

## RESPONSE OF COMMON BEAN (*PHASEOLUS VULGARIS*) TO ITALIAN POPULATIONS OF FOUR SPECIES OF *MELOIDOGYNE*

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**Summary.** The reactions of lines and cultivars of common bean (*Phaseolus vulgaris*) to Italian populations of races 1 and 2 of *Meloidogyne incognita*, *M. javanica*, race 2 of *M. arenaria* and *M. hapla* were evaluated in a glasshouse. Groups of ten pregerminated seeds of each line or cultivar of common bean were sown in trays of 12 dm<sup>3</sup> filled with a steam sterilized sandy soil artificially infested with 10,000 eggs and juveniles of each nematode population per plant. The lines Alabama #1 and PI 165426 were resistant to all populations of *Meloidogyne* spp. tested. The line A 445 was resistant to *M. incognita* race 2 and *M. javanica*, and segregated for resistance to *M. incognita* race 1. The inbred line ISCI 197/151-5 was resistant to *M. incognita* race 1, *M. arenaria* race 2 and *M. hapla*. The remaining lines and cultivars were susceptible to all four species of root-knot nematodes.

Common bean (*Phaseolus vulgaris*) is the most widely cultivated legume in the world. In 2003, about 27,149,000 ha were cultivated, with a dry seed production of 19,038,000 t (FAO, 2003). Several species of nematodes have been reported to damage the crop (Sikora and Greco, 1990), with an annual yield loss of about 11% at world level (Sasser and Freckman, 1987). The root-knot nematodes *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are among the most common and damaging nematodes of the crop in several countries. These nematodes are also widespread in Italy (Di Vito *et al.*, 2004), where common bean is one of the most important food legumes and is often damaged by these pathogens under both field and greenhouse conditions. The control of root-knot nematodes by crop rotation is rather difficult because of their wide host range. Soil solarization and nematicides would, as alternative to crop rotation, effectively control root-knot nematodes (Sikora and Greco, 1990; Greco *et al.*, 1998), but they may not always be feasible, as they are expensive and nematicides may cause pollution. Therefore, the use of resistant cultivars would seem to be a sound approach to control these nematodes. However, although several cultivars of common bean that are tolerant (McSorley and Phoronezny, 1981) or resistant (Fassuliotis *et al.*, 1970; Mullin *et al.*, 1991; Sydenham *et al.*, 1996) are available, unfortunately they are not suitable for the Italian market.

In order to tackle this problem, a breeding programme was recently started in Italy. Preliminary results on the introgression of resistance into Italian common bean main types (borlotta, cannellino and stringless) for fresh consumption or freezing, with either dwarf or climbing growth-habits were very encouraging (Parisi *et al.*, 2004). Therefore, the aim of this study was to screen lines and cultivars of common bean to identify sources of resistance to Italian populations of *Meloidogyne* spp. to be used in future breeding programmes.

## MATERIALS AND METHODS

The lines and cultivars of common bean tested are listed in Tables I, II, III and IV. The Italian populations of root-knot nematodes used were *Meloidogyne incognita* (Kofoid *et al.* White) Chitw. host race 1 (Taylor and Sasser, 1978; Di Vito and Cianciotta, 1991) from sugarbeet at Castellaneta (province of Taranto), *M. incognita* host race 2 from tobacco at Lecce, *M. javanica* (Treub) Chitw. from tomato at Pozzallo (province of Ragusa), *M. arenaria* (Neal) Chitw. host race 2 from peach at Bolone (province of Verona) and *M. hapla* Chitw. from sugarbeet at Lesina (province of Foggia). These nematode populations were reared on tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers in a glasshouse at 26 ± 2 °C. When large egg masses were formed, the eggs were extracted from the roots using the sodium hypochlorite method (Hussey and Barker, 1973).

Groups of pregerminated seeds of each line or cultivar of common bean were sown in 12-dm<sup>3</sup> trays (forty seeds per tray) filled with a steam sterilized sandy soil (sand 88%, silt 5%, clay 7% and organic matter 2.5%). Seven days later, ten seedlings of each line or cultivar were inoculated with 10,000 eggs and juveniles per plant of each root-knot nematode population. Tomato cv. Rutgers was also used in order to ascertain that growing conditions were suitable for nematode infection and reproduction. The trays were randomly arranged on benches in a glasshouse maintained at 26 ± 2 °C.

Forty five days after inoculation, the plants were uprooted and the roots gently washed free of the adhering soil. Then the gall (GI) and egg mass (EI) indices of the root systems were assessed according to a 0-5 scale, where 0 = no gall and/or egg mass, 1 = 1-2 galls and/or egg masses, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = more than 100 galls and/or egg masses (Taylor and Sasser, 1978). Lines and cultivars were considered resistant

when the average gall (GI) and/or egg mass (EI) index was  $\leq 2$  (Taylor and Sasser, 1978). All resistant genotypes were re-tested to confirm their resistance.

The data of gall (GI) and egg mass (EI) index were statistically analyzed by ANOVA and compared using Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

The glasshouse conditions during the experiments

favoured the development of the common bean lines and cultivars and the nematode populations. As a result, the roots of the susceptible tomato cv. Rutgers revealed very high gall (GI) and egg mass (EI) indices (about 5).

Great differences were observed in both gall and egg mass indices of the tested genotypes. Alabama #1 and PI 165426 were completely free of galls when inoculated with races 1 and 2 of *M. incognita*, *M. javanica*, race 2 of *M. arenaria* and *M. hapla* (Table II). No gall was ob-

**Table I.** Origin of lines and cultivars of the common bean tested and their agronomical traits.

Genotype	Breeder*	Seed origin	Growth-habit	Types of reference and main use
Alabama #1	1	USA	climbing	stringless with filet green pods for fresh market
A 445	2	USA	climbing	dry seed harvest for fresh market
PI 165426	3	USA	climbing	dry seed harvest for fresh market
Black Turtle II	4	Canada	half-runner	dry seed harvest for fresh market
Blanco Laran	5	Peru	dwarf	dry or waxy seed harvest for fresh market
ISCI 112/94-27	6	Italy	climbing	stringless with filet green pods for fresh market
ISCI 197/151-5	6	Italy	climbing	stringless with filet green pods for fresh market
ISCI 213/28-4	6	Italy	climbing	stringless with filet green pods for fresh market
ISCI 481/16-9	6	Italy	climbing	stringless with filet green pods for fresh market
Bat 93	2	USA	climbing	dry seed harvest for fresh market
Lingua di Fuoco	7	Italy	dwarf	dry seed harvest for fresh market
Kondor	6	Italy	climbing	dry or waxy seed harvest for fresh market
Helda	8	The Netherlands	climbing	String less with flat green pods for fresh market
Kaimano	6	Italy	climbing	String less with flat yellow pods for fresh market
King	6	Italy	dwarf	dry or waxy seed harvest for fresh market and freezing
Jalo EPP 558	9	USA	climbing	dry seed harvest for fresh market
Luxor	6	Italy	dwarf	dry or waxy seed harvest for fresh market and freezing
Talento	6	Italy	dwarf	dry or waxy seed harvest for fresh market and freezing

\* 1=Alabama Agricultural Experiment Station, Auburn, AL, USA; 2=Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia; 3=USDA, Vegetable Breeding Station, Charleston, SC, USA; 4=New York State Agricultural Experiment Station, Geneva, NY, USA; 5=Instituto Nacional de Investigación Agraria, La Molina, Peru; 6=Istituto Sperimentale per le Colture Industriali, Bologna, Italy; 7=Società Agricola Italiana Sementi spa, Cesena, Italy; 8=Nunhems Zaden BV, AA Haelen, The Netherlands; 9=Estação Experimental de Patos de Minas, MG, Brazil.

**Table II.** Root gall indices (GI) of common bean inoculated with host races 1 (Mi1) and 2 (Mi2) of *Meloidogyne incognita*, *M. javanica* (Mj), host race 2 of *M. arenaria* (Ma2) and *M. hapla* (Mh).

Line and cultivar	Root gall index (0 - 5)				
	Mi1	Mi2	Mj	Ma2	Mh
Alabama #1	0 a*	0 a	0 a	0 a	0 a
A 445	2.5 c	0 a	0 a	3 c	3.5 c
PI 165426	0 a	0 a	0 a	0 a	0 a
Black Turtle II	4.6 f	5 e	4 c	4.7 fgh	5 g
Blanco Laran	3.4 d	3.5 b	3 b	0.5 b	0 a
ISCI 112/94-27	5 g	5 e	4.8 ef	4.9 gh	4.3 e
ISCI 197/151-5	0.5 b	4.1 cd	3.9 c	0.1 a	1.4 b
ISCI 213/28-4	4.7 fg	4.2 cd	4 c	4.3 de	3.7 cd
ISCI 481/16-9	4.7 fg	4.4 d	4.4 d	4.6 efg	4.8 fg
Bat 93	5 g	5 e	5 f	4.7 fgh	4.8 fg
Lingua di fuoco	5 g	5 e	4 c	4.5 ef	5 g
Kondor	4.5 f	4 c	5 f	5 h	5 g
Helda	5 g	5 e	5 f	5 h	5 g
Kaimano	5 g	5 e	4.5 de	5 h	3.9 d
King	4 e	4 c	3.9 c	5 h	4.8 fg
Jalo EPP 558	5 g	5 e	5 f	4 d	3.9 d
Luxor	5 g	5 e	5 f	4.9 gh	4.8 fg
Talento	5 g	5 e	5 f	5 h	5 g
Tomato "Rutgers"	4.8 fg	4.9 e	5 f	5 h	5 g

\* Means sharing a common letter are not significantly different according to Duncan's Multiple Range Test ( $P = 0.05$ ).

served also on the line A 445 inoculated with race 2 of *M. incognita* or *M. javanica*, while a few and no galls were observed on the roots of cv. Blanco Laran when inoculated with race 2 of *M. arenaria* and *M. hapla*, respectively. The inbred line ISCI 197/151-5 showed mean gall indices of 0.5, 0.1 and 0 when inoculated with race 1 of *M. incognita*, race 2 of *M. arenaria* and *M. hapla*, respectively. The average egg mass indices were also low or nil for all Italian populations tested on the lines Alabama #1 and PI 165426 (Table III). On the line A445, the indices were low when inoculated with races 1 and 2 of *M. incognita* and *M. javanica* and high when inoculated with the other nematode populations. A few egg masses were observed on the inbred line ISCI 197/151-5 inoculated with race 1 of *M. incognita*, *M. arenaria* and *M. hapla*. The remaining genotypes showed high gall and egg mass indices. Therefore, Alabama #1 and PI 165426 can be considered highly resistant to Italian populations of races 1 and 2 of *M. incognita*, *M. javanica*, race 2 of *M. arenaria* and *M. hapla* (Table III). The line A 445 showed resistance only to race 2 of *M. incognita* and *M. javanica* and segregated for resistance to race 1 of *M. incognita*. The inbred line ISCI 197/151-5 was resistant only to race 1 of *M. incognita*, race 2 of *M. arenaria* and *M. hapla*. The remaining geno-

types were susceptible to all nematode populations tested. The cv. Blanco Laran, when inoculated with race 2 of *M. arenaria* and *M. hapla*, despite showing a low gall index (0-0.5) (Table II), allowed these nematodes to produce many egg masses filled with eggs (Table III); therefore, it was considered susceptible (Table IV). The resistant reactions of the above lines and cultivars were confirmed by the second test.

The presence of resistance to races 1 and race 2 of *M. incognita*, *M. javanica*, race 2 of *M. arenaria* and *M. hapla* in Alabama #1 and PI 165426 of common bean is of great interest as these sources of resistance can easily be transferred to Italian common bean types.

The results of these studies confirmed the occurrence of resistance to the major root-knot nematode species and races in common bean observed by Fassuliotis *et al.* (1970) and Omwega *et al.* (1989). However, there are some discrepancies in the resistant response if we compare our results with those obtained by other authors (Omwega *et al.*, 1989). In our tests, the lines Alabama #1 and PI 165426 were resistant to all Italian populations of root-knot nematodes tested while Omwega *et al.* (1989) found them resistant only to race 2 of *M. incognita* and *M. hapla*. Moreover, the line A 445 was resistant to races 1 and 2 of *M. incognita*, race 2 of *M. are-*

**Table III.** Egg mass indices (EI) of common bean inoculated with host races 1 (Mi1) and 2 (Mi2) of *Meloidogyne incognita*, *M. javanica* (Mj), host race 2 of *M. arenaria* (Ma2) and *M. hapla* (Mh).

Line and cultivar	Egg masses index (0 - 5)				
	Mi1	Mi2	Mj	Ma2	Mh
Alabama #1	0.3 a*	0 a	0.9 b	0.6 b	0.4 a
A 445	2.1 d	0 a	0.3 a	4 e	3.5 c
PI 165426	1 b	0 a	0 a	0 a	0.5 a
Black Turtle II	5 f	5 d	4 d	4.4 f	5 e
Blanco Laran	5 f	5 d	3 c	2.6 d	4 d
ISCI 112/94-27	5 f	5 d	4.8 fg	5 g	5 e
ISCI 197/151-5	1.7 c	5 d	3.9 d	1.3 c	1.9 b
ISCI 213/28-4	5 f	4.9 cd	4 d	5 g	5 e
ISCI 481/16-9	5 f	4.8 c	4.4 e	5 g	5 e
Bat 93	5 f	5 d	5 g	5 g	5 e
Lingua di fuoco	5 f	5 e	4 g	4.9 g	5 e
Kondor	4.8 f	5 d	5 g	5 g	5 e
Helda	5 f	5 d	5 g	5 g	5 e
Kaimano	5 f	5 d	5 g	5 g	5 e
King	4 e	4.4 b	5 g	5 g	5 e
Jalo EPP 558	5 f	5 d	4 d	4 e	4 d
Luxor	5 f	5 d	5 g	5 g	5 e
Talento	5 f	5 d	5 g	5 g	5 e
Tomato "Rutgers"	4.8 f	4.8 c	5 g	4.8 g	5 e

\* Means sharing a common letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

**Table IV.** Reaction type of common bean to host races 1 (Mi1) and 2 (Mi2) of *Meloidogyne incognita*, *M. javanica* (Mj), host race 2 of *M. arenaria* (Ma2) and *M. hapla* (Mh), based on results in Tables II and III.

Line and cultivar	Reaction type				
	Mi1	Mi2	Mj	Ma2	Mh
Alabama #1	R*	R	R	R	R
A 445	R/S	R	R	S	S
PI 165426	R	R	R	R	R
Black Turtle II	S	S	S	S	S
Blanco Laran	S	S	S	S	S
ISCI 112/94-27	S	S	S	S	S
ISCI 197/151-5	R	S	S	R	R
ISCI 213/28-4	S	S	S	S	S
ISCI 481/16-9	S	S	S	S	S
Bat 93	S	S	S	S	S
Lingua di fuoco	S	S	S	S	S
Kondor	S	S	S	S	S
Helda	S	S	S	S	S
Kaimano	S	S	S	S	S
King	S	S	S	S	S
Jalo EPP 558	S	S	S	S	S
Luxor	S	S	S	S	S
Talento	S	S	S	S	S
Tomato "Rutgers"	S	S	S	S	S

\* R = resistant, gall and/or egg mass index  $\leq 2$ ; S = susceptible, gall and/or egg mass index  $> 2$ ; R/S = segregated for resistance.

*naria* and *M. hapla* and susceptible to *M. javanica* Italian nematode populations, while Omwega *et al.* (1989) reported this line as resistant to all races and species tested except race 2 of *M. incognita*. These discrepancies in resistance response could be linked with different origins, pathogenicity and virulence of the nematode populations used. This conclusion is supported by Chen and Roberts (2003), who also found different responses of different lines to different *M. hapla* populations.

These results provide a basis for starting a breeding programme to introduce resistance to root-knot nematodes into some Italian common bean types. However, further investigations are necessary to confirm the number of genes involved in the observed sources of resistance, their inheritance and heat stability.

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## LITERATURE CITED

- Chen P. and Roberts P.A., 2003. Virulence in *Meloidogyne hapla* differentiated by resistance in common bean (*Phaseolus vulgaris*). *Nematology*, 5: 39-47.
- Di Vito M. and Cianciotta V., 1991. Identificazione delle razze in popolazioni italiane di nematodi galligeni (*Meloidogyne* spp.). *Informatore Fitopatologico*, 41(11): 54-55.
- Di Vito M., Parisi B. and Catalano F., 2004. Effect of population densities of *Meloidogyne incognita* on common bean. *Nematologia Mediterranea*, 32: 81-85.
- FAO, 2004. *Faostat Database Collections*. (<http://faostat.fao.org/faostat/collections?subset=agriculture>).
- Fassuliotis G., Deakin J.R. and Hoffman J.C., 1970. Root-knot nematodes resistance in snap beans: breeding and nature of resistance. *Journal of American Society for Horticulture Science*, 95: 640-645.
- Greco N., Lamberti F., Brandonisio A. and De Cosmis P., 1998. Control of root-knot nematodes on zucchini and string beans in plastic house. *Nematologia Mediterranea*, 26 (supplement): 39-44.
- Hussey R.S. and Barker K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*, 57: 1025-1028.
- Mullin B.A., Abawi G.S., Pasto-Corrales M.A. and Kornegay J.L., 1991. Reactions of selected bean pure lines and accessions to *Meloidogyne* species. *Plant Disease*, 75: 1212-1216.
- Omwega C.O., Thomason I.J., Roberts P.A. and Waines J.G., 1989. Identification of new sources of resistance to root-knot nematodes in *Phaseolus*. *Crop Sciences*, 29: 1436-1468.
- Parisi B., Baschieri T., Del Bianco F., Di Vito M., Carboni A., Ranalli P., 2004. Breeding for nematodes resistance in common bean (*Phaseolus vulgaris* L.). Proceedings of XLVIII Annual Conference of Italian Society of Agriculture Genetics. Lecce, Italy, 15-18 September 2004, 177-178.
- Sasser J.N. and Freckman D.W., 1987. A world perspective on nematology: the role of the society. Pp. 7-14. *In: Vistas on Nematology* (Veech J.A. and Dickson D.W., eds.). E.O. Painter Printing Co., DeLeon Springs, USA.
- Sikora R.A. and Greco N., 1990. Nematode parasites of food legumes. Pp. 181-235. *In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (Luc M., Sikora R.A. and Bridge J., eds). CAB International, Wallingford, U.K.
- Sydenham G.M., McSorley R. and Dunn R.A., 1996. Effects of resistance in *Phaseolus vulgaris* on development of *Meloidogyne* species. *Journal of Nematology*, 28: 485-491.
- Taylor A.L. and Sasser J.N., 1978. *Biology, Identification and Control of Root-knot Nematodes* (*Meloidogyne* species). North Carolina State University Graphics, Raleigh, USA, 105 pp.

