OCCURRENCE AND DISTRIBUTION OF SPECIES OF THE *HETERODERA AVENAE* GROUP IN SYRIA AND TURKEY

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Summary. A survey of cyst nematodes showed that 69.9% and 80% of cereal fields were infested, respectively, in Syria and Turkey. Based on morphometrics, three species belonging to the *Heterodera avenae* group were identified. In Syria, *Heterodera latipons* was the dominant species, being found in 96% of the cereal fields; *Heterodera avenae* has a very limited distribution, being found in only three fields of northern and central regions. *Heterodera filipjevi* was detected for the first time in one barley field in Northern Syria near the Turkish border. In Turkey, *H. filipjevi* and *H. latipons* were widely distributed in the Central Anatolian Plateau occurring as single species in 37.5% and 33.3% of infested fields, respectively, and as species mixtures in 29.2% of infested fields. Phylogenetic and phylogeographic research prospects in this group of cyst nematodes and control methods based on the use of resistance genes in cereals are discussed.

Cereals are the most extensively grown crops in Syria and Turkey but have low average yields of around 2 t/ha. Over five million hectares of cereals in Turkey and 2.26 M ha in Syria are under rainfed and limited irrigation. In these countries, cereal monoculture prevails and this provides the main sources of food security for both human and animals. Under these marginal growing conditions the combined effects of abiotic (mainly drought) and biotic (soil-borne diseases) stresses are known to affect cereal productivity (Sikora, 1988; FAO, 1999; Scholz, 2001; Nicol *et al*., 2004a). Disease caused by cyst nematodes belonging to the *Heterodera avenae* group is one of the most important soil-borne diseases, along with root rots caused by fungi (van Leur and Bailey, 2000; Nicol *et al*., 2004a). The main cyst nematode species attacking cereals are *Heterodera avenae* Wollenweber, *Heterodera filipjevi* (Madzhidov) Stelter and *Heterodera latipons* Franklin (Rivoal and Cook, 1993; Evans and Rowe, 1998; Nicol, 2002).

Heterodera avenae occurs in most cereal growing areas of the world (Meagher, 1977), and is known as a major production constraint of cereals in Europe (Rivoal and Cook, 1993), Australia (Brown, 1984), India (Khan *et al*., 1990), North America (Miller, 1986) and in several countries of North Africa and West Asia (Sikora, 1988; Al-Yahya *et al*., 1998). *Heterodera latipons* has been found in the Mediterranean region (Franklin, 1969; Tacconi, 1976; Romero, 1980; Sikora and Oostendorp, 1986; Philis, 1988; Greco, 1994; Enneli *et al*., 1994), but it has also been found in the temperate continental climates of Southern Russia, Ukraine, Central Asian Republics (Subbotin *et al*., 1996), Iran (Talatschian *et al*., 1976), Europe (Stoyanov, 1982; Sabova *et al*., 1988), and Canada (Sewell, 1973). *Heterodera filipjevi* has been reported in the former USSR (Subbotin *et al*., 1996), Iran (Sturhan, 1996), Turkey (Rumpenhorst *et al*., 1996), and in several European countries (Bekal *et al*., 1997; Rivoal *et al*., 2003; Subbotin *et al*., 2003; Holgado *et al*., 2004).

Considerable yield damage of economic importance can be caused by these cyst nematodes, especially in temperate climates and, more particularly, in semi-arid regions, where they can increase drought stress (Rivoal and Cook, 1993). Yield losses caused by *H. avenae* reached 20% on barley and 23-50% on wheat in Australia (Meagher, 1972) and ranged between 15 and 20% on wheat in Pakistan (Maqbool, 1988), 40-92% on wheat and 17-77% on barley in Saudi Arabia (Ibrahim *et al*., 1999). In Cyprus, *H. latipons* decreased barley yields by 50% (Philis, 1988). In Syria, grain and straw losses caused by *H. latipons* could be an important constraint on barley and durum wheat production in semi-arid regions (Scholz, 2001). The economic importance of *H. filipjevi* on winter wheat was determined under rainfed conditions in Turkey, which showed that this nematode is one of the major causes of yield loss in wheat (Öztürk *et al*., 1999; Nicol *et al*., 2004b).

The three main species of the *H. avenae* group have spread throughout the cereal growing regions from the Fertile Crescent. Thus it is important to accurately identify the different species, determine their relative distribution and possible association with different cereal crops and environmental conditions, and develop appropriate and effective integrated control strategies. Our study was carried out in the major cereal growing

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Table I. Occurrence of cyst nematodes of the *Heterodera avenae* group in wheat and barley fields surveyed throughout Syria and Turkey.

Provinces and locations	Number of fields surveyed[a]		*Heterodera* species	Range of infestation (cysts per 100 g of soil)	
	Wheat	Barley		Wheat	Barley
Syria					
Darra	18(15)	3(3)	*H. latipons*	1 to 24	4 to 18
Damascus	2(2)	na	*H. latipons*	7 to 22	na
Homs	3(2)	1(0)	*H. latipons*	1 to 2	na
Hama	7(4)*	14(13)	*H. latipons*	1 to 126	1 to 319
			H. latipons+*H. avenae*	29+15	na
Lattakia	6(2)	na	*H. latipons*	1 to 4	na
Tartous	2(0)	na		0	na
Idleb	1(1)	5(5)	*H. latipons*	15	17 to 91
Aleppo	16(12)	22(16)*	*H. latipons*	1 to 56	1 to181
			H. avenae	na	161
			H. latipons+*H. avenae*	na	18+36
			H. latipons+*H. filipjevi*	na	30+4
Raqqa	7(2)	13(12)	*H. latipons*	1	1 to 166
Der Ezzor	3(1)	na	*H. latipons*	1	na
Hassakeh	15(10)	5(0)	*H. latipons*	1 to 30	0
Total	80(51)	63(49)			
Turkey					
Osmaniye	1(1)*	na	*H. filipjevi*+*H. latipons*	2+2	na
Ceyhan	1(1)*	na	*H. filipjevi*+*H. latipons*	7+2	na
Erefli	1(0)	na		0	na
Karapinar	1(0)	na		0	na
Konya	2(1)	1(1)	*H. filipjevi*	4	47
Cumra	3(2)	na	*H. filipjevi*	2 to 111	na
Sarayonu	1(1)	na	*H. latipons*	4	na
Llgin	2(1)*	na	*H. filipjevi*+*H. latipons*	1+7	na
Cay	1(0)	na		0	na
Emiradag	1(1)*	na	*H. filipjevi*+*H. latipons*	2+14	na
Cifteler	1(1)	na	*H. filipjevi*	147	na
Hamidiye	na	1(1)*	*H. filipjevi*+*H. latipons*	na	18+3
Eskisehir	3(3)*	na	*H. filipjevi*	40	na
			H. filipjevi+*H. latipons*	22+3	na
			H. latipons	27	na
Alpu	2(2)	na	*H. filipjevi*	16	na
			H. latipons	13	na
Topkaya	1(1)	na	*H. filipjevi*	66	na
Karakaya	1(1)	na	*H. latipons*	7	na
Polatli	1(1)	1(1)	*H. latipons*	25	34
Temelli	1(1)	na	*H. latipons*	4	na
Haymana	3(3)*	na	*H. latipons*	4	na
			H. latipons+*H. filipjevi*	49+12	na
			H. filipjevi	30	na
Total	27(21)	3(3)			

[a] Numbers in brackets refer to infested fields; * presence of mixed species; na: data not available; +: indicates the number of cysts for each species, respectively.

areas of Syria and Turkey to identify the cyst nematodes found in barley and wheat fields and to determine their distribution and infestation levels.

MATERIALS AND METHODS

Surveys of cyst nematodes were conducted over a 4-year period (2000-2003) in the major cereal growing areas (Fig. 1), including 80 wheat and 63 barley fields representing 11 provinces in Syria and 27 wheat and three barley fields from 19 locations in the Central Anatolian Plateau of Turkey (Table I). Approximately two kilograms of soil were collected from each field by taking soil to a depth of 20 cm from at least five points 50 m apart and arranged in a zigzag fashion across the field during the period of grain filling to post-harvest. Soil samples were passed through a coarse sieve, to eliminate stones and straw residues, and thoroughly homogenised.

Cyst nematodes were extracted from 200 g sub-samples of soil from each field sample. The sub-samples were processed through a modified Fenwick can, following the procedure described for the Kort Elutriator (Kort, 1960). The cysts and soil residues were collected on a

250 µm pore sieve, transferred to cones of filter paper and stored dry at laboratory temperature. Cysts and soil residues were later floated on water in a vessel two-thirds filled with tap water and covered on its vertical interior sides with a band of filter paper. To help the cysts and soil debris move to the periphery of the vessel, a drop of liquid soap was added to the water surface. The filter paper band was then gently removed along with the attached cysts and soil debris and laid on a plastic sheet for examination under a stereomicroscope. The cysts were separated from other residues with a brush, and transferred to a moist filter paper in a Petri dish.

Cysts of *H. avenae*, *H. filipjevi* and *H. latipons* were identified according to their colour, and the presence or absence of bullae and an underbridge in the vulval cone (Fig. 2). When necessary, cysts were opened to observe these morphological characteristics. Total numbers of full and empty cysts were recorded to estimate average infestations per 100 g of soil (Table I).

RESULTS

Field infestation

Cyst nematodes occurred in 51 wheat fields (64%) and in 49 barley fields (78%) in Syria, and in 21 wheat fields (78%) and in all 3 barley fields in Turkey (Table I). Infested fields showed patches of stunted plants that varied in size, and cysts covered with a subcrystalline layer were found attached to the root systems.

In Syria, high infestation levels were found on barley in the major cereal growing areas represented by central, northern and northeastern regions in Hama, Aleppo and Raqqa provinces, respectively. The highest levels of infestation were found in the Hama-Bary Sharky, Aleppo-Bouider and Raqqa-Jurn Aswad locations, where the total number of cysts per 100 g of soil reached 319 (151), 181 (53) and 166 (75), respectively (numbers of full cysts in brackets). The north-western region represented by Idleb province also showed a high infestation level on barley, particularly at the Saraqeb location with 91 (5) cysts per 100 g of soil (Table I). Infestations in wheat fields generally were at lower levels than those in barley fields but high infestation levels were found on wheat in central, northern and southern regions represented by Hama, Aleppo and Darra provinces, respectively. Locations in Hama-Salamiah, Aleppo-Breda and Darra-Jellin showed the highest levels, where the total number of cysts per 100 g of soil reached 126 (13), 56 (8) and 24 (6), respectively. Other sites in these provinces and the rest of the sites sampled in eastern and coastal regions had very low infestation levels of cyst nematodes and some fields were almost free of them.

In Turkey, cyst nematodes were detected in almost all the fields sampled, which were predominantly cropped with wheat. In several fields, high infestation levels were recorded, with total numbers of cysts per 100 g of soil of 147 (23) at Cifteler, 111 (13) at Cumra, 66 (22) at Topkaya, 61 (10) at Haymana, 40 (7) at Eskisehir and 25 (9) at Polatli (Table I).

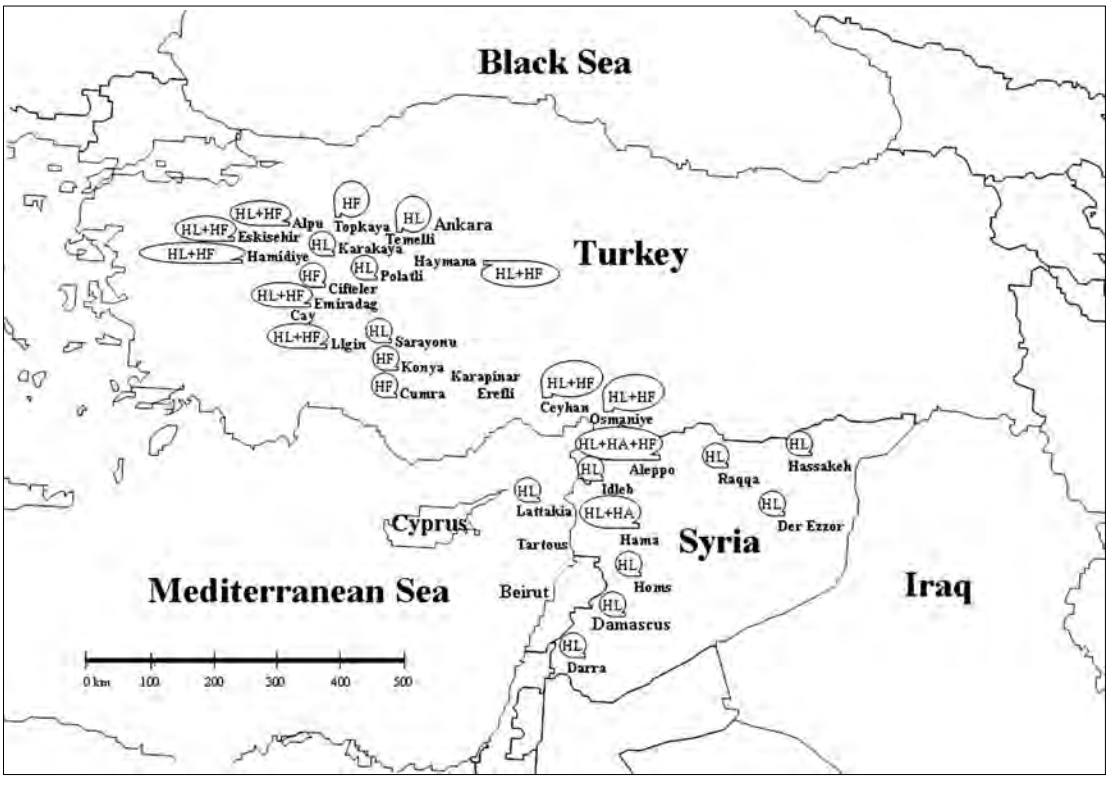

Fig. 1. Distribution of cyst nematodes belonging to the *Heterodera avenae* group in Syria and Turkey from surveys over a 4-year period (2000-2003): HA: *H. avenae*; HF: *H. filipjevi*; HL: *H. latipons*.

Identification of species

Root systems had a bushy knotted appearance with cysts grouped in each knot in fields infested specifically with *H. avenae*. Cysts extracted from samples were typically ovoid to lemon-shaped, and the main morphological characteristics of the cyst vulval cone that allowed the separation of the three species, *H. avenae*, *H. filipjevi* and *H. latipons*, are shown in Fig. 2.

Heterodera avenae has large cysts of dark-brown to black colour, heavy prominent bullae and no underbridge in the vulval cone (Fig. 2A). Cysts of *H. filipjevi* looked smaller, of yellow to light-brown colour. They had light bullae and a distinct underbridge close to the vulval bridge, thick in the middle and thin at the ends; a bifurcated terminus was not always seen at the cone periphery (Fig. 2B). *Heterodera latipons* had cysts of different sizes, brown in colour and with a round vulval cone top. No bullae could be found but a very strong underbridge with a pronounced enlargement in the middle facing the vulval slit was seen deeper in the vulval cone, and it was clearly bifurcated at both ends. The fenestration area of cysts belonging to this species was characterized by two distinct circular semi-fenestrae separated by a distance clearly greater than the semi-fenestra diameter. The fenestration area of cysts of the two other species showed two oval semi-fenestrae separated by a distance shorter than the semi-fenestra diameter (Fig. 2C).

Distribution of the cyst nematodes

The survey of cyst nematodes in Syria showed that 100 fields out of 143 were infested by one or more species of the *H. avenae* group (Fig. 1). *Heterodera latipons* was the most common and was found in 96 fields distributed throughout the major wheat and barley areas. *Heterodera avenae* was found in three fields: one cropped with barley at the Bayat El-Danish site (Aleppo province) and with a very high infestation level (161 cysts/100 g of soil). This species was also found mixed with *H. latipons* at two other sites: a barley field located at Bouider (Aleppo province) with 36 *H. avenae* and 18 *H. latipons* cysts per 100 g of soil, and a wheat field at Musyaf (Hama province) with 15 and 29 cysts per 100 g of soil, respectively. *Heterodera filipjevi* was present in one barley field at the Gandoura site (Aleppo province) in northern Syria close to the Turkish border (Fig. 1). Few cysts of *H. filipjevi* were found and these were mixed with *H. latipons* (4 and 30 cysts/100 g soil, respectively).

In Turkey, *H. filipjevi* and *H. latipons* were commonly distributed in wheat and barley fields. Nine infested

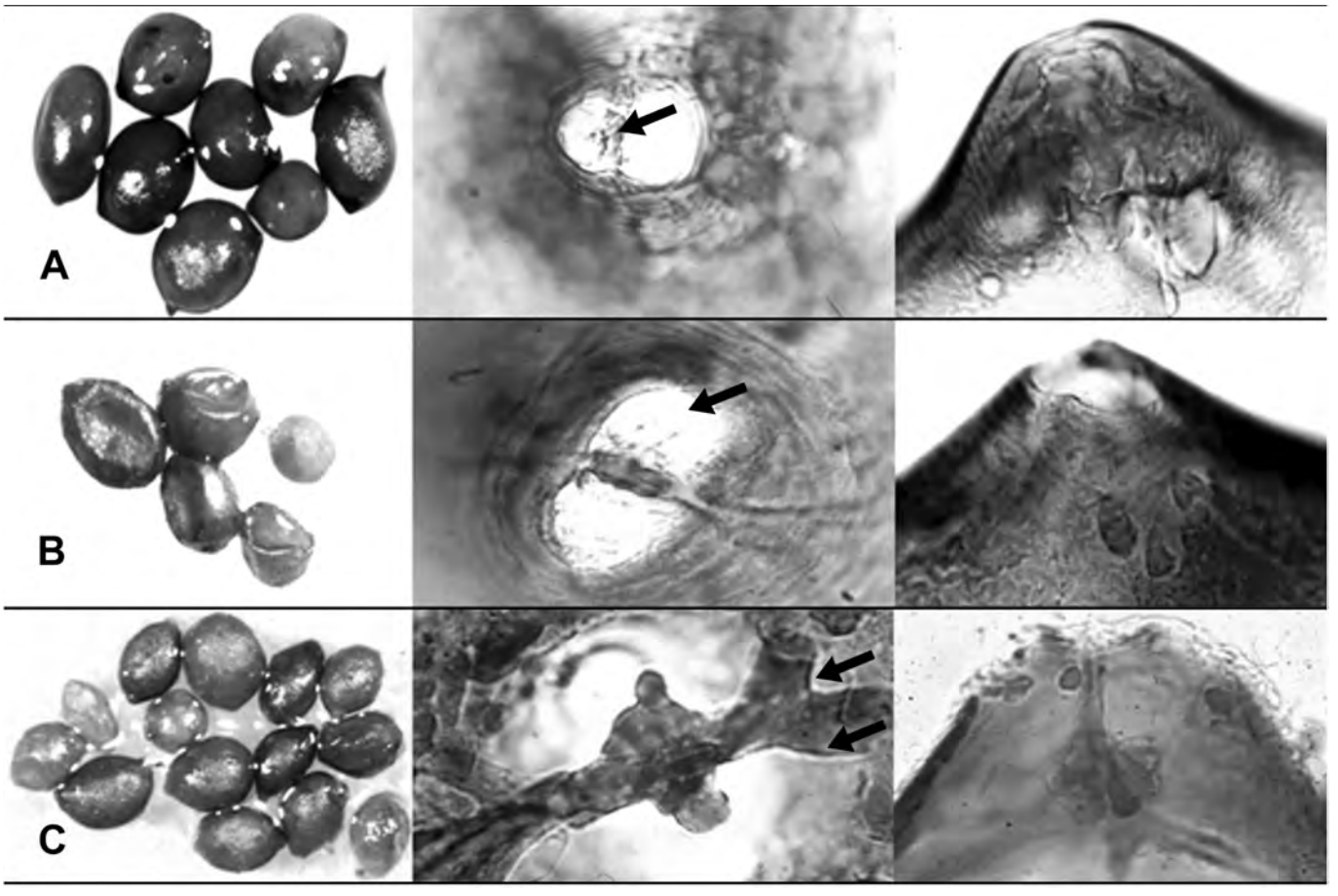

Fig. 2. Views of cyst nematodes belonging to the *Heterodera avenae* group. A: *H. avenae*, with arrow on the vulval slit; B: *H. filipjevi* with arrow on the oval semi-fenestrae; C: *H. latipons*, with arrow on the clear underbridge. For each species, the cysts are at the left, the internal vulval cone *en face* view is in the centre, and the in profile view is at the right.

fields out of 24 had pure *H. filipjevi* populations, found at seven sites (Konya, Cumra, Cifteler, Eskisehir, Alpu, Topkaya and Haymana), and eight fields were infested by *H. latipons* only (at the Sarayonu, Eskisehir, Alpu, Karakaya, Polatli, Temelli and Haymana sites). Both species were found in mixtures in seven fields representing seven different sites of the Central Anatolian Plateau (Osmaniye, Ceyhan, Ligin, Emiradag, Hamidiye, Eskisehir and Haymana) (Fig. 1). *Heterodera avenae* was not found in this survey in Turkey.

DISCUSSION

Our surveys showed that most agricultural regions of Syria and a great part of the Central Anatolian Plateau of Turkey were commonly infested by cyst nematodes belonging to the *H. avenae* group. Though only suggestive, due to the sampling procedure followed and the time at which they were obtained, the data showed that large numbers of full cysts were present, thus suggesting that damaging infestation levels of these nematodes may have occurred in many cereal fields of Syria and Turkey. Our results are in agreement with the studies previously cited and those of Saxena *et al.* (1988) and Scholz (2001), who stated that heavy infestations of cyst nematodes, especially *H. latipons,* could be found, most commonly on barley cultivated in the dry areas of Syria with less than 300 mm annual rainfall. Synergistic interactions between *H. latipons* and common root rot caused by *Bipolaris sorokiniana* (Sacc. in Sorok) Shoemaker were also suspected to increase yield loss in barley (Scholz, 2001; Abidou *et al.*, 2002). As reviewed by Nicol (2002), many surveys of the more intensively studied species *H. avenae* have, in various parts of the world, assessed the economic threshold of the host crops under field conditions. Therefore, studies on the population dynamics under field conditions are necessary to estimate the economic thresholds of cereals to both *H. filipjevi* and *H. latipons* before we can understand the economic significance of these nematodes.

The main outcome of these investigations is the rather distinct distribution of these cyst nematodes in these West Asian regions. *Heterodera latipons* is the most widely distributed species, found in different agroecological zones of cereal production in Syria and almost in all the sampled sites of winter cereal production areas in the Central Anatolian Plateau of Turkey. Its distribution is wider than previously reported (Rumpenhorst *et al.*, 1996; Öztürk *et al.*, 1998). *Heterodera filipjevi* occurred mainly in Turkey but it was also found in Northern Syria near the border with Turkey.

Even though Yuksel (1973) recorded *H. avenae* in Eastern Anatolia of Turkey, our investigation found this species sporadically only in northern regions of Syria. Moreover, mixtures of two species were often observed: *H. filipjevi* and *H. latipons* were found in the same field in Turkey and northern Syria, and *H. avenae* and *H. latipons* in Syria.

Syria and Turkey cover a part of the Fertile Crescent that is generally considered as the birthplace of agriculture where wild *Graminaceae* were domesticated to produce wheat and related wheat wild relatives (Bell *et al.*, 1987). We may hypothesize that the cyst nematodes belonging to the *Heterodera avenae* group also originated from these Near East regions along with their obligatory domesticated and wild hosts. Combined morphological and molecular data based on PCR-RFLP and sequences of the ribosomal DNA demonstrated that these species belong to two different phylogenetic lineages, the H. avenae group sensu-stricto containing *H. avenae* and *H. filipjevi*, and the *H. latipons* group (Subbotin *et al.*, 1999; Rivoal *et al.*, 2003; Abidou *et al.*, 2005). Further studies on the phylogenetic relationships in these two groups will allow the time of their divergence to be estimated and perhaps their specific ancestry as achieved on einkorn wheat by Heun *et al.* (1997). In addition, according to the apparent radiating distribution of these three species from the Fertile Crescent to neighbour European, Mediterranean and Asiatic regions, it would be also worth to develop some phylogeography approaches to establish the principles and processes determining the geographical distribution of these differentiating lineages.

To maintain the population densities of these species of nematodes below damaging levels, appropriate management measures, such as rotational schemes and the use of resistant varieties, are necessary. A number of resistance sources for breeding purposes have been found in domestic cereals and their wild relatives, acting against both *H. avenae* and *Pratylenchus* spp. (Nicol *et al.*, 2004b). However, the efficiency of the genes involved differs according to the virulence of the populations (pathotypes) of *H. avenae*, although preliminary studies indicate that several resistance genes of barley or wheat, are to some extent also resistant to populations of *H. filipjevi* or *H. latipons* originating from different sites in North Africa, Europe and Asia (Bekal *et al.*, 1998; Rivoal *et al.*, 2001; Mokabli *et al.*, 2002). Further investigations are necessary to identify suitable resistance sources to be used in cereal breeding programmes.

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