GENETICS AND INTROGRESSION OF RESISTANCE TO ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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Summary. Genetics studies on the resistance to *Meloidogyne* spp. in common bean (*Phaseolus vulgaris* L.) were carried out in a glasshouse. F_1 hybrids and F_2-F_4 progenies, derived from crosses of the Mesoamerican resistant accessions A#445 and PI#165426 with CRA-ISCI lines 212/28 and 15/7-70, were evaluated for resistance to Italian populations of *Meloidogyne incognita* race 1 and *M. javanica*. All F_1 hybrids were resistant to both nematode species. Ninety-eight, 17 and 136 F_2 lines were resistant, susceptible and segregant to the nematodes, respectively, and 1909 and 655 plants were resistant and susceptible, respectively. The results suggest a monogenic dominant control of the resistance, with a ratio 3:1 of resistant to susceptible plants. Of the 52 selected F_4 lines tested for their reaction to race 1 of *M. incognita,* 22 were resistant, 24 segregant and six susceptible. Of the 52 F_4 lines tested against *M. javanica*, 31, 11 and 10 were resistant, segregant and susceptible, respectively.

Key words: Inheritance, *Meloidogyne incognita*, *M. javanica*, resistance, string bean.

Several species of nematodes have been reported to damage common bean (*Phaseolus vulgaris* L.) (Sikora *et al*., 2005), with an annual yield loss of about 11% at world level (Sasser and Freckman, 1987). Probably the more widespread and detrimental for this crop are *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw. These nematodes are also widespread in Italy, where common bean is one of the most important food legumes and is often affected by these root-knot nematodes (Di Vito *et al*., 2004, 2007). The control of root-knot nematodes by crop rotation is rather difficult because of their wide host range. Soil solarization and nematicides could, as alternatives to crop rotation, control these pathogens (Greco *et al*., 1998; Sikora *et al*., 2005), but they may not always be feasible as they are expensive and there is the added problem that nematicides may cause pollution. Therefore, the use of resistant cultivars appears to be the most appropriate strategy to control these nematodes. However, although several tolerant or resistant cultivars of common bean are available (Fassuliotis *et al*., 1970; Mullin *et al*., 1991; Sydenham *et al*., 1996), these are not suitable for the Italian market.

Preliminary results on the introgression of resistance genes into the main types of Italian common bean (borlotto, cannellino and stringless) for the fresh market or freezing, with either dwarf or climbing growth-habits, were very encouraging (Parisi *et al*., 2004, 2007). Therefore, the aim of this investigation was to study the genetics of this resistance and to continue the introgression of resistance to *M. incognita* race 1 and *M. javanica* in new lines of common bean suitable for the Italian market.

MATERIALS AND METHODS

Common bean material. F_1 hybrids and F_3 and F_4 lines of common bean (Tables III, IV, V and VI) were obtained by crosses and inverse crosses and selection of the Mesoamerican lines A#445 and PI#165426 with two inbred lines (ISCI 212/28 and ISCI 15/7-70) of the CRA-ISCI collection (Tables III, IV, V and VI). In a previous experiment, A#445 and PI#165426 were resistant to *M. javanica* and *M. incognita* race 1 and 2 and *M. arenaria* race 2, respectively (Table I). One hundred and twenty-five and 126 F_2 progenies of the lines 28/1-1(3d) and 28/1-2(1d), respectively, were obtained by crossing the cv. Paulista with PI#165426 (Table II).

Nematode populations. Root-knot nematode populations used in the screenings were: *M. incognita* host race 1 (Taylor and Sasser, 1978; Di Vito and Cianciotta, 1991), collected from sugar beet (*Beta vulgaris* L. cv. *saccharifera* Alef.) at Castellaneta (Taranto, Apulia region, Italy), and *M. javanica*, collected from peach [*Prunus persica* (L.) Batsch.] at San Ferdinando di Puglia (Foggia, Apulia region, Italy). These nematode populations were reared on tomato (*Lycopersicum esculentum* Mill.) cv. Rutgers in a glasshouse at 26 ± 2 °C. When large egg masses had formed, the eggs were extracted from the roots using the sodium hypochlorite method (Di Vito *et al*., 2005).

Inoculation. Groups of pre-germinated seeds of each line or cultivar of common bean were sown in 12-dm^3 trays (forty seeds per tray) filled with a steam sterilized sandy soil (sand 88%, silt 5%, clay 7%, organic matter 2.5%). There were separate trays for the two nematode species. Seven days later, ten seedlings of each line or cultivar were inoculated with 10,000 eggs and juveniles per plant of either root-knot nematode population. The common bean cv. Talento was used as a susceptible host in order to ascertain that growing conditions were suit-

Table I. Sources of genetic resistance to the four species of root-knot nematodes, *Meloidogyne incognita* race 1 (Mi1) and race 2 (Mi2), *M. javanica* (Mj), *M. arenaria* race 2 (Ma2) and *M. hapla* (Mh), available in two Mesoamerican bean genotypes utilized in the breeding programme and for the studies on inheritance of the resistance.

Genotype	Origin*	Reaction type**				
		Mi1	Mi2	Mj	Ma2	Mh
A#445	CIAT, Colombia	S	S	R	S	S
PI#165426	USDA, USA	R	R	S	R	R

* Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia; USDA, Vegetable Breeding Station, Charleston, SC, USA (modified from Di Vito *et al.*, 2005).
** R = resistant, gall and/or egg mass index ≤ 2; S = susceptible, gall and/or egg mass index > 2.

Table II. Responses of two F_2 breeding lines of common bean, obtained from the cross cv. Paulista × PI#165426, to *Meloidogyne incognita* host race 1 in a glasshouse at 26 ± 2 °C.

Breeding line	Number of lines*				Resistant (%)	Number of plants*			Resistant (%)
	Tested	R	S	R/S		Tested	R	S	
28/1-1(3d)	125	48	2	75	38	1341	1054	287	78.5**
28/1-2(1d)	126	50	15	61	40	1224	855	368	69.8**

* R = resistant, gall and/or egg mass index ≤ 2; S = susceptible, gall and/or egg mass index > 2; R/S = segregant.
** = χ^2 statistically significant for ratio 3:1.

able 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 ≤ 2 (Taylor and Sasser, 1978). All resistant genotypes were replanted to obtain seeds for future studies.

The data of gall (GI) and egg mass (EI) indices were analyzed by ANOVA and compared using Duncan's Multiple Range Test; a χ^2 test was also performed.

RESULTS AND DISCUSSION

The glasshouse conditions during the experiments favoured the development of the common bean lines and cultivars and the nematode populations. In fact, the roots of the susceptible common bean cv. Talento revealed very high GI and EI (about 5).

All inoculated F_1 plants were resistant to both nematode populations.

Of the F_2 progenies (125 plants) of the line ISCI 28/1-1(3d), obtained by crossing the susceptible cv. Paulista with the resistant accession PI#165426, 48 were resistant, 2 susceptible and 75 segregating to *M. incognita* race 1 (Table II); 126 progenies of the line ISCI 28/1-2(1d), from the same cross, showed a different reaction as 50, 15 and 61 were resistant, susceptible or segregating to the same nematode, respectively. But, when the results for all 1341 F_2 plants of the progenies of line ISCI 28/1-1(3d) were combined, 1054 (78.5%) were resistant. Similar re-

Table III. Responses of F_3 breeding lines of common bean, obtained from the cross A#445 × PI #165426 and its reverse, to *Meloidogyne incognita* host race 1 and *M. javanica* in a glasshouse at 26 ± 2 °C.

Line	*M. incognita*		*M. javanica*	
	Tested	Resistant (%)[$]	Tested	Resistant (%)[$]
ISCI A2*	38	84	38	87
ISCI A4*	36	97	36	94
ISCI P2**	38	95	38	81
ISCI P14**	37	97	38	92

[$]R = resistant, gall and/or egg mass index ≤ 2.
* Cross = A#445 × PI#165426.
** Cross = PI#165426 × A#445.

Table IV. Responses of F_4 breeding lines of common bean, obtained from A#445 × PI#165426 crosses, to attack by *Meloidogyne incognita* race 1 and *M. javanica* in glasshouse at 26 ± 2 °C.

Line and cultivar	*Meloidogyne incognita*					*Meloidogyne javanica*				
	Plants tested	Gall index (0-5)*	Egg mass index (0-5)*	Reaction type**	% plants resistant	Plants tested	Gall index (0-5)*	Egg mass index (0-5)*	Reaction type**	% plants resistant
A2-1	8	4.4	4	S	-	4	0	0	R	100
A2-3	10	0.8	0.8	R/S	80	10	0.2	0.1	R	100
A2-6	10	0	0	R	100	10	0	0	R	100
A2- 11	9	1.1	1.2	R/S	78	9	0.6	1.2	R/S	78
A2-12	7	1	0.8	R/S	86	-	-	-	-	-
A2-15	10	0	0	R	100	7	1.3	1.4	R/S	71
A2-18	10	4.1	4.5	S	-	9	0.7	1.4	R/S	78
A2-19	10	0	0	R	100	10	1.1	1.5	R/S	70
A2-23	10	0	0	R	100	10	3.9	5	S	-
A2-24	10	2	2	R/S	50	10	0.7	1	R/S	80
A2-26	4	0.8	0.9	R/S	75	6	0	0	R	100
A2-28	10	0	0	R	100	10	4.5	5	S	-
A2-38	9	0	0	R	100	10	1.8	2.2	R/S	50
A2-39	9	4.1	4.8	S	-	10	0	0	R	100
A4-2	10	0	0	R	100	10	3.1	3.7	R/S	33
A4-5	5	0	0	R	100	4	0	0	R	100
A4-6	9	0	0	R	100	10	0.4	0.5	R	100
A4-8	8	0.8	1	R/S	87	9	0	0	R	100
A4-12	12	3.5	3.7	R/S	11	10	4.9	5	S	-
A4-13	10	0	0	R	100	10	4.1	5	S	-
A4-14	10	1.4	1.3	R/S	60	10	2.4	5	S	-
A4-17	8	3.7	3.5	S	-	10	0	0	R	100
A4-19	10	0	0	R	100	10	0	0	R	100
A4-20	10	0	0	R	100	9	1.6	1.9	R/S	55
A4-28	9	1.8	2	R/S	67	5	0	0	R	100
A4-34	-	-	-	-	-	6	0	0	R	100
A4-36	10	0	0	R	100	10	4.8	5	S	-
A4-37	10	0	0	R	100	10	1	1	R/S	80
A4-39	10	0.8	0.8	R/S	80	10	0.5	0.9	R/S	90
cv. Talento (check)	10	5	5	S	-	10	5	5	S	-
LSD $P \leq 0.05$		0.95	0.98				1.05	1.16		
$P \leq 0.01$		1.25	1.29				1.39	1.53		

* 0 = 0 gall and/or egg mass, 1 = 1-2 galls and/or egg masses, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 > 100 galls and/or egg masses.

** R = resistant, gall and/or egg mass index ≤ 2; S = susceptible, gall and/or egg mass index > 2; R/S = segregant.

Table V. Responses of F_4 breeding lines of common bean, obtained from PI#165426 × A#445 crosses, to attack by *Meloidogyne incognita* race 1 and *M. javanica* in glasshouse at 26 ± 2 °C.

Line and cultivar	*Meloidogyne incognita*					*Meloidogyne javanica*				
	Plants tested	Gall index (0-5)*	Egg mass index (0-5)*	Reaction type**	% plants resistant	Plants tested	Gall index (0-5)*	Egg mass index (0-5)*	Reaction type**	% plants resistant
P2-3	10	0	0	R	100	10	0	0	R	100
P2-7	9	0.9	1	R/S	78	7	0.2	0.2	R	100
P2-14	10	0	0	R	100	8	0	0	R	100
P2-18	8	2.2	3	R/S	37	10	0	0	R	100
P2-19	6	0.6	1	R/S	83	10	0	0	R	100
P2-23	10	1.7	2.5	R/S	50	10	4.5	4.5	S	-
P2-28	2	0	0	R	100	5	0	0	R	100
P2-30	8	4.3	4.3	R/S	12	10	0	0	R	100
P2-33	10	0	0	R	100	10	3.7	4	S	-
P2-35	3	2	2	R/S	67	4	0	0	R	100
P14-1	10	0.8	0.8	R/S	80	10	0	0	R	100
P14-4	6	0	0	R	100	10	0	0	R	100
P14-8	9	1	1	R/S	67	10	0	0	R	100
P14-10	8	0.3	0.3	R/S	87	8	0	0	R	100
P14-11	9	0.9	1	R/S	78	10	0	0	R	100
P14-13	8	0	0	R	100	10	0	0	R	100
P14-23	9	3.7	4	S	-	10	0	0	R	100
P14-24	10	0.7	1	R/S	80	10	5	5	S	-
P14-26	6	0.2	0.5	R/S	67	9	1	1	R/S	78
P14-30	10	0	0	R	100	10	5	5	S	-
P14-32	10	0	0	R	100	7	0	0	R	100
P14-34	10	0.8	1	R/S	80	8	0.3	0.5	R	100
P14-35	9	1.7	2	R/S	62	7	0.3	0.5	R/S	86
P14-38	10	3.9	4	S	-	10	0	0	R	100
cv. Talento (check)	10	5	5	S	-	10	5	5	-	-
LSD $P \leq 0.05$		1.09	1.24				0.45	0.51		
$P \leq 0.01$		1.44	1.64				0.6	0.68		

* 0 = 0 gall and/or egg mass, 1 = 1-2 galls and/or egg masses, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 > 100 galls and/or egg masses.

** R = resistant, gall and/or egg mass index ≤ 2; S = susceptible, gall and/or egg mass index > 2; R/S = segregant.

sults were obtained with the progenies of the F_2 line ISCI 28/1-2(1d), when 855 (69.8%) out of the 1224 plants tested were resistant. These percentages of resistant plants are highly significant confirmation for a 3:1 resistant/susceptible ratio. Therefore, the resistance in the line of common bean 'PI#165426' to race 1 of *M. incognita* seems to be controlled by a monogenic dominant gene.

The F_3 lines ISCI A2 and ISCI A4, obtained by crossing A#445 with PI#165426, showed high percentages of resistance of 84% and 97% to race 1 of *M. incognita*, respectively (Table III), and 87% and 94% to *M. javanica*. The F_3 lines ISCI P2 and ISCI P14, obtained from the reverse cross (PI#165426 × A#445), showed 95 and 97% of plants to be resistant to *M. incognita*, respectively, and 81 and 92% of plants to be resistant to *M. javanica* (Table III).

Among the 257 plants of the 28 selected F_4 families, obtained by crossing A#445 with PI#165426 and tested with race 1 of *M. incognita*, 133 plants (14 lines) were resistant, with gall and egg mass indices less than 2 (Table IV); 89 plants (10 lines) were segregant with a high number of resistant plants; and the remaining 25 plants (4 lines) were susceptible, with an infestation degree similar to the susceptible cv. Talento used as control. Of the 248 plants of the 28 lines, obtained from the same cross and selection and tested with *M. javanica*, 93 plants (13 lines) were resistant to the nematode; 85 plants (9 lines) were segregant and the remaining 70 plants (6 lines) were susceptible to this nematode (Table IV).

From the reciprocal cross PI#165426 × A#445, the reactions of 200 plants from 24 F_4 families to race 1 of *M. incognita* were as follows: 66 plants (8 lines) were resistant (Table V), 115 plants (14 lines) were segregant and 19 (2 lines) were susceptible. Of the 213 plants (24 lines), obtained from the same cross but inoculated with *M. javanica*, 157 plants (18 lines) were resistant (Table V), 16 plants (2 lines) were segregant; and 40 plants (4 lines) were susceptible and similar in their reaction to that of the control cv. Talento (Table V).

Of the 28 F_4 lines, obtained from the cross A#445 × PI#165426, and inoculated with race 1 of *M. incognita* and *M. javanica*, 50 and 47% of the lines were resistant, 36 and 32% segregant and 14 and 21% susceptible, respectively (Table VI). Of the 24 F_4 lines obtained from the reciprocal cross and tested against the same nematodes, 34 and 75% were resistant, 58 and 8% segregant and 8 and 17% susceptible, respectively (Table VI).

Our results confirm and give more insights into the resistance of common bean to root-knot nematodes.

The two accessions PI#165426 and A#445, resistant to root-knot nematodes, were efficient sources for the introgression of resistance genes to the Italian population of race 1 of *M. incognita* and *M. javanica*, respectively. The genes involved in this resistance show a dominant character as all F_1 progenies obtained from crosses of these two accessions with cultigens of common bean are resistant. These results for the F_1 are confirmed by the segregation response of the F_2 progenies derived from the cross of the susceptible cv. Paulista with the resistant line PI#165426, because the ratio of resistant/susceptible plants was 3:1 and highly significant (Table 2). These data were also confirmed by preliminary results of the development of molecular markers associated with the 'PI#165426' resistance and designed on putative Resistance Gene Analogs. The PCR SCAR markers also showed a 3:1 ratio, like the segregating progenies (Del Bianco, 2007; Del Bianco *et al.*, 2007).

Our results also show that these two resistant genes are independent and that they do not interfere with each other in the resistance response (Table VI). However, the resistance of common bean to *Meloidogyne* spp. is a very complex system, probably linked to numerous secondary genes responsible for the regulation of the resistance at race level (Fassuliotis *et al.*, 1970; Omwega *et al.*, 1989, 1990; Omwega and Roberts, 1992). This conclusion is supported by Chen and Roberts (2003), who also found different responses of different lines to different *M. hapla* populations.

This work provides a basis for continuing the breeding programme to introduce resistance to root-knot nematodes into Italian common bean cultigens. However, further investigations are necessary to confirm the number and type of genes involved in the control of the resistance, their inheritance and their heat stability.

Table VI. Evaluation of resistance to *Meloidogyne incognita* host race 1 and *M. javanica* in new Italian breeding lines of common bean derived from A#445 × PI#165426 crosses and the inverse.

Cross	*Meloidogyne incognita* host race 1				*Meloidogyne javanica*			
	Lines tested	Percentage*			Lines tested	Percentage*		
		R	R/S	S		R	R/S	S
A#445 × PI#165426	28	50	36	14	28	47	32	21
PI#165426 × A#445	24	34	58	8	24	75	8	17

* R = resistant, gall and/or egg mass index ≤ 2; S = susceptible, gall and/or egg mass index > 2; R/S = segregant

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