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Heritability and gain from selection for field resistance against multiple root-knot nematode species (*Meloidogyne incognita* race 1 and *M. javanica*) in carrot

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Summary

Heritability and gain from selection of traits associated with field resistance to multiple root-knot nematode species were estimated in carrot (*Daucus carota* L.). Experimental plots were uniformly and simultaneously infested with *Meloidogyne incognita* race 1 and *M. javanica*. Forty-seven half-sib families derived from the cv. 'Brasília' were evaluated for: (1) percentage of commercial roots with non-galling symptoms (%RNG); (2) percentage of commercial roots with gall symptoms (%RGS) and (3) percentage of non-commercial roots (%NCR). The cultivars 'Brasília' (resistant), 'Carandaí' (intermediate) and 'Nova Kuroda' (susceptible) were employed as standards. Broad-sense heritability estimates were 61.9 % for %RNG; 30.6 % for %RGS and 67.9 % for %NCR. However, the gains from selection were very small for all traits. The genotypic correlation between %RGS and %NCR was –0.38 and the correlation between %RNG and %RGS was 0.13. Selection on increasing %RNG resulted in a negative correlated response on %NCR (genotypic correlation = –0.99) indicating repulsion phase linkage(s) between the genetic factor(s) controlling these traits. The phenotypic correlation of –0.88 between %RNG and %NCR suggested that incomplete penetrance and dosage effects might be involved in the expression of these traits. Selection of superior genotypes (i.e. high %RNG, low %RGS, and low %NCR) can be achieved. 'Brasília' was among the genotypes selected for the following cycle of recombination reinforcing the notion that this cultivar is one of the most promising sources of stable, wide-spectrum field resistance to *Meloidogyne* species in *D. carota*.

Introduction

The root-knot nematode species belonging to the genus *Meloidogyne* Goeldi may cause severe yield and quality reductions in carrot (*Daucus carota* L.). The 'cosmetic injuries', forking, and gall formation resulting from nematode infection on the tap root may reduce drastically its commercial value for fresh market consumption (Simon et al., 2000). The root-knot nematode species of worldwide economic importance for carrot cultivation are *Meloidogyne hapla*, *M. javanica* and *M. incognita*. Infection by *M. javanica* and *M. incognita* are more prevalent in tropical and subtropical areas of the world (Rubatzky et al., 1999). Control of the root-knot nematode species employing cultural management alone is difficult because these pathogens are very persistent in the soil and they have very wide host ranges, which limit options of crop rotation.

Resistance genes have been identified to *Meloidogyne* species in *Daucus* spp. germplasm and some of these genetic factors are now being incorporated into commercial cultivars (for review see Boiteux & Simon, 2002). Effective selection for resistance to *M. javanica* has been reported in carrot populations derived from the cultivar 'Brasília' (Vieira et al., 1983). Resistance to *M. javanica* in 'Brasília' was associated with delayed nematode penetration, slow nematode development and egg production with few nematodes completing their life cycles (Huang, 1986).

Breeding efforts have been also carried out in order to develop carrot populations with resistance to multiple *Meloidogyne* species. A collection of 384 carrot breeding lines was evaluated in Brazil and progenies with combined resistance to *M. incognita* race 1 and *M. javanica* were identified (Charchar & Vieira, 1994). In California, field evaluations conducted at two sites (one infested with *M. incognita* and the other infested with *M. javanica*) were able to identify lines derived from the cross 'Brasília 1252' × 'B6274' with resistance to both *M. javanica* and *M. incognita* (Simon, 1999).

Studies have been conducted to determine the genetic basis as well as the genetic parameters associated with the resistance to *Meloidogyne* species in carrot. Narrow sense heritability estimates of resistance to *M. javanica* were in the range of 0.48 ± 0.07 for primary root galling and 0.35 ± 0.08 for egg mass production in 'Brasília' (resistant), and 0.16 ± 0.11 for primary root galling and 0.31 ± 0.09 for egg mass production in 'Kuronan' (rated as tolerant) (Huang et al., 1986). Greenhouse experiments were also conducted to investigate the inheritance of resistance to *M. javanica* in one inbred line derived from the open-pollinated cultivar 'Brasília' (Simon et al., 2000). In crosses involving 'Brasilia 1252' (resistant) and 'B6274' (susceptible), the segregation data indicated that either a single dominant gene or two closely linked dominant genes might be associated with the resistant response to *M. javanica*. The genomic region encompassing the *M. javanica* resistance locus from 'Brasília' has been characterized with molecular markers and has been named the *Mj-1* locus (Boiteux et al., 2000). However, formal reports on inheritance and/or heritability studies for 'field resistance' (*sensu* Nelson, 1973) to *M. incognita* as well as for simultaneous field resistance to *M. incognita* and *M. javanica* are still scarce in carrot.

Determination of heritability of traits associated with multiple field resistance to *Meloidogyne* species is crucial in defining either the most appropriate breeding strategy or to estimate the expected improvement due to selection. The present work was designed to estimate heritability, phenotypic and genotypic correlations among resistance traits, and expected gain from selection in a population derived from the cultivar 'Brasília', which was segregating for field resistance against two root-knot nematode species: *M. incognita* race 1 and *M. javanica*.

Material and methods

Plant material

A total of 47 half-sib progenies were obtained from the cv. 'Alvorada' (a population derived from the resistant cultivar 'Brasília'). The half-sib families were obtained by harvesting seeds from vernalized roots of individual plants that were selected within a field of 'Alvorada' whose individual roots had no gall symptoms after cultivation in a soil naturally infested with *M. javanica* in Brasília-DF in 1998.

Experimental conditions

These 47 half-sib progenies were then evaluated under field conditions for root yield and simultaneous resistance to two root-knot nematodes (*M. javanica* and *M. incognita* race 1) during the hot summer season (November, 15th 1999–March, 15th 2000). Seeds had no chemical treatment and were planted by hand. The assay was conducted at the experimental farm of the CNPH/Embrapa Hortaliças (16°S and 1020 m above sea level) in a randomized complete block design with four replications. Soil was uniformly and simultaneously infested by a previous cultivation of okra (*Abelmoschus esculentus* L.) cultivar 'Santa Cruz 47' (a highly susceptible host of both nematode species). The soil type was a typical dark-red clay dystrophic latosol. Plots were sprinkler irrigated in order to keep the soil close to water saturation. Irrigation regime was determined by monitoring the levels of the water in soil with a tensiometer. The average air temperature during the assay was 25 °C with a range of 18 °C to 35 °C. The average soil temperature (taken at 10 cm below the ground level) was 24.2 °C with a range of 22.3 °C to 25.8 °C during the assay. The cultivars 'Brasília' (resistant), 'Carandaí' (intermediate) and 'Nova Kuroda' (susceptible) were employed as controls to check for root yield, inoculum pressure and uniformity of the inoculation across the experimental field. These controls were also employed for statistical and biometric comparisons with the half-sib progenies under evaluation. Plots were 2 m^2 beds with four rows (20 plants/row) and row spacing of 20 cm. Two rows were planted in each plot with seeds from one of the progenies under evaluation and the other two rows were planted with seeds of the susceptible control 'Nova Kuroda'. This arrangement provided local control of inoculum pressure and also allowed for visual comparisons of the root attributes between progenies and the commercial standard (control).

Table 1. Average percentage of commercial roots with non-galling symptoms (%RNG), percentage of commercial roots with gall symptoms (%RGS) and percentage of non-commercial roots (%NCR) of 47 carrot half-sib families and three standard cultivars evaluated for field resistance to *Meloidogyne incognita* race 1 and *M. javanica* in Brasília-DF, Brazil

Accession	% RNG	% RGS	% NCR	Accession	% RNG	% RGS	% NCR
981288/1	55.75	4.53	39.72	**981287/15**	75.61	8.12	16.27
981288/2	56.65	5.60	37.75	**981287/35**	58.18	15.17	26.64
981288/3	53.60	0.00	46.40	**981287/23**	76.57	5.32	18.12
981288/4	75.60	2.80	21.60	**981287/33**	74.50	9.97	15.53
981289/1	59.60	0.00	40.40	**981287/22**	69.37	11.70	18.93
981287/03	77.06	5.45	17.49	**981287/06**	62.98	9.30	27.72
***981287/04**	79.03	4.49	16.48	**981287/11**	70.21	5.29	24.50
981287/47	71.91	7.57	20.53	**981287/20**	54.58	9.06	36.36
981287/08	71.62	12.06	16.32	**981287/13**	63.20	12.26	24.54
981287/43	76.76	4.10	19.13	**981287/21**	53.90	7.04	39.06
981287/26	69.56	12.55	17.89	**981287/36**	70.43	5.40	24.17
981287/09	64.10	4.82	31.08	**981287/10**	64.55	5.32	30.13
981287/38	74.78	3.18	22.04	**981287/12**	57.25	14.47	28.28
***981287/34**	82.05	1.42	16.54	**981287/29**	65.50	7.73	26.76
981287/39	71.45	5.20	23.36	***981287/44**	79.06	6.72	14.22
981287/02	78.19	5.76	16.05	**981287/19**	57.10	16.56	26.34
981287/28	69.52	5.13	25.35	**981287/14**	74.42	8.24	17.35
981287/48	58.23	4.76	37.01	**981287/30**	73.66	11.90	14.43
981287/37	68.74	9.01	22.24	***981287/32**	79.61	1.76	18.63
981287/01	70.28	9.46	20.26	**981287/16**	69.91	8.46	21.63
***981287/45**	78.45	1.19	20.36	**981287/40**	68.74	8.39	22.88
981287/05	77.77	2.45	19.78	**981287/18**	75.18	5.70	19.12
***981287/07**	79.72	5.17	15.11	***Brasília**	79.04	0.00	20.96
***981287/31**	78.43	6.84	14.73	**Kuroda**	30.60	15.23	54.17
981287/27	57.66	6.43	35.90	**Carandaí**	63.70	2.27	34.03

* Selected genotype for a new recombination cycle.

Trait evaluation and genetic analysis

Observations were made for three traits: percentage of commercial roots with non-galling symptoms (%RNG); percentage of commercial roots with galling symptoms (%RGS) and percentage of non-commercial roots (%NCR). All the 40 roots in each plot were evaluated for these three traits. The evaluation of %RNG and %RGS was based on visual inspection for the presence/absence of galls on the taproot and taproot extension. Therefore, the presence of a single visible gall in the taproot resulted in the classification of a given root in the class RGS. Egg mass production was not evaluated due to the difficulty in removing intact secondary (feeder) root system especially during harvesting time under field conditions. In addition, for the specific case of carrot breeding, the presence/absence of galls in the taproot is by far the most important and practical criterion when selecting roots for fresh market consumption. The following genetic parameters were estimated for each trait under study: broad sense heritability, genetic and phenotypic correlations, coefficient of environmental variation and coefficient of genetic variation. In addition, we calculated gain from selection [GS $\text{direct}_{(x)} = i * h_x * s_{gx}$] and correlated response to selection [GS $\text{indirect}_{(y/x)} = i * h_x * s_{gx} * rg_{x,y}$] where i = selection intensity; h_x = square root of heritability; s_{gx} = genotypic standard deviation and $rg_{x,y}$ = genotypic correlation between characters x and y. The data set was analyzed using the software ‘Genes’ (Cruz, 1998) as described in detail by Cruz & Regazzi (1994).

Results and discussion

Average values for the traits %RNG, %RGS, and %NCR of the 47 half-sib families and also three stand-

Table 2. Estimate of genetic parameters of 47 carrot progenies derived from the cv. Alvorada and three standard cultivars evaluated for field resistance to *Meloidogyne javanica* and *M. incognita* race 1 in Brasília-DF, Brazil

Genetic parameters	% commercial root		% non-commercial
	without gall	with gall	roots
Phenotypic variance (average)	0.0024	0.0082	0.0102
Environmental variance (average)	0.0009	0.0057	0.0033
Genotypic variance (average)	0.0015	0.0025	0.0069
Heritability (%) (selection unit = family mean)	61.8981	30.6217	67.9126
Coefficient genetic variation -CGV (%)	2.0524	4.2181	5.5022
Coefficient environmental variation -CEV (%)	3.2204	12.6974	7.5642
CGV/CEV Ratio	0.6373	0.3322	0.7274

Table 3. Matrix of phenotypic (above bold diagonal) and genotypic (below bold diagonal) correlations among three traits: percentage of commercial roots with non-galling symptoms (%RNG); percentage of commercial roots with gall symptoms (%RGS) and percentage of non-commercial roots (%NCR). These traits were evaluated in 47 progenies derived from the cv. 'Alvorada' and three standard cultivars tested for field resistance to *Meloidogyne javanica* and *M. incognita* race 1 in Brasília-DF, Brazil

	%RNG	%RGS	%NCR
%RNG	**1.00**	–0.22	–0.88
%RGS	0.13	**1.00**	–0.22
%NCR	–0.99	–0.38	**1.00**

Table 4. Estimates of the gain from selection for percentage of commercial roots with non-galling symptoms (%RNG) and the correlated response to selection for percentage of commercial roots with gall symptoms (%RGS) and percentage of non-commercial roots (%NCR). These estimates were obtained employing 47 progenies derived from 'Alvorada' and three control cultivars evaluated for field resistance to *Meloidogyne javanica* and *M. incognita* race 1 in Brasília-DF, Brazil

Traits	Original population mean	Mean of selected progenies	Gain from selection (%)
% RNG	1.89	1.95	2.06
% RGS	1.19	1.11	–2.06
% NCR	1.52	1.42	–4.23

ard cultivars are presented in Table 1. The half-sib family values ranged from 53.60–82.05 % for RNG, 0.00–16.56 % for RGS and 14.22–46.40 % for NCR. 'Brasília' (the resistant standard cultivar) had no root with visible galling symptoms but had 20.96 % of NCR. High %NCR may be due to either a genetic attribute of the family *per se* or a result of the *Meloidogyne* spp. infection process. In our experimental conditions (with high inoculum pressure), it is possible that a fraction of this %NCR could be explained by direct (e.g. root forking) and indirect (reduced root development) consequences of *Meloidogyne* spp. attack. An illustrative example is the reaction of the susceptible standard cultivar ('Nova Kuroda') which had 2.6 times higher %NCR than the resistant standard 'Brasília' (Table 1).

Broad-sense heritability estimates were 61.9 % for %RNG; 30.6 % for %RGS and 67.9 % for %NCR (Table 2). The coefficient of environmental variation was low (indicating good environmental control in the experiment) but its relationship with the coefficient of genetic variation was also very low reinforcing that this population has, indeed, high levels of inbreeding at least for the traits under study. Genotypic correlation between %RGS and %NCR was negative and higher (–0.38), in absolute terms, than the genotypic correlation between %RNG and %RGS (0.13) (Table 3). Consequently, a direct selection on %RNG will cause a negative correlated response to selection on %RGS and %NCR especially because of the high negative genotypic correlation (–0.99) observed between %RNG and %NCR. Even though the values of heritability were relatively high, the gains from selection were very small for all traits (Table 4). This probably results from the very small genotypic variances observed for this population. This is somewhat an expected result since this population has been improved for all traits under study for as long as two decades. These relatively high levels of inbreeding on the base population resulted in very small gains from selection with values ranging below 5 % (Table 4). These gains from selection were estimated based upon the direct and indirect selection method using as a major criterion, %RNG. These expected gains from selection would be the ones obtained from the recombination of the following high performance genotypes: '981287/34', '981287/32', '981287/07', '981287/04', '981287/44', 'Brasília', '981287/45' and '981287/31' (Table 1). The index of selection 'genotype-idiotype distance' as developed by Cruz (1998) as well as the method of 'desired gains' developed by Pesek & Baker (1969) were also employed, but they resulted in lower values of genetic gains.

The genotypic correlation values also indicate that probably some of the genetic factors controlling the three traits are in the same linkage group. The development of a dense genomic map covering all the linkage groups of this base population will allow a genome-wide search to confirm and quantify the genetic associations among these quantitative traits. The high and negative genotypic correlation between %RNG and %NCR (–0.99) suggest the presence of linkage disequilibrium (in repulsion) between major genetic factors controlling expression of these traits. In addition, the phenotypic correlation value of –0.88 suggests that incomplete penetrance and dosage effects rather than pleiotropy might be influencing the phenotypic expression of these traits. Incomplete penetrance has been observed at soil temperatures above 28 °C in lines carrying the *Mj-1* locus for resistance to *M. javanica* (Simon et al., 2000). Under our experimental conditions, the highest soil temperature (observed at 10 cm below the ground level) was 25.8 °C. However, it is possible that the temperature in more superficial layers of the soil could reach around 28 °C during some warm/sunny days. This could result in lower levels of expression of resistance in roots carrying this resistance locus, especially on the first days after seed germination. In addition, it is interesting that dosage-dependent effects of the major *Mj-1* locus have been observed in F_2:F_3 progenies of derived crosses involving one 'Brasília' inbred line (Simon et al., 2000) and has been corroborated via analysis with codominant flanking markers (Boiteux, 2000).

Our results indicate that selection of genotypes combining all characteristics of interest (i.e. high %RNG and low %RGS and %NCR) could be achieved. However, depending upon family structure and selection unit these gains from selection could be maximized. It is interesting that the cultivar 'Brasília' (employed as resistant standard) was indicated by our analysis as one of the genotypes to be included in the following cycle of recombination (Table 1). This result clearly indicates that the genetic variability of this population for resistance to multiple nematode species under field conditions is far from depleted. In fact, our data as well as all the previous results obtained so far reinforce the notion that breeding for field resistance to multiple *Meloidogyne* species employing 'Brasília'-derived populations/lines as a basic germplasm might be successful. Combined resistance to *M. incognita* race 1 and *M. javanica* was identified in 384 breeding lines derived from the cultivar 'Brasília' evaluated under field conditions in Brazil. After four selection cycles, six lines were selected having infestation rates ranging from 3.7–25.2%. In a second series of experiments, 27 lines were selected with infection rates of 5.8–23.7% (Charchar & Vieira, 1994). Likewise, in California, 18 of 66 lines (derived from the cross 'Brasília 1252' × 'B6274') as well as the cultivars 'Brasília' and 'Londrina' were resistant to *M. javanica* and also highly resistant to *M. incognita* (Simon, 1999). Sources of multiple resistance to *M. javanica*, *M. incognita* and also *M. arenaria* were identified in inbred lines selfed from the original 'Brasília' population (Roberts, 1998). These results highlight the importance of 'Brasília'-derived germplasm as one of the most promising sources of stable, wide-spectrum field resistance to root-knot nematodes species in carrot.

References

Boiteux, L.S., 2000. Characterization of the *Meloidogyne javanica* Resistance Locus Employing Molecular Markers and Isolation of Candidate Disease Resistance Loci in the Carrot (*Daucus carota* L.) Gemome. Ph.D. Thesis. University of Wisconsin-Madison, Madison-WI, USA, 454 pp.

Boiteux, L.S. & P.W. Simon, 2002. Breeding for Disease Resistance in Carrots. In: Compendium of Umbelliferous Crop Diseases. APS Press, Saint Paul-MN, USA, pp. 7–8.

Boiteux, L.S., J.G. Belter, P.A. Roberts & P.W. Simon, 2000. RAPD linkage map of the genomic region encompassing the root-knot nematode (*Meloidogyne javanica*) resistance locus in carrot. Theor Appl Genet 100: 439–446.

Charchar, J.M. & J.V. Vieira, 1994. Seleção de cenoura com resistência a nematóides de galhas (*Meloidogyne* spp.). Hort Bras 12: 144–148.

Cruz, C.D., 1998. Programa Genes – Aplicativo computacional em estatística aplicada à genética. Genet Mol Biol 21: 135–138.

Cruz, C.D. & A.J. Regazzi, 1994. Modelos Biométricos Aplicados ao Melhoramento Genético. Universidade Federal de Viçosa – Imprensa Universitária, Viçosa, Minas Gerais, Brazil.

Huang S.P., 1986. Penetration, development, reproduction and sex ratio of *Meloidogyne javanica* in three carrot cultivars. J Nematol 18: 408–412.

Huang, S.P., P.T. DellaVecchia & P.E. Ferreira, 1986. Varietal response and estimates of heritability of resistance to *Meloidogyne javanica* in carrots. J Nematol 18: 496–501.

Nelson, R.R., 1973. Breeding Plants for Disease Resistance – Concepts and Applications. Pennsylvania State University Press.

Pesek, J. & R.J. Baker, 1969. Desired improvement in relation to selected indices. Can J Plant Sci 49: 803–804.

Roberts, P.A., 1998. Identification of gene sources for resistance to root-knot nematodes attacking carrots in California. Annual Report of the California Fresh Carrot Advisory Board, pp. 39–41.

Rubatzky, V.E., C.F. Quiros & P.W. Simon, 1999. Carrots and Related Vegetable *Umbelliferae*. Crop Production Science in Horticulture Vol. 10. CABI Publishing, CAB Int, Wallingford, UK, 294 pp.

Simon, P.W., 1999. Carrot breeding to develop and introduce improved cultivars for California producers, pp. 7–21. Annual Report of the California Fresh Carrot Advisory Board.

Simon, P.W., W. Matthews & P.A. Roberts, 2000. Evidence for simply inherited dominant resistance to *Meloidogyne javanica* in carrot. Theor Appl Genet 100: 735–742.

Vieira, J.V., P.T. DellaVechia & H. Ikuta, 1983. Cenoura 'Brasília'. Hort Bras 1: 42.