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Response of potato gametoclones to infection of four root-knot nematode (*Meloidogyne*) species

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Summary. Forty-six potato gametoclones were obtained from three anther donor genotypes (H_2258, AH 78/8015.37a and $F_1$15). The response of the anther-derived clones to infection of four root-knot nematode species (*Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*) was evaluated in a glasshouse experiment. Nematode reproduction rates on different gametoclones was estimated by evaluating egg-masses and galling index for each nematode species. In resistant gametoclones, examined microscopically 21 and 60 days after inoculation, infection sites were absent, or when present exhibited necrotic tissues and giant cells, but were undersized resulting in suppression of nematode development. In the roots of susceptible gametoclones, the nematode completed its development and the egg-laying females fed on well developed giant cells that had numerous hypertrophied nuclei and granulated cytoplasm. Thirteen anther-derived lines (28%) were resistant to at least one species of root-knot nematode. The resistance to root-knot nematodes was retained through the anther culture process, even when the ploidy level was reduced, indicating that the resistance mechanism is not influenced by the number of alleles involved. Variation for resistance among the gametoclones may be explained by the induction of *de novo* variability during the regeneration process.

Key words: *Solanum tuberosum*, anther culture, breeding, gametoclonal variation, resistance.

Vegetable crops, including potato, are extensively damaged by one or more nematode species of the genus *Meloidogyne*, with estimated annual losses reaching 25% (Hooker, 1981). Anther culture has been used in potato for production of primary dihaploid lines from tetraploid cultivars and of monohaploid plants from dihaploid lines (Rokka, 1998). Homozygous genotypes can be obtained in a single generation by doubling the chromosome complement of monohaploids (Sonnino *et al.*, 1988; Fleming *et al.*, 1992). The genetic variation, referred to as gametoclonal variation (Evans *et al.*, 1984), observed among androgenetic plants (Morrison & Evans, 1988; Grammatikaki, 1996) can be derived either from genetic variability present in the gametes prior to anther culture, or from the regeneration process itself (Veilleux, 1998). The possibility of using gametoclonal variation for production of potato clones resistant to viruses has been reported by several authors (Wenzel & Uhrig, 1981; Grammatikaki *et al.*, 1998), and to cyst and root knot nematodes (Uhrig, 1983; Vovlas *et al.*, 1994).

The objective of this study was to assess the variation of behaviour of root-knot nematodes present in an anther-derived potato population. The ability of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* to develop on anther-derived haploid potato (*Solanum tuberosum* L.) lines from three different genotypes was assessed.

MATERIAL AND METHODS

Three genotypes of *Solanum tuberosum* L. H_2258 (tetraploid), AH 78/8015.37a (homozygous diploid), and $F_1$15 (diploid hybrid derived from the cross AH 78/8015.37a x 381320.23, resistant to root-knot nematodes) (Vovlas *et al.*, 1994), were used as anther donor plants. Anther culture was carried out according to the procedure described by Sopory *et al.*, (1978).

Macroscopic structures (embryoids) obtained from the anthers were cultured in a regeneration medium which contained MS macro and micronutrients (Murashige & Skoog, 1962) plus 0.3 mg/l zeatin riboside, 10% coconut water and 3% sucrose. The cultures were incubated at 24 °C, in a 16h day light regime with a Photosynthetic Photon Flux (PPF) of 200 μm^{-2} s^{-1}. After three weeks the converted embryoids were transferred to MS minimal medium with 3% sucrose to promote root and shoot development. When plantlets developed root and shoot systems they were transferred to sterile soil in pots. Immediately after transfer from *in vitro* culture, the plantlet containers were covered with plastic lids to prevent rapid dehydration. The ploidy level of anther-derived plantlets was estimated by counting chromosomes in root tips, and chloroplasts on stomatal guard cells (Grammatikaki, 1996).

Forty-six potato gametoclones obtained from the three potato anther donor plants were used for root-knot nematode resistance tests. Ten plants for each gametoclonal line were grown singly in 500 cm^3 plastic pots, containing sandy loam soil and maintained under glasshouse conditions at 26-28 ±2°C. Each plant received a 10 ml aliquot of *M. incognita*, or *M. javanica*, or *M. arenaria* or *M. hapla* eggs and juveniles suspension, placed into 3-5 cm deep holes in the soil near the roots to give a total inoculum population density of 10,000 eggs and juveniles per plant. All populations of root-knot nematodes used were reared on tomato (*Lycopersicum esculentum* Mill. cv. Rutgers.) in glasshouse cultures. A small amount of roots from each gametoclonal line was harvested 3 weeks after inoculation for histological observation and study, and the remainder were collected 60 days after inoculation and their infestation assessed. Galling of each root system was evaluated according to the 0-5 scale of Taylor and Sasser (1978). Root systems were then immersed in phloxin B solution to stain egg-masses (Dickson & Benstruble , 1965) which were counted and rated for galling index.

Root segments were harvested from the plants at 21 and 60 day post inoculation and fixed in formaldehyde-acetic acid solution, dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Sections, 12 μm thick, were stained with safranin and fast-green, mounted permanently in Dammar xylene and examined microscopically (Johansen, 1941).

RESULTS AND DISCUSSION

Based on reproduction rates (egg mass index) and on histological results (necrotic tissues near the nematode body, undersized giant cells and consequent suppression of nematode development), the gametoclones with an egg-mass index < 2, and a hypersensitive reaction, were considered resistant to each *Meloidogyne* species, and those with an index > 2 susceptible. (Tables 1-5).

Histological observations of root tissues 21 and 60 days after inoculation showed that *Meloidogyne* spp. juveniles penetrated the root tissues of all lines tested. In the resistant lines juveniles were immobilized and surrounded by necrotic tissue, and although they initiated a permanent feeding site they failed to form functional giant cells. Nematodes associated with these atypical feeding sites did not develop into egg-laying adults.

On the remaining lines (egg-masses and/or a galling index > 2) evident swellings at infection sites were observed, being the result of active hyperplastic and hypertrophied formations. Each adult female was surrounded by 3 to 8 large giant cells with granulated cytoplasm and numerous hypertrophied nuclei and nucleoli. Sixty days after inoculation, in contrast to the ungalled roots or those showing only slight swellings, large egg-masses protruded from the galls of the susceptible lines. Histological examination of these galled roots revealed well-established permanent feeding sites induced by nematode feeding.

The resistance to *M. incognita* found in gametoclones derived from the genotype $F_1$15 confirms that this character is retained through the anther culture process, as previously reported by Vovlas *et al.* (1994).

From the anther donor genotype $H_2$258, 1 of 13 gametoclone was resistant to *M. arenaria*, 2 of 12 were resistant to *M. javanica* and 2 of 8 were resistant to *M. hapla*. Six of the 7 gametoclones derived from $F_1$15 were resistant to *M. incognita*. With genotype AH78/8015.37a 2 of 6 gametoclones appeared resistant to *M. incognita*. Thirteen anther derived lines (28%) apparently were resistant to at least one species of root-knot nematode, whilst the remaining lines were considered susceptible.

Variation in resistance among the gametoclones derived from AH78/8015.37 (Table 5) may be due to induction of *de novo* variability during the regeneration process. Genotype AH78/8015.37 is a homozygous diploid and consequently should provide genetically uniform gametes. This may also apply to the variation found with the gametoclones derived from $H_2$258. This clone is a heterozygous tetraploid, but should be homozygous recessive at the three specific loci involved in the resistance genes (Mendosa & Jatala, 1984). In this case the variation observed could not originate from recombination occurring before anther excision, or by possible formation of SDR unreduced microspores.

Table 1. Galling index and type of reaction to *Meloidogyne arenaria* on gametoclones of the genotype H_2258.

Gametoclone	Ploidy level	Gall and egg-mass index	Reaction type
H_2258-1G.C.	(2x)	3,4	S
H_2258-2G.C.	(2x)	4	S
H_2258-6G.C.	(2x)	3	S
H_2258-8G.C.	(2x)	3.8	S
H_2258-11G.C.	(2x)	3	S
H_2258-14G.C.	(2x)	4	S
H_2258-15G.C.	(2x)	4	S
H_2258-16G.C.	(4x)	2.2	S
H_2258-17G.C.	(2x)	3	S
H_2258-23G.C.	(2x)	3.2	S
H_2258-25G.C.	(2x)	5	S
H_2258-26G.C.	(2x)	3.2	S
H_2258-54G.C.	(4x)	0	R

Table 2. Galling index and type of reaction to *Meloidogyne javanica* on gametoclones of the genotype H_2258.

Gametoclone	Ploidy level	Gall and egg-mass index	Reaction type
H_2258-30G.C.	(2x)	4	S
H_2258-31G.C.	(2x)	3	S
H_2258-39G.C.	(2x)	2.1	S
H_2258-41G.C.	(4x)	2.2	S
H_2258-42G.C.	(4x)	2.6	S
H_2258-43G.C.	(4x)	4	S
H_2258-44G.C.	(4x)	3	S
H_2258-45G.C.	(4x)	1	R
H_2258-46G.C.	(4x)	3.5	S
H_2258-48G.C.	(2x)	3	S
H_2258-50G.C.	(4x)	2.2	S
H_2258-53G.C.	(4x)	1	R

Table 3. Galling index and type of reaction to *Meloidogyne hapla* on gametoclones of the genotype H_2258.

Gametoclone	Ploidy level	Gall and egg-mass index	Reaction type
H_2258-7-G.H.	(2x)	3	S
H_2258-6-G.H.	(2x)	3	S
H_2258-8-G.H.	(4x)	2.3	S
H_2258-10-G.H.	(4x)	2.3	S
H_2258-2-O.A.	(2x)	1	R
H_2258-4-O.A.	(4x)	2.3	S
H_2258-5-O.A.	(2x)	2.2	S
H_2258-6-O.A.	(2x)	1	R

Table 4. Galling index and type of reaction to *Meloidogyne incognita* on gametoclones of the genotype $F_1$15.

Gametoclone	Ploidy level	Gall and egg-mass index	Reaction type
$F_1$15-2-G.C.	(1x)	0.2	R
$F_1$15-3-G.C.	(2x)	0.6	R
$F_1$15-10-G.C.	(1x)	0	R
$RF_1$15-12-G.C.	(2x)	0	R
$RF_1$15-13-G.C.	(2x)	0	R
$F_1$15-14-G.C.	(2x)	0	R
$RF_1$15-3-O.A.	(2x)	4	S

R = Resistant: Egg-masses index <2; S = Susceptible: Egg-mass index >2.
Scale of evaluation of egg-mass index: 0= no galls or egg-masses per plant; 1 = 1-2 galls and/or egg-masses; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more then 100 galls and/or egg-masses per plant.

Table 5. Galling index and type of reaction to *Meloidogyne incognita* on gametoclonal lines of the genotype AH 78/8015.37a.

Gametoclone	Ploidy level	Gall and egg-mass index	Reaction type
AH 78/8015.37a-1 O.A.	(2x)	3	S
AH 78/8015.37a-8 O.A.	(2x)	3.2	S
AH 78/8015.37a-3 O.A.	(1x)	3	S
AH 78/8015.37a-4 O.A.	(1x)	2.2	S
AH 78/8015.37a-5 O.A.	(2x)	1.6	R
AH 78/8015.37a-10 O.A.	(?)	1	R

R = Resistant: Egg-masses index <2; *S* = Susceptible: Egg-mass index >2.
Scale of evaluation of egg-mass index: 0= no galls or egg-masses per plant; 1 = 1-2 galls and/or egg-masses; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more then 100 galls and/or egg-masses per plant.

Resistance to *Meloidogyne incognita* was found at the diploid level, as reported by Watanabe *et al.* (1994), and also at the monoploid level (Table 4). Therefore, the resistance mechanism seems not to be influenced by the number of alleles involved. The complex inheritance of the resistance to *Meloidogyne* species, which is controlled by three major complementary genes (Mendoza & Jatala, 1982), and the low number of gametoclones tested, prevented examination of the patterns of segregation.

In conclusion, our results support the concept that anther culture is helpful in potato breeding for resistance to root-knot nematodes. However, the use of gametoclones for genetic analysis and for drawing genetic maps seems unreliable due to the induction of new variation which skews segregation patterns in anther-derived populations (Rivard *et al.*, 1996).

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Grammatikaki G., Vovlas N., Kaltsikes P.J., Sonnino A. Реакция гаметоклонов картофеля на заражение четырьмя видами галловых нематод (*Meloidogyne*).

Резюме. На основе трех генотипов растений ($H_2$258, АН 78/8015.37а и $F_1$15) были получены 46 гаметоклонов растений. В тепличном эксперименте исследовали воздействие заражения 4-мя видами галловых нематод *(Meloidogyne incognita, M. javanica, M. arenaria* и *M. hapla*) на эти клоны, полученные из тычиночной ткани. Степень размножения нематод на различных гаметоклонах оценивали по формированию скоплений яиц и индексу галлообразования для каждого из видов. На корнях устойчивых гаметоклонов, как показало микроскопическое изучение, через 21 и 60 дней после заражения места питания обычно отсутствовали. В корнях восприимчивых гаметоклонов нематоды завершали свое развитие, яйцекладущие самки питались из гигантских клетках с гипертрофированными ядрами и гранулированной цитоплазмой. У 13 линий гаметоклонов обнаружили устойчивость по крайней мере к одному из видов галловых нематод. Устойчивость к нематодам сохранялась в процессе получения клонов из тычиночного материала, даже если уровень плоидности растений понижался. Это показывает, что число вовлеченных аллелей не влияет на механизм устойчивости. Различия по уровню устойчивости среди полученных гаметоклонов объясняется воздействием новых факторов изменчивости, появляющихся в процессе регенерации.