

## RESISTANCE OF NEW ITALIAN POTATO BREEDING CLONES TO CYST AND ROOT-KNOT NEMATODES

N. Greco<sup>1</sup>, M. Di Vito<sup>1</sup>, B. Parisi<sup>2</sup>, P. Ranalli<sup>2</sup>, A. Brandonisio<sup>1</sup> and F. Catalano<sup>1</sup>

<sup>1</sup> CNR-Istituto per la Protezione delle Piante, Sezione di Bari, 70126 Bari, Italy

<sup>2</sup> CRA-Centro di Ricerca per le Colture Industriali, 40128 Bologna, Italy

**Summary.** Screening tests were conducted in glass-houses in 2006 and 2007 to assess the reaction of 27 new Italian potato (*Solanum tuberosum*) breeding clones to the potato cyst nematode, *Globodera rostochiensis* pathotypes Ro1 and Ro2, and to root-knot nematodes, *Meloidogyne incognita* host race 1 and *M. javanica*. Seven potato cultivars, known to be resistant to cyst nematodes, were also screened for their response to root-knot nematodes. The tests were conducted in 1 dm<sup>3</sup> clay pots containing steam sterilized sandy soil infested with 10-15 eggs/cm<sup>3</sup> of either pathotype of *G. rostochiensis* or 10,000 eggs and second stage juveniles/pot of either root-knot nematode species. The glass-houses were maintained at 20 ± 2 °C for *G. rostochiensis* and at 26 ± 2 °C for *Meloidogyne* spp. The clones CS 8617 and MN 3-1469 R2 were resistant to all three nematode species, and the clone AND 97-15, known to be resistant to all European pathotypes of *G. rostochiensis* and *G. pallida*, was also resistant to *M. incognita* and moderately resistant to *M. javanica*. Fourteen of eighteen clones tested in 2006 and seven of the nine clones tested in 2007 were resistant to both pathotypes of *G. rostochiensis*. Two clones were resistant to both root-knot nematode species in 2006 and one clone moderately resistant to *M. incognita* and one moderately resistant to *M. javanica* in 2007. Some of the clones resistant to *G. rostochiensis* also possess good agronomic traits and have potential for registration as new cultivars.

**Key words:** *Globodera rostochiensis*, *Meloidogyne incognita*, *M. javanica*, multiple resistance, *Solanum tuberosum*.

Potato (*Solanum tuberosum* L.) is one of the most widely cultivated crop plants in the world and about 70,000 ha (FAO, 2007) are devoted to the crop in Italy, in nearly all regions. In the centre and north of Italy, potato is mainly planted from late winter to early spring while in the south, because of the mild winter temperatures, along the coast it is also planted from mid-autumn to mid-winter, to be harvested from early to late spring, or at the end of summer to be harvested in December.

As in most potato growing countries (Brodie *et al.*, 1993; Marks and Brodie, 1998; Trudgill *et al.*, 1998; Scurrah *et al.*, 2005), potato can be severely damaged in Italy by the cyst-forming nematodes, *Globodera rostochiensis* (Behrens) Woll. and *G. pallida* (Behrens) Stone when their soil population densities exceed 1.9 eggs/g soil (Greco *et al.*, 1982; Seinhorst, 1982). Moreover, root-knot nematodes, *Meloidogyne* spp., are also common in Italy and Mediterranean countries where severe infestations (Lamberti, 1979; Vovlas *et al.*, 2005) and damage (Russo *et al.*, 2007) have been observed on potato planted from late summer to early autumn.

Crop rotation is very effective for potato cyst nematode control, but if it were the only means of control it would limit the frequency of potato cultivation in areas where this crop is the main source of farmer income; rotation is problematic for control of root-knot nematodes because of their wide host ranges. The use of nematicides (Whitehead and Turner, 1998; Scurrah *et al.*, 2005) and soil solarization (Greco *et al.*, 2000) is very effective at controlling these nematodes but can be rather expensive and, in the case of nematicides, also

polluting. Because of increasing awareness regarding the quality of agricultural produce and concerns over the use of chemicals to control plant pathogens, the consumer is increasingly seeking organic agriculture products. In Italy, about 1,000,000 ha are managed on organic agriculture protocols and this area is expected to increase in Italy and elsewhere. The use of resistant cultivars is non-polluting, cheap and a very effective means of controlling nematodes and, therefore, very desirable, especially in organic agriculture. Many cultivars of potato resistant to one or several pathotypes of *G. rostochiensis* and/or partially resistant to pathotypes of *G. pallida* are available for cultivation in Europe. However, most of them are not suitable for Italian potato production and the Italian market. Moreover, in Europe only clone CS 8617, derived from the University of Naples potato breeding programme (Greco *et al.*, 2002; Di Vito *et al.*, 2003), is so far available with resistance to the major species of root-knot nematodes. This genotype can be problematic as a donor parent because it possesses some unsuitable morphological traits, such as long stolons and deep eyes. To fill this gap, in the last decade breeding research projects have been funded by the Italian Ministry of Agriculture to produce potato cultivars suitable for Italian production conditions and the Italian and European markets. Among the traits required for any new cultivar is resistance to the major nematode pests of potato occurring in Italy. Therefore, potato lines possessing resistance to cyst and root-knot nematodes were used as parents in the breeding programmes and the most promising lines were evaluated for their response to the most common pathotypes of *G.*

*rostochiensis* occurring in Italy and the root-knot nematodes *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw. During 2006 and 2007, 34 potato cultivars and clones (Table I) were evaluated and their responses to nematodes are reported in this paper.

## MATERIALS AND METHODS

*Tests with G. rostochiensis pathotypes Ro1 and Ro2.* One population each of pathotypes Ro1 and Ro2, from the collection of the first author, were reared in clay pots containing 3 dm<sup>3</sup> steam sterilized sandy soil (about 89% sand) inoculated with about 20 eggs per g of either nematode pathotype and planted with one or two sprouting potato tubers of the susceptible potato cv. Spunta. The pots were maintained in a glass-house at 20 ± 2 °C, irrigated whenever required (every two or three days) and fertilized every two weeks with a 20-20-20 NPK fertilizer. Insecticides and fungicides were also applied, but only when considered necessary. Supplementary light was given from fall to mid-spring. About three months after plant emergence, when most of the nematodes on the roots had matured into cysts, the potatoes were uprooted, the roots shaken and removed and the soil thoroughly mixed. To estimate the nematode population density of the soil, three 200-g sub-samples of dried soil were processed with the Fenwick can, the cysts further separated from soil debris by flotation in alcohol (Seinhorst, 1974), picked up with a fine brush under a stereo-microscope, counted, crushed according to Bijloo's modified method (Seinhorst and den Ouden, 1966) and their egg content estimated using 1-ml Peter's counting slides. Then, appropriate amounts of the infested soil were thoroughly mixed in a concrete mixer with the same type of steam sterilized sandy soil, to obtain soil infested with 10-15 eggs of either pathotype per g. Three 200-g dried soil sub-samples were taken from this soil to re-check the nematode population densities before planting. The mean was considered the initial population density (Pi) and used to calculate the reproduction rate (final population density/initial = Pf/Pi) of the nematode at the end of the test. The inoculated soil was used to fill 14-cm-diameter clay pots (about 900 cm<sup>3</sup> soil), and each was planted with a tuber of the clone to be tested. There were five pots for each clone and pathotype combination and they were arranged on benches in a randomised block design. The pots were treated as mentioned before except that irrigation was every day or every other day. After 40-50 days, the root ball of each pot was examined for the presence of nematodes. Starting from when most of the nematodes on the roots of all pots were already yellow females, the plants were allowed to growth for about 20 days more and then cut at ground level and irrigation discontinued. After a week, the soil of each pot was mixed, left to dry and nematode population densities estimated by pro-

cessing 200-g sub-samples as mentioned above. The reproduction rate of the nematode and numbers of eggs per cyst were also calculated. Based on these results, a clone was considered resistant when it supported a nematode reproduction rate of less than 1.

Eighteen clones were tested in 2006 (Table II). Their reaction was compared with that of the susceptible cv. Spunta and of the resistant cv. Kuroda. The nine clones produced in 2006 were tested in 2007 (Table III) and compared with the same susceptible cultivar.

*Tests with M. incognita host race 1 and M. javanica.* One population each of *M. incognita* host race 1, 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), were increased on tomato (*Lycopersicon esculentum* Mill.) in a glass-house at 26 ± 2 °C. When large egg masses had formed on the roots, the tomatoes were uprooted, the roots gently washed in tap water and cut into 0.5-1 cm long pieces. Eggs and juveniles of the nematodes were extracted by shaking aliquots of the roots for 3 minutes in a 1% sodium hypochlorite solution and then sieving the nematode suspension through a 70 µm sieve mounted on a 5 µm sieve; water was sprayed on the sieves to wash away the chemical (Hussey and Barker, 1973). Eggs and juveniles remaining on the 5 µm sieve were collected in a beaker, diluted and their total numbers estimated by making three counts per nematode populations using 1 ml Peter's counting slides. Ten thousand eggs and juveniles of either nematode population were inoculated per pot at potato emergence by making four holes in the rhizosphere of the potatoes and pouring in the appropriate amount of nematode suspension. Growing conditions for the potato clones were as mentioned for cyst nematodes, except that the temperature of the glass-house was maintained at 26 ± 2 °C. The potato clones tested were the same, in both years, as those used in the screening with *G. rostochiensis* (Tables IV and V). However, in 2006 the cvs Kuroda, Kuras, Signum, Arnika, Quadriga and Aiko and in 2007 the clone AND 97-15, known to be resistant to some pathotypes of *G. rostochiensis* and/or *G. pallida*, were also tested because they are being used as donor parents in the new Italian potato breeding programme to obtain new genotypes suitable for organic production. In both years, tomato cv. Rutgers was used as a susceptible control to be sure that the experimental conditions were suitable for nematode infestation.

Forty to fifty days after inoculation, all potato and tomato plants were uprooted. The roots were washed free of adhering soil and their gall indices estimated on a 0 to 5 scale, in which 0 = no gall, 1 = 1-5 small galls, 2 = 5-20 small galls, 3 = more than 20 galls, 4 = root system reduced and deformed with large galls, and 5 = root system completely deformed and altered with a few

**Table I.** Description of potato cultivars and clones tested for their reaction to cyst and root-knot nematodes.

Genotype	Breeder	Cross	Resistance to nematodes		Res./Tol. to other pests and diseases <sup>1</sup>	Use
			Cyst	Root-knot		
ISCI 2/9-99	CRA-ISCI, IT	ISCI 1215 × ISCI 3119	unknown	unknown	-	w <sup>2</sup>
ISCI 4F88	CRA-ISCI, IT	Pioneer × AC Belmont	unknown	unknown	<i>Fusarium</i> dry rot	w
ISCI 87/3-99	CRA-ISCI, IT	ISCI 1212 × ISCI 3119	unknown	unknown	-	w
ISCI 69/05-1	CRA-ISCI, IT	CS 8617 × Kuroda	unknown	unknown	-	w
ISCI 69/05-2	CRA-ISCI, IT	CS 8617 × Kuroda	unknown	unknown	-	w
ISCI 69/05-3	CRA-ISCI, IT	CS 8617 × Kuroda	unknown	unknown	-	w
ISCI 2/03-1	CRA-ISCI, IT	CS 8617 × InnovatorCS Presto	unknown	unknown	-	w
ISCI 5/03-1	CRA-ISCI, IT	Marabel × Desiree	unknown	unknown	-	w
ISCI 5/93-33	CRA-ISCI, IT	Lady Rosetta × Element	unknown	unknown	-	c
ISCI 10/03-1	CRA-ISCI, IT	Kuroda × Agria	unknown	unknown	-	w
ISCI 21/01	CRA-ISCI, IT	PR 90/20 × K 2811	unknown	unknown	-	c
ISCI 33/03-1	CRA-ISCI, IT	Kuroda × ISCI 2813	unknown	unknown	-	w
ISCI 96/25-101	CRA-ISCI, IT	Vivaldi × Asterix	unknown	unknown	-	w, ff
CS 8617	University of Naples, IT	DTO 14 × W 879	Ro2	Mi1 + Mj + Mh + Ma2	-	w, ff
CS 99-2-8	University of Naples, IT	Mondial × Carmine	unknown	unknown	-	w
CS 99-6-5	University of Naples, IT	Monalisa × Alcmaria	unknown	unknown	-	w
CS 99-11-28	University of Naples, IT	Agria × Atzimba	unknown	unknown	-	w
S 99-4-20	University of Naples, IT	Mondial × Blondy	unknown	unknown	-	w
CS 99-6-3	University of Naples, IT	Monalisa × Alcmaria	unknown	unknown	-	w
MN 1404 O5	ASTRA, U.O. "M.Neri", IT	Spunta × MN 1326 G2	unknown	unknown	-	w
MN 1501 R5	ASTRA, U.O. "M.Neri", IT	Novita × Donald	unknown	unknown	-	w
MN 1503 R2	ASTRA, U.O. "M.Neri", IT	Novita × MN 356	unknown	unknown	-	w
MN 1511 R3	ASTRA, U.O. "M.Neri", IT	Liseta × MN 356	unknown	unknown	-	w
MN 2-1577 S1	ASTRA, U.O. "M.Neri", IT	Fabula × Bonell	unknown	unknown	-	w
MN 3-1469 R2	ASTRA, U.O. "M.Neri", IT	Dali × MN 356	unknown	unknown	-	w
MN 3-1500 R8	ASTRA, U.O. "M.Neri", IT	Novita × Teodora	unknown	unknown	-	w
Q 115-6	Cornell University, USA	L 227 × 790-82	unknown	unknown	potato tuber moth	unknown
AND 97-15	HZPC, NL	Agria × Innovator	Ro1 to Ro5 Pa2 + Pa3	unknown	-	s
Kuras	AGRICO, NL	PG 285 × AR 69-491	Ro1	unknown	late blight on foliage and on tuber, wart	s
Signum	AGRICO, NL	SI 87-27-723 × AR 85-096-13	Ro1 + Ro4	unknown	wart	s
Arnika	SOLANA, D	Granola × MPI 71.241/50	Ro1 to Ro5	unknown	-	s
Quadriga	SOLANA, D	Dinamo × Walli	Ro1 to Ro5	unknown	late blight on foliage, rhizoctonia, blackleg	s
Aiko	SOLANA, D	Assia × Juliver	Ro1 to Ro5	unknown	-	w
Kuroda	AGRICO, NL	AR 76-199-3 × KO 80-1407	Ro1 + Ro2	unknown	-	w, ff

<sup>1</sup> Information as given by breeding companies.<sup>2</sup> Abbreviations: w = ware potatoes; s = starch production; ff = french fries industry; c = chipping industry.

**Table II.** Reactions of potato clones to pathotypes Ro1 and Ro2 of *Globodera rostochiensis* tested in a glass-house at 20 ± 2 °C in 2006.

Genotype	Pf (eggs/g soil)		Eggs/cyst		Pf/Pi		Reaction type <sup>1</sup>
	Ro1	Ro2	Ro1	Ro2	Ro1	Ro2	
ISCI 2/9-99	9.7	3.8**	216	38**	0.7	0.3**	R
ISCI 4F88	207	449	237	268	15.5	32.2	S
ISCI 87/3-99	185.4	516**	335	362	13.8	38.2**	S
ISCI 69/05-1	9.1	2.3**	205.6	20**	0.5	0.2	R
ISCI 69/05-2	6.1	0.6**	166.8	6**	0.4	0.04**	R
ISCI 69/05-3	7.9	0.7**	170.6	7**	0.6	0.04**	R
Q 115-6	3.2	0.4	89.4	5**	0.2	0	R
CS 8617	6	2.7	172.6	29	0.4	0.2	R
CS 99-2-8	206.4	662.2**	191	272**	15.4	49.0**	S
CS 99-6-5	9.5	10	191	97	0.7	0.7	R
CS 99-11-28	12.2	9.4	217	71**	0.9	0.7	R
MN 1404 O5	94.6	586.4**	195	256	7.0	42.4**	S
MN 1501 R5	7.7	3.7	159	30**	0.6	0.3	R
MN 1503 R2	12.9	3.1**	246	30**	1.0	0.2**	R
MN 1511 R3	10	1.7**	195	17**	0.7	0.1**	R
MN 2-1577 S1	13.1	4.4	204	46**	1.0	0.3	R
MN 3-1469 R2	8.9	4.8	200	50**	0.7	0.3	R
MN 3-1500 R8	314.4	851**	280	298	23.4	63.0**	S
Kuroda (resistant check)	5.3	0.9**	157.4	9**	0.3	0.1	R
Spunta (susceptible check)	187	727**	246	256	13.9	53.8**	S
LSD P ≤ 0.05	51.65	77.92	55.08	46.19	3.85	5.69	
P ≤ 0.01	68.48	103.32	73.03	61.24	5.11	7.54	

Eggs and juveniles at planting (Pi) = 13.5/g soil for both Ro1 and Ro2. Eggs per cyst at planting 269 for Ro1 and 125 for Ro2.

Pf = nematode population (eggs/g soil) at end of the test.

<sup>1</sup>R = resistant, Pf/Pi ≤ 1; S = susceptible, Pf/Pi > 1.

\*\* Mean of Ro2 differing significantly from that of Ro1 at P ≤ 0.01, according to Student's *t* test.

very large galls (Di Vito *et al.*, 1979). Thereafter, each root system was dipped for 15-20 minutes in a 0.15% phloxine B solution to highlight the egg masses (Dickson and Ben Struble, 1966) and then the egg mass index was estimated, also on a 0-5 scale, where 0 = 0 egg mass, 1 = 1-2 egg masses, 2 = 3-10, 3 = 11-30, 4 = 31-100, and

5 > 100 egg masses (Taylor and Sasser, 1978). Potato plants were considered resistant when both gall and egg mass indices were no larger than 2.

*Statistical analysis.* Analysis of variance (ANOVA) was performed on all the data and means were com-

**Table III.** Reactions of potato clones to pathotypes Ro1 and Ro2 of *G. rostochiensis* tested in a glass-house at  $20 \pm 2$  °C in 2007.

Genotype	PF (eggs/g soil)		Eggs/cyst		Pf/Pi		Reaction type <sup>1</sup>
	Ro1	Ro2	Ro1	Ro2	Ro1	Ro2	
ISCI 96/25-101	3.28	0.7**	82	39.2	0.52	0.1**	R
ISCI 21/01	2.54	0.86	113.2	31.2	0.4	0.1	R
ISCI 5/03-1	1.78	0.96	59.4	44	0.3	0.1	R
ISCI 10/03-1	2.64	1.1	102.4	47.2*	0.22	0.1*	R
ISCI 33/03-1	6.6	0.86**	140.6	35.8	1.1	0.1**	R
ISCI 5/93-33	1229.8	717	452.4	498.4	201.6	93	S
ISCI 2/03-1	1.7	1.96	44.8	79	0.26	0.2	R
S 99-4-20	2.1	1.96	76	101.2	0.34	0.3	R
CS 99-6-3	1020.6	876.6	452	437.2*	167.2	114	S
Spunta (susceptible check)	821.4	952.2	342.6	436.4	134.6	123.8	S
LSD P ≤ 0.05	228.36	211.87	61.77	75.76	37.52	27.45	
P ≤ 0.01	306.11	283.51	82.66	100.46	50.21	36.73	

Eggs and juveniles at planting (Pi) = 6.1/g soil Ro1 and 7.7/g for Ro2. Eggs per cyst = 307 for pathotype Ro1, 463 for pathotype Ro2. Pf = nematode population (eggs/g soil) at end of the test.

<sup>1</sup>R = resistant, Pf/Pi ≤ 1; S = susceptible, Pf/Pi > 1.

Mean of Ro2 differing significantly from that of Ro1, according to Student's *t* test: \* at P ≤ 0.05 and \*\* at P ≤ 0.01.

pared by least significant difference (LSD). Also, for each infestation parameter and clone, the average values of the two pathotypes of *G. rostochiensis* were compared by Student's *t* test.

## RESULTS

*Tests with G. rostochiensis pathotypes Ro1 and Ro2.* In 2006 (Table II), the control cvs Spunta and Kuroda confirmed their susceptibility and resistance, respectively, to *G. rostochiensis*. Of the new clones of potato, thirteen out of seventeen appeared resistant to pathotypes Ro1 and Ro2 of the nematodes while the remaining four clones were highly susceptible. However, while all clones gave the same type of reaction to both pathotypes, significant differences of reaction to the two pathotypes were observed among both resistant and susceptible clones.

Among the susceptible clones inoculated with pathotype Ro1, the nematode population densities at the end of the test were similar to that of the control cv. Spunta (187 eggs/g soil) in three clones (ISCI 4F88, ISCI 87/3-99 and CS 99-2-8), larger (314.4 eggs/g soil) in clone

MN 3-1500 R8 and significantly less (94.6 eggs/g soil) in clone MN 1404 O5. The final population densities of the nematode in the pots planted to resistant clones ranged from 3.2 to 13.1 eggs/g soil, and was lower than or similar to the nematode density at planting (13.5 eggs/g soil). The average numbers of eggs per cyst of pathotype Ro1 was 246 on the susceptible cv. Spunta and similar in most of the susceptible and resistant clones, except that it was larger (335) on the susceptible clone ISCI 87/3-99 and less (89.4-172.6) on the resistant clones ISCI 69/05-2, ISCI 69/05-3, Q 115-6, MN 1501 R5 and the resistant control cv. Kuroda. The least number of eggs per cyst occurred on clone Q 115-6. The reproduction rate of pathotype Ro1 on the susceptible clones was similar to that on the control cv. Spunta (13.9) on three clones (ISCI 4F88, ISCI 87/3-99 and CS 8617), significantly less (7) on clone MN 1404 O5 and significantly larger (23.4) on clone MN 3-1500 R8.

With pathotype Ro2, the nematode soil population densities in the pots planted to susceptible clones, except for clone ISCI 4F88 and the control cv. Spunta, were significantly larger than those inoculated with pathotype Ro1. On the same clones, the number of eggs per cyst also was larger than that of pathotype Ro1, but

the observed differences were significant only on clone CS 99-2-8. Pathotype Ro2 reproduced much more than Ro1 on the susceptible clones and the control cv. Spunta and the observed differences, except on clone ISCI 4F88, were highly significant ( $P \leq 0.01$ ). On the other hand, the nematode soil population densities, eggs per cysts and reproduction rate on the resistant clones of pathotype Ro2 were similar or significantly much less than those observed for pathotype Ro1.

Of the nine new clones tested in 2007 (Table III), seven were resistant and two susceptible to both pathotypes of *G. rostochiensis*. Population densities of pathotype Ro1 on the susceptible clones and on the control cv. Spunta were in the range 821-1230 eggs/g soil; the eggs per cyst were 343-452, and the reproduction rate 135-201. On the resistant clones the values were much less, namely 1.8-3.3 eggs/g soil, 45-141 eggs per cyst, and reproduction rate of 0.3-1.1. In general, the final populations and reproduction rates for pathotype Ro2 were similar to those of Ro1, but significantly less on clones ISCI 96/25-101 and ISCI 33/03-1. The numbers of eggs per cyst were also similar for both pathotypes but with significant differences occurring with clones ISCI 10/03-1 and CS 99-6-3, in which they were less for Ro1 than Ro2.

*Tests with M. incognita host race 1 and M. javanica.* In 2006 (Table IV), on tomato cv. Rutgers the root gall indices were 4.8 and 4.5 and the egg mass indices 5 and 5, for *M. incognita* and *M. javanica*, respectively, indicating that glass-house conditions were suitable for both nematode species. On the potato clones, root gall and egg mass indices were 0 only on CS 8617. However, gall indices of 1.4-1.5 and egg mass index of 0.6 were observed on clone MN 3-1469 R2, which, therefore, was rated as resistant to both nematode species. On the other clones, root gall and egg mass indices were in the range 3-5 and, although some significant differences were observed among them, they were all considered susceptible to both nematodes. The cvs Aiko, Arnika, Kuroda, Kuras, Quadriga and Signum, known to be resistant to potato cyst nematodes, were susceptible to the root-knot nematodes as they also showed root gall and egg mass indices of 3-5.

In 2007 (Table V), root infestation by the nematodes was also severe on tomato cv. Rutgers and generally significantly less on the new clones. However, of the new clones only two were of some interest. Of them, clone ISCI 2/03-1 showed a gall index of 2.6 and an egg mass index of 3.2, and clone S 99-4-20 a gall index of 3.8 and an egg mass index of 2.5 to the two nematode species; thus they could be considered as moderately resistant to both nematodes. Of more interest was the clone AND 97-15 as it had root gall and egg mass indices of 1.8 caused by host race 1 of *M. incognita* and of 2.4 by *M. javanica* and, therefore, was classified as resistant to only the first nematode species and segregant to *M. javanica* (Table V).

## DISCUSSION

The results show that most of the new Italian potato clones were resistant to *G. rostochiensis* pathotypes Ro1 and Ro2, having inherited the resistance of their parents. Whenever a cross involved a parent resistant to *G. rostochiensis*, the resulting clones were also resistant, thus confirming the dominant nature of the resistance. The only exception was with clone CS 99-2-8, derived from cvs Mondial (resistant)  $\times$  Carmine (susceptible). Clone CS 8617 has confirmed resistance to pathotype Ro2 (Greco *et al.*, 2002) and has demonstrated its resistance also to pathotype Ro1. The resistance to both pathotypes may be due to introgression of the major gene conferring resistance to pathotype Ro1 and accumulation of minor genes from either or both parents conferring resistance also to pathotype Ro2. Although differences were observed in the reproduction of the two pathotypes of *G. rostochiensis*, they were not always significant and, therefore, they could depend on both the virulence of the pathotype and the response of the genotype. Two clones were resistant and two moderately resistant to root-knot nematodes. It is not possible, from our results and information on background of the parents, to infer the nature of resistance to root-knot nematodes. However, as observed by Gomez *et al.* (1983), the same gene confers resistance to *M. incognita* and *M. javanica* in clone CS 8617 and MN 3-1469 R2, while the gene occurring in cv. AND 97-15 also seems to confer resistance to both nematodes but to a much lesser degree. Of the four clones having as a parent the clone CS 8617 (Table I), previously found also resistant to *M. arenaria* and *M. hapla* (Di Vito *et al.*, 2003), only ISCI 2/03-1 showed some resistance to *M. incognita* and *M. javanica* (Tables IV and V). It is worthwhile to note that clone CS 8617 is highly resistant to both pathotypes of *G. rostochiensis* and both root-knot nematode species tested and also to *M. arenaria* (Neal Chitw. and *M. hapla* Chitw. (Greco *et al.*, 2002; Di Vito *et al.*, 2003), and is now under Italian examination trials necessary for its registration in the Italian national list of varieties. Multiple resistance was also observed in clone MN 3-1469 R2 to both nematodes tested, in clone ISCI 2/03-1 to *G. rostochiensis* and *M. incognita*, and in clone S 99-4-20 to *G. rostochiensis* and *M. javanica*. The breeding clone AND 97-15, a new clone from HZPC seed company, besides being resistant to all European pathotypes of *G. rostochiensis* and *G. pallida*, was also resistant to *M. incognita* and moderately resistant to *M. javanica*. All these genotypes could be usefully considered in future breeding programmes for the introgression of multiple nematode resistance into new potato cultivars.

The good agronomic traits observed during two-year (2005 and 2006) trials in Italy with the new Italian clones MN 1503 R2 (salad type), ISCI 96/25-101 (ware and fries industry) and ISCI 21/01 (chipping industry), resistant to Ro1 and Ro2 of the potato golden nematode, offer promise for their registration as new cultivars. Also,

**Table IV.** Reaction of new clones of potato to *Meloidogyne incognita* race 1 and *M. javanica* tested in a glass-house at  $26 \pm 2$  °C in 2006.

Genotype	Root gall index (0 - 5) <sup>1</sup>		Egg mass index (0 - 5) <sup>2</sup>		Reaction type <sup>3</sup>
	<i>M. incognita</i>	<i>M. javanica</i>	<i>M. incognita</i>	<i>M. javanica</i>	
ISCI 2/9-99	4.4	3.8	4.0	3.6	S
ISCI 4F88	4.0	3.6	3.4	4.0	S
ISCI 87/3-99	4.4	4.0	4.8	4.6	S
ISCI 69/05-1	3.5	3.8	4.4	4.4	S
ISCI 69/05-2	3.8	4.0	4.8	4.2	S
ISCI 69/05-3	4.5	4.3	4.8	4.8	S
Q 115-6	3.0	3.0	3.4	4.8	S
CS 99-2-8	4.4	4.0	4.4	4.0	S
CS 99-6-5	4.2	4.0	4.4	4.4	S
CS 99-11-28	4.2	3.4	4.0	3.4	S
MN 1404 O5	4.2	3.8	4.2	3.4	S
MN 1501 R5	4.4	3.8	4.2	4.2	S
MN 1503 R2	4.2	3.0	4.6	3.8	S
MN 1511 R3	4.2	3.8	4.2	4.4	S
MN 2-1577 S1	5.0	4.0	4.8	4.2	S
MN 3-1469 R2	1.5	1.4	0.6	0.6	R
MN 3-1500 R8	3.8	4.0	4.2	4.6	S
Kuroda	3.2	3.4	3.4	4.2	S
Kuras	4.0	4.1	3.4	3.4	S
Signum	3.7	3.5	3.6	3.8	S
Arnika	3.0	3.4	4.2	3.6	S
Quadriga	3.8	3.6	4.2	4.8	S
Aiko	3.6	3.8	4	4.0	S
CS 8617 (resistant check)	0	0.1	0	0	R
Tomato "Rutgers"	5.0	5.0	5.0	5.0	S
LSD P ≤ 0.05	0.93	0.68	0.91	0.61	
P ≤ 0.01	1.24	0.91	1.22	0.81	

<sup>1</sup>0 = no gall, 1 = 1-5 small galls, 2 = 6-20 small galls, 3 = more than 20 galls, 4 = root apparatus reduced and deformed with large galls, and 5 = root system completely deformed and altered with a few very large galls.

<sup>2</sup>0 = no egg mass; 1 = 1-2 egg masses, 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more than 100 egg masses.

<sup>3</sup>R = resistant, gall and/or egg mass index ≤ 2; S = susceptible, gall and/or egg mass index > 2.

**Table V.** Reaction of new clones of potato to *M. incognita* race 1 and *M. javanica* tested in a glass-house at  $26 \pm 2$  °C in 2007.

Genotype	Root gall index (0 - 5) <sup>1</sup>		Egg mass index (0 - 5) <sup>2</sup>		Reaction type <sup>3</sup>
	<i>M. incognita</i>	<i>M. javanica</i>	<i>M. incognita</i>	<i>M. javanica</i>	
ISCI 2/03-1	2.6	3.2	2.6	3.2	R/S
ISCI 5/03-1	3.4	3.0	3.3	3.0	S
ISCI 5/93-33	3.8	3.6	3.9	3.6	S
ISCI 10/03-1	4.2	4.0	4.1	4.0	S
ISCI 21/01	4.2	3.4	4.0	3.6	S
ISCI 33/03-1	3.6	4.0	3.3	4.0	S
ISCI 96/25-101	3.6	3.2	3.6	3.2	S
S 99-4-20	3.8	2.5	3.8	2.5	R/S
CS 99-6-3	4.2	3.8	4.0	3.4	S
AND 97-15	1.8	2.4	1.8	2.4	R/S
Tomato "Rutgers"	5.0	5.0	5.0	5.0	S
LSD	P ≤ 0.05	0.93	0.68	0.91	0.61
	P ≤ 0.01	1.24	0.91	1.22	0.81

<sup>1</sup>0 = no gall, 1 = 1-5 small galls, 2 = 6-20 small galls, 3 = more than 20 galls, 4 = root apparatus reduced and deformed with large galls, and 5 = root system completely deformed and altered with a few very large galls.

<sup>2</sup>0 = no egg mass; 1 = 1-2 egg masses, 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more than 100 egg masses.

<sup>3</sup>S = susceptible, gall and/or egg mass index > 2; and R/S = segregant.

as most Italian populations of the nematodes are pathotype Ro2 (Greco *et al.*, 2007), hopefully the resistance observed in these clones would last for long time.

## ACKNOWLEDGEMENTS

We wish to thank the Italian companies MENARINI & C. srl and SOLPAT srl for providing the foreign potato clones and cultivars tested. This work has been conducted within the framework of the research project "Costituzione di varietà di patata adatte alle produzioni biologiche (VAPABIO)", supported by the Italian Ministry of Agriculture.

## LITERATURE CITED

- Brodie B.B., Evans K. and Franco J., 1993. Nematode parasites of potato. Pp. 87-132. *In: Plant Parasitic Nematodes in Temperate Agriculture* (Evans K., Trudgill D.L. and Webster J.M., eds). CAB International, Wallingford, UK.
- Dickson D.W. and Ben Struble F., 1966. A sieving-staining technique for extraction of egg masses of *Meloidogyne incognita* from soil. *Phytopathology*, 55: 497 (abstr.).
- Di Vito M., Greco N., Carputo D. and Frusciante L., 2003. Response of wild and cultivated potato clones to Italian populations of root-knot nematodes *Meloidogyne* spp. *Nematologica*, 33: 65-72.
- Di Vito M., Lamberti F. and Carella A., 1979. La resistenza del pomodoro nei confronti dei nematodi galligeni: prospettive e possibilità. *Rivista di Agronomia*, 13: 313-322.
- FAO, 2007. Faostat, FAO, Rome, Italy (<http://faostat.fao.org/site/336/default.aspx>).
- Gomez P.L., Plaisted R.L. and Brodie B.B., 1983. Inheritance of the resistance of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* in potatoes. *American Potato Journal*, 60: 339-351.
- Greco N., Di Vito M., Brandonisio A., Giordano I. and De Marinis G., 1982. The effect of *Globodera pallida* and *G. rostochiensis* on potato yield. *Nematologica*, 28: 379-386.
- Greco N., Brandonisio A. and Dangelico A., 2000. Control of the potato cyst nematode, *Globodera rostochiensis*, with soil solarization. *Nematologia Mediterranea*, 28: 93-99.
- Greco N., Di Vito M. and Carputo D., 2002. Patotipi di nematodi cisticoli della patata presenti in Italia e fonti di resistenza a questi nematodi ed a quelli galligeni in nuovi cloni di patata. *Rivista di Agronomia*, 36: 61-65.
- Greco N., Brandonisio A. and De Cosmis P., 2007. Pathotypes and heterogeneity of Italian populations of *Glo-*



- bodera rostochiensis* and *G. pallida*. *Nematologia Mediterranea*, 35: 137-142.
- 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.
- Lamberti F., 1979. Economic importance of *Meloidogyne* spp. in subtropical and Mediterranean climates. Pp. 341-357. *In: Root-knot nematodes (Meloidogyne species) - Systematics, Biology and Control* (Lamberti F. and Taylor C.E., eds). Academic Press, London, UK.
- Marks R.J. and Brodie B.B., 1998. Introduction: potato cyst nematodes – an international pest complex. Pp. 1-4. *In: Potato Cyst nematodes: Biology, Distribution and Control* (Marks R.J. and Brodie B.B., eds). CAB International, Wallingford, UK.
- Russo G., Greco N., d'Errico F.P. and Brandonisio A., 2007. Impact of the root-knot nematode, *Meloidogyne incognita*, on potato during two different growing seasons. *Nematologia Mediterranea*, 35: 29-34.
- Scurrah M.M., Björn N. and Bridge J., 2005. Nematode Parasites of *Solanum* and sweet potatoes. Pp. 193-219. *In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture - Second Edition* (Luc M. Sikora R.A. and Bridge J., eds). CABI Publishing, Wallingford, UK.
- Seinhorst J.W., 1974. Separation of *Heterodera* cysts from organic debris using ethanol. *Nematologica*, 20: 367-369.
- Seinhorst J.W., 1982. The relationship in field experiments between population density of *Globodera rostochiensis* before planting potatoes and yield of potato tubers. *Nematologica*, 28: 277-284.
- Seinhorst J.W. and Ouden H. den, 1966. An improvement of Bijloo's method for determining the egg content of *Heterodera* cysts. *Nematologica*, 12: 170-171.
- Taylor A.L. and Sasser J.N., 1978. *Biology, Identification and Control of Root-knot Nematodes (Meloidogyne species)*. North Carolina State University Graphics, Raleigh, NC, USA, 111 pp.
- Trudgill D.L., Evans K and Phillips M.S., 1998. Potato cyst nematode: damage mechanisms and tolerance in the potato. Pp. 117-133. *In: Potato Cyst nematodes: Biology, Distribution and Control* (Marks R.J. and Brodie B.B., eds). CAB International, Wallingford, UK.
- Vovlas N., Mifsud D., Landa B.B. and Castillo P., 2005. Pathogenicity of the root-knot nematode *Meloidogyne javanica* on potato. *Plant Pathology* 54: 657-664.
- Whitehead A.G. and Turner S.J., 1998. Management and regulatory control strategies for potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*). Pp. 135-152. *In: Potato Cyst nematodes: Biology, Distribution and Control* (Marks R.J. and Brodie B.B., eds). CAB International, Wallingford, UK.

