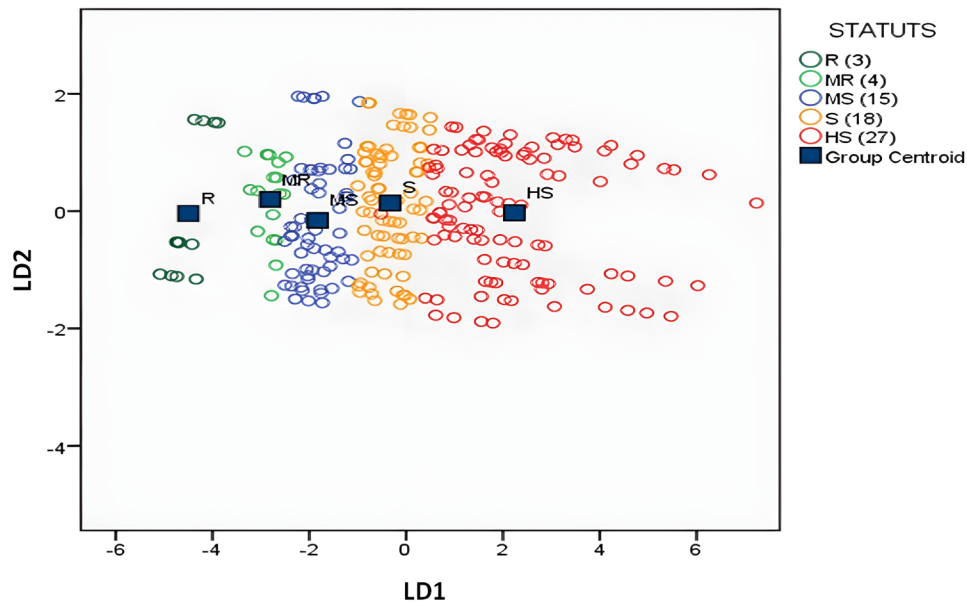


Figure 2. Linear Discriminant Analysis (LDA) showing the population structure for a set of 67 lines of wheat based on RF values (final population density/initial population density); LDA displaying resistance reaction (RR) ranking to *Pratylenchus thornei*. Abbreviations stand for: R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible, HS = Highly Susceptible.

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