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Broad *Meloidogyne* Resistance in Potato Based on RNA Interference of Effector Gene *16D10*

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Abstract: Root-knot nematodes (*Meloidogyne* spp.) are a significant problem in potato (*Solanum tuberosum*) production. There is no potato cultivar with *Meloidogyne* resistance, even though resistance genes have been identified in wild potato species and were introgressed into breeding lines. The objectives of this study were to generate stable transgenic potato lines in a cv. Russet Burbank background that carry an RNA interference (RNAi) transgene capable of silencing the *16D10 Meloidogyne* effector gene, and test for resistance against some of the most important root-knot nematode species affecting potato, i.e., *M. arenaria*, *M. chitwoodi*, *M. hapla*, *M. incognita*, and *M. javanica*. At 35 days after inoculation (DAI), the number of egg masses per plant was significantly reduced by 65% to 97% ($P < 0.05$) in the RNAi line compared to wild type and empty vector controls. The largest reduction was observed in *M. hapla*, whereas the smallest reduction occurred in *M. javanica*. Likewise, the number of eggs per plant was significantly reduced by 66% to 87% in *M. arenaria* and *M. hapla*, respectively, compared to wild type and empty vector controls ($P < 0.05$). Plant-mediated RNAi silencing of the *16D10* effector gene resulted in significant resistance against all of the root-knot nematode species tested, whereas $R_{Mc1(blb)}$, the only known *Meloidogyne* resistance gene in potato, did not have a broad resistance effect. Silencing of *16D10* did not interfere with the attraction of *M. incognita* second-stage juveniles to roots, nor did it reduce root invasion.

Key words: Effector, host–parasitic relationship, *Meloidogyne arenaria*, *M. chitwoodi*, *M. hapla*, *M. incognita*, *M. javanica*, potato (*Solanum tuberosum*), resistance, RNA interference.

Root-knot nematodes (*Meloidogyne* spp.) pose serious problems to potato (*Solanum tuberosum*) production in both temperate and tropical climates (Brodie et al., 1993; Sikora and Fernández, 2005). In addition to direct losses resulting from reduced tuber yields, *Meloidogyne* spp. can render potatoes unmarketable because of quality defects caused by gall formation on the tuber surface (Finley, 1981). Some species are quarantine pests and interfere with international trade. Even though *M. chitwoodi* is present in most major potato-producing areas, its detection in tubers can lead to the rejection of entire shipments (Ingham et al., 2000; Elling, 2013). To date, no commercially available potato cultivars with resistance to root-knot nematodes exist. Current nematode control strategies in potato heavily rely on synthetic nematicides. This practice is not only costly but also potentially harmful to the environment and faces increasing restrictions from regulatory agencies in many countries.

Knowledge about root-knot nematode resistance genes in potato is sparse (Gebhardt and Valkonen, 2001; Sanchez-Puerta and Masuelli, 2011). There is no known root-knot nematode resistance gene in the cultivated potato *S. tuberosum*, but several genes have been found in wild potato species (Brown et al., 1991a, 1995; Janssen et al., 1996; Brown et al., 2004; Williamson and Kumar, 2006). The best characterized root-knot nematode resistance gene in *Solanum* sect. *Petota* is $R_{Mc1(blb)}$ from *S. bulbocastanum*, a gene that is effective against some races of *M. chitwoodi* (Brown et al., 2009). The resistance mechanism of $R_{Mc1(blb)}$ is based on a hypersensitive response and involves calcium signaling (Davies et al., 2015). Recent studies suggest that *M. chitwoodi* resistance in different species of *Solanum* is based on the same gene, thereby limiting the diversity of available resistance (Brown et al., 2014).

Resistance based on plant-mediated RNA interference (RNAi) is emerging as a promising new disease control tactic. RNAi was first discovered in *Caenorhabditis elegans* but is now regarded as a widespread phenomenon in virtually all eukaryotes (Fire et al., 1998). RNAi is based on the ability of a cell to detect and degrade double-stranded RNA (dsRNA). Dicer, a ribosome III-like enzyme, catalyzes the cleavage of long dsRNA into small interfering RNA (siRNA) segments of about 21 nt in length. Double-stranded siRNA molecules are unwound and separated into single strands. While the passenger strand is degraded, the guide strand is loaded into a large complex, RISC (RNA-induced silencing complex). If the guide strand binds to a complementary region in the mRNA of a target gene, the respective gene is silenced (Hammond et al., 2001).

Endogenous nematode genes can be silenced by expressing dsRNA that is complementary to nematode genes *in planta*. Since root-knot nematodes are able to ingest molecules of up to 140 kDa (Zhang et al., 2012), there is an opportunity for oral delivery of dsRNA or siRNA into the nematode. During feeding, the nematodes take up plant cytoplasm containing dsRNA or siRNA that may then silence the respective target gene in the parasite (Lilley et al., 2012; Elling and Jones, 2014). For RNAi applications in an agricultural setting, it is essential to avoid off-target effects that could silence

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genes in the host plant or nontarget animals or humans. Nematode effector genes represent a very specific and therefore attractive target. Effector genes are expressed in the esophageal gland cells of plant–parasitic nematodes and form the basis for the molecular interactions between the parasite and its host (Mitchum et al., 2013). The *16D10* effector gene was initially cloned in *M. incognita*, but orthologs have since been identified in *M. arenaria*, *M. chitwoodi*, *M. hapla*, and *M. javanica* (Huang et al., 2006a, 2006b; Dinh et al., 2014a). The nucleotide sequence of *16D10* is 95% to 98% identical between *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* (Huang et al., 2006a). In contrast, the *M. chitwoodi* ortholog is only 70% identical to *M. incognita* (Dinh et al., 2014a). Previous studies have shown that plant-mediated silencing of the *Meloidogyne* effector gene *16D10* can lead to a dramatic increase in resistance (Huang et al., 2006a; Dinh et al., 2014a, 2014b). However, these reports were based either on *Arabidopsis* or made use of only a single species of *Meloidogyne*. The objective of this study was to select a high-performing stable transgenic *16D10* RNAi line with the genetic background of a commercial potato cultivar and then evaluate the resistance of this line against a broad range of *Meloidogyne* spp. found in temperate and tropical potato-producing regions.

Materials and Methods

Plant transformation: Stable transgenic lines of potato 'Russet Burbank' were generated as described by Dinh et al. (2014a). Briefly, potato stem segments (about 1 cm in length) obtained from plants growing under axenic conditions were coincubated with *Agrobacterium tumefaciens* strain GV3101 carrying silencing vector pART27(16D10i-2) (Huang et al., 2006a) or empty vector pART27 as control. Incubation was done for 3 d in the dark at 19°C on CIM media (MS basal salts, 0.25 ppm folic acid, 0.05 ppm D-biotin, 2 ppm glycine, 0.5 ppm nicotinic acid, 0.5 ppm pyridoxine HCl, 0.4 ppm thiamine HCl, 0.01% myo-inositol, 3% D-sucrose, 1 ppm 6-benzylaminopurine, 2 ppm 1-naphthaleneacetic acid, 0.6% Daishin agar, pH 5.6). The stem segments were transferred every 2 wk for up to 3 mon to fresh 3C5ZR media (MS basal salts, 0.5 ppm nicotinic acid, 0.5 ppm pyridoxine HCl, 1 ppm thiamine HCl, 0.01% myo-inositol, 3% D-sucrose, 0.5 ppm indole-3-acetic acid, 3 ppm zeatin ribose, 0.5 g/liter timentin, 70 μg/ml kanamycin sulfate, 0.6% Daishin agar, pH 5.9), supplemented with 50 μg/ml kanamycin sulfate and 50 μg/ml timentin (Sheerman and Bevan, 1988; Brown et al., 1991b; Dinh et al., 2014a). Potato plantlets that regenerated on 3C5ZR media were maintained and propagated on propagation media (MS basal salts, 3% D-sucrose, 50 μg/ml kanamycin sulfate, 50 μg/ml timentin, 0.6% Daishin agar, pH 5.7). Wild type 'Russet Burbank' and advanced breeding line PA99N82-4 were maintained on propagation media without kanamycin sulfate and timentin. PA99N82-4 carries the $R_{Mc1(blb)}$ resistance gene from *S. bulbocastanum* (Brown et al., 2009).

Southern and northern blotting: DNA was extracted from potato leaves ground in liquid nitrogen with TPS buffer (100 mM Tris-HCl pH 8, 100 mM EDTA pH 8, 1 M KCl), precipitated with isopropanol, treated with RNase, and purified with chloroform/isopropanol precipitation. The DNA pellets were resuspended in 100 μl sterile water before being used for Southern blots. For each plant line, 15 μg DNA was digested with 50 U Xba I (New England Biolabs, Ipswich, MA) for 16 hr at 37°C, then separated on a 0.8% agarose gel at 70 V for 16 hr and transferred to a GeneScreen Plus nylon membrane (PerkinElmer, Boston, MA) in 10× saline sodium citrate (SSC) buffer (1.5 M NaCl, 0.15 M sodium citrate, pH 7). The membrane was UV cross-linked and hybridized for 16 hr at 42°C with a [α-^{32}P] dATP labeled 16D10i-2 probe (Dinh et al., 2014b) in hybridization buffer (50% deionized formamide, 0.1 mg/ml salmon sperm DNA, 1% sodium dodecyl sulfate (SDS), 1 M NaCl, 10% dextran sulfate). Following hybridization, the membrane was washed twice with 2× SSC buffer for 5 min at 42 °C, three times with 2× SSC plus 1% SDS for 20 min at 65°C, and three times with 0.1× SSC plus 1% SDS for 20 min at 42°C. After washing, the membrane was exposed to X-ray film for 2 d at −70°C.

For northern blots, total RNA enriched for small RNA was extracted from 1 g potato leaves using the mirVana miRNA isolation kit (Ambion, Austin, TX) according to the manufacturer's instructions. Twenty micrograms denatured total RNA of each potato line was separated on a 1% agarose gel at 120 V for 1 hr, transferred to a Nytran N nylon membrane (Sigma-Aldrich, St. Louis, MO) for 16 hr, UV cross-linked, and hybridized with [α-^{32}P] dATP labeled 16D10 and U6 probes (Dinh et al., 2014b). After 16 hr of hybridization at 25°C in hybridization buffer (50% deionized formamide, 3× SSC, 0.1 mg/ml salmon sperm DNA, 1% SDS, 0.05 M phosphate buffer, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.2% Ficoll), the membrane was washed three times for 20 min at 46°C with 2× SSC plus 0.2% SDS and exposed to X-ray film for 1 to 5 d at −70°C. Details about probes used for Southern and northern blots were described previously by Dinh et al. (2014a, 2014b).

Nematode culture and extraction: Meloidogyne arenaria, *M. chitwoodi* isolate WAMC1, *M. hapla*, *M. incognita* isolate OP-50, and *M. javanica* were maintained on tomato (*S. lycopersicum*) 'Rutgers' grown in autoclaved sand under greenhouse conditions. Nematode eggs were extracted from roots 3 mon postinoculation. To release the eggs, roots were cut into 1- to 3-cm pieces and agitated in a 0.5% NaOCl solution following the method of Hussey and Barker (1973). The suspension was poured over nested sieves (850-, 75-, 25-μm pore size from top to

bottom) and eggs collected on the 25-μm pore size sieve. Eggs were purified on a 70% sucrose gradient (Elling and Jones, 2014) before being used for infection assays. To obtain *M. incognita* infective second stage juveniles (J2) for attraction assays, eggs were hatched in modified Baermann pans (Dinh et al., 2014a).

Nematode infection assays: Single nodes of each potato line were grown in propagation media for 30 d before being transferred to a sterilized sand/soil mixture (3 parts sand : 1 part soil). Plants were supplied with 20–10–20 N–P–K liquid fertilizer every 2 d for the duration of the experiment. All infection assays were set up in randomized complete block designs with host genotype as the main effect.

The initial infection assay screened for highly resistant potato lines. Five plants each of 22 transgenic 16D10i-2 lines and wild type 'Russet Burbank' were grown in separate 6-in clay pots and inoculated with 1,200 *M. chitwoodi* eggs per pot after the plants were acclimated to greenhouse conditions (16-hr light, 22°C, and 8-hr dark, 20°C) for 10 d. Roots were harvested at 55 days after inoculation (DAI), washed to remove soil particles, and processed for egg extraction as described above. The total number of eggs was estimated by counting three 100-μl aliquots of a 50-ml suspension per root system under a Stemi 2000C stereomicroscope (Zeiss, Jena, Germany). The reproductive efficiency R was calculated as the ratio between the initial inoculated number of eggs (Pi) and the final number of eggs at harvest (Pf).

For subsequent infection assays, the best performing transgenic line D21 was tested for resistance against *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica*, with empty vector line E34, wild type PA99N82-4, and 'Russet Burbank' controls. Plantlets were transferred to Ray Leach SC10U Cone-tainers (Stuewe and Sons, Tangent, OR) and acclimated to a growth chamber for 10 d before being inoculated with nematode eggs. The infection assay was performed under 16-hr light, 24°C, and 8-hr dark, 22°C conditions in a growth chamber. For each line, 20 plants were inoculated with 2,000 eggs per nematode species. At 35 DAI, 10 root systems were harvested to count egg masses and at 55 DAI an additional 10 root systems were harvested to count the number of eggs, respectively, for each plant line and nematode combination. At 35 DAI, roots were washed, fresh weight per root system determined, and roots were stained for 15 min with 0.15 g/liter phloxine B (Fisher Scientific, Fair Lawn, NJ) to visualize egg masses. At 55 DAI, root fresh weight was determined and eggs extracted as described above. The infection assay was conducted twice for each plant line and nematode combination.

Nematode attraction assays: The attraction of *M. incognita* J2 to *16D10i-2* potato line D21 and PA99N82-4, empty vector E34, and wild type 'Russet Burbank' controls was investigated following the methods of Wang et al. (2009). Briefly, potato plantlets grown for 15 d from single nodes in propagation media were placed on microscope glass slides. The root systems were coated with 1 ml of 23% pluronic gel PF-127 (Sigma-Aldrich) containing 300 *M. incognita* J2. For each potato line, 15 replicates with one plantlet each were prepared on separate glass slides and then covered with cover slips. After exposure in a moist chamber for 6 hr at 25°C, the numbers of J2 touching the terminal 10 mm of each root tip were counted. Following, samples were kept in a moist chamber for an additional 4 d before the roots were stained with acid fuchsin (Acros Organics, Morris Plains, NJ). For staining, roots were soaked in 0.5% NaOCl for 3 min, washed under running tap water for 10 min, and boiled in acid fuchsin solution (0.35% acid fuchsin, 25% acetic acid) for 5 min (Byrd et al., 1983). Stained roots were kept in distilled water at 4°C until parasitic J2 inside roots were counted. Nematode attraction and invasion were visualized with a SteREO Discovery.V8 stereomicroscope, AxioCam ICc1 digital camera, and ZEN imaging software (Zeiss) at 6 h after inoculation and 4 DAI and staining, respectively.

Data analysis: Average numbers of eggs and egg masses and SE were calculated using Microsoft Excel. A Student's *t*-test (LSD) at alpha level 0.05 (SAS 9.2 software; SAS Institute, Cary, NC) was used to estimate statistical significant differences between treatments.

Results

Screening of 16D10i-2 stable transgenic potato lines for resistance against M. chitwoodi: Silencing construct pART27(16D10i-2) was introduced into 'Russet Burbank' potato to generate stable transgenic RNAi lines. Twenty-two independent lines, none of which showed any overt phenotypical changes compared to wild type plants, were chosen for further analysis. Southern blots indicated that lines D3, D5, D11, D21, D24, and D25 carried single copies of the *16D10i-2* transgene. Lines D2, D16, and D20 had double insertions, and the remaining lines carried more than two copies of *16D10i-2* (Fig. 1). For the 22 transgenic RNAi lines and wild type control plants inoculated with *M. chitwoodi* eggs, the number of eggs at 55 DAI and Pf/Pi ratios revealed that lines D1, D9, D21, D25, D33, and D36 were most resistant against *M. chitwoodi*. The average number of eggs per plant at 55 DAI in the resistant lines ranged from 263 in D21 to 5,633 in D25, and had corresponding Pf/Pi ratios from 0.22 to 4.69. The reduction was significant ($P < 0.05$) compared to wild type plants, which yielded on average 98,882 eggs per plant and had a Pf/Pi ratio of 82.4 (Fig. 2; Table 1). D21 emerged as the most highly resistant line in this screening assay and was chosen for further analysis. Northern blots confirmed a strong expression of *16D10*-specific small RNAs in line D21 (Fig. 3).

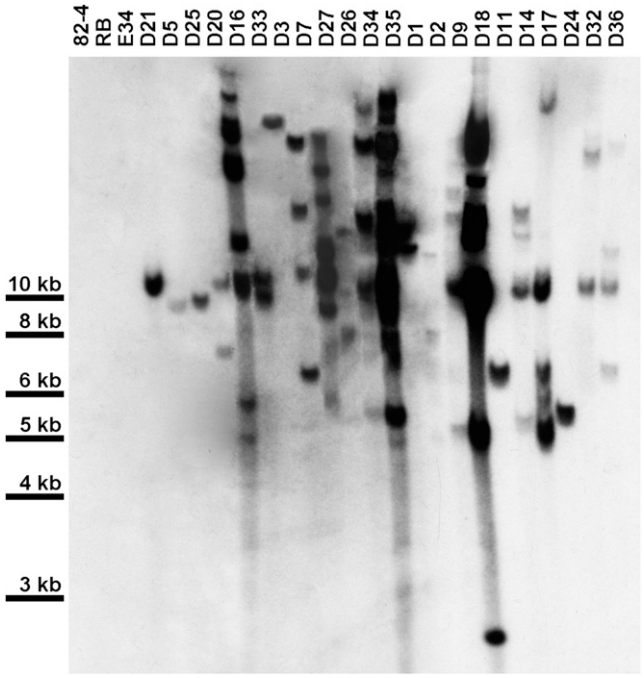

FIG. 1. Southern blot showing copy numbers of the *16D10i-2* RNAi transgene in transformed potato lines and controls. For each plant line, 15 µg DNA was digested with 50 U Xba I and separated on a 0.8% agarose gel. 82-4, PA99N82-4 advanced breeding line; RB, wild type 'Russet Burbank'; E34, empty vector control; D1 to D36, *16D10i-2* transgenic lines.

Broad Meloidogyne resistance in stable transgenic 16D10i-2 potato line D21: When challenged with *M. arenaria*, *M. hapla*, *M. incognita*, or *M. javanica*, line D21 showed strong resistance regardless of root-knot nematode species. At 35 DAI, the number of egg masses per plant was significantly reduced ($P < 0.05$) in D21 compared to wild type 'Russet Burbank', empty vector, and PA99N82-4 control plants by 65% to 97% (Figs. 4,5). The greatest reduction in the number of egg masses was achieved against *M. hapla*, whereas *M. javanica* showed the smallest reduction. Comparable levels of resistance were observed when the number of egg masses was analyzed on a per gram root basis (Fig. 4A,B). PA99N82-4 control plants, which carried the $R_{Mc1(blb)}$ resistance gene, had significantly fewer egg masses for *M. arenaria* and *M. incognita* on a per plant basis, but not when the data were adjusted for root fresh weight ($P < 0.05$). At 55 DAI, the number of eggs per plant remained significantly lower in line D21 for all root-knot nematodes tested ($P < 0.05$). Compared to wild type and empty vector controls, egg production in line D21 was reduced from 66% in *M. arenaria* to 87% in *M. hapla* on a per plant basis (Fig. 4C). Similar levels of resistance were achieved when the number of eggs were expressed on

TABLE 1. Reproductive efficiency of *Meloidogyne chitwoodi* isolate 'WAMC1' on wild type and *16D10i-2* RNAi transgenic 'Russet Burbank' potato lines at 55 days after inoculation.

Line	R = Pf/Pi ± SE[a]	Line	R = Pf/Pi ± SE[a]
RB	82.4 ± 13.14	D20	43.45* ± 8.16
D1	3.76* ± 0.50	D21	0.22* ± 0.16
D2	7.71* ± 2.13	D24	11.31* ± 3.27
D3	35.66* ± 12.89	D25	4.69* ± 0.93
D5	31.54* ± 13.66	D26	28.24* ± 9.34
D7	24.96* ± 5.93	D27	37.86* ± 9.08
D9	2.15* ± 0.93	D32	6.22* ± 1.47
D11	20.75* ± 6.39	D33	4.38* ± 0.86
D14	34.28* ± 3.34	D34	22.49* ± 17.81
D16	70.08 ± 4.45	D35	64.67 ± 21.65
D17	8.8* ± 3.34	D36	2.84* ± 1.47
D18	46.22* ± 6.00		

[a] Nematode reproduction R is shown as the ratio of the number of eggs at final harvest Pf to the initial egg inoculum Pi, (R = Pf/Pi) with SE.

RB = 'Russet Burbank'; * = significant differences compared to RB wild type according to Student's *t*-test ($P < 0.05$).

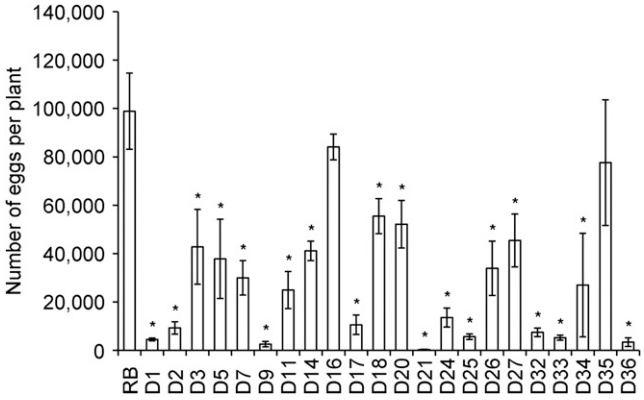

FIG. 2. Number of *Meloidogyne chitwoodi* 'WAMC1' eggs per plant in wild type and transgenic RNAi potato lines at 55 days after inoculation. RB, wild type 'Russet Burbank'. Each bar represents the mean of five plants per independent line with SE. Asterisk indicates statistically significant differences compared to RB controls using a Student's *t*-test ($P < 0.05$).

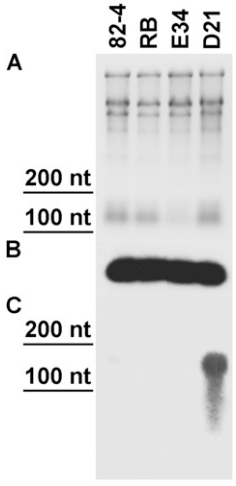

FIG. 3. Northern blot for *16D10i-2* RNAi transgene. A. Total RNA loading control. B. U6 small nuclear RNA (snRNA) loading control. C. *16D10i-2*-specific small RNA (smRNA) using probe 16D10 (Dinh et al., 2014a). 82-4, PA99N82-4 advanced breeding line; RB, wild type 'Russet Burbank'; E34, empty vector control; D21, *16D10i-2* transgenic line D21.

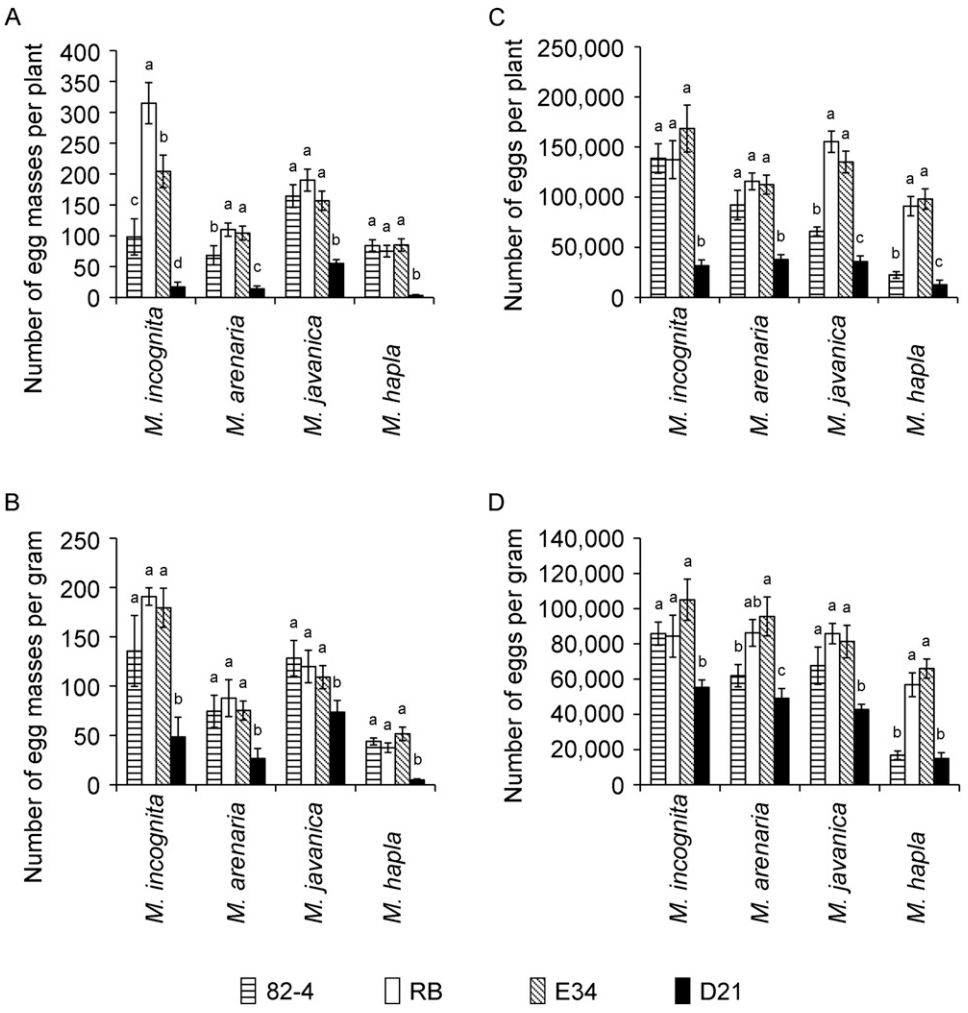

Fig. 4. Reproductive success of *Meloidogyne* spp. in potato lines with and without *16D10i-2* RNAi transgene. Each bar represents the mean of 10 plants per independent line and time point with SE. Letters indicate statistically significant differences using a Student's *t*-test ($P < 0.05$). 82-4, PA99N82-4 advanced breeding line; RB, wild type 'Russet Burbank'; E34, empty vector control; D21, *16D10i-2* transgenic line D21. A and B. Reproductive success of nematodes as number of egg masses per plant and per gram root fresh weight at 35 days after inoculation (DAI). C and D. Reproductive success of nematodes as number of eggs per plant and per gram root fresh weight at 55 DAI.

a per gram root basis (Fig. 4D). Line PA99N82-4 significantly lowered the number of eggs on a per plant and per gram root basis for *M. hapla*, but not for the other root-knot nematodes tested.

Plant-mediated 16D10i-2 resistance does not affect Meloidogyne attraction to host roots: To test whether *16D10i-2* alters the nematodes' ability to find and invade roots, an attraction assay was performed. There was no statistically significant difference between D21 and PA99N82-4, 'Russet Burbank', and empty vector controls when the number of *M. incognita* infective J2 or parasitic J2 were counted at 6 hr after inoculation or 4 DAI, respectively. No overt phenotypical differences were observed between the roots of either line (Fig. 6).

Discussion

This study demonstrates that plant-mediated RNAi resistance can protect potato against important root-knot nematode pathogens. Stable transgenic lines of potato expressing the RNAi transgene *16D10i-2* showed significant resistance against *M. arenaria*, *M. chitwoodi*, *M. hapla*, *M. incognita*, and *M. javanica*. The *16D10* gene is widely conserved in *Meloidogyne* spp., but sequence divergence can reach about 30% between some species. Importantly, all orthologs found so far carry a highly conserved region of about 21 nt that is targeted by the *16D10i-2* silencing construct (Dinh et al., 2014a). Thus, statistically significant increases in resistance compared to wild type and empty vector controls could be achieved for all root-knot nematode species tested here. The number of RNAi transgene insertions into the host genome was not a reliable indicator of the resistance level. This finding confirms previous results and underscores the dominating role of position effects (Barrell et al., 2013; Dinh et al., 2014b). Furthermore, one or more copies of promoters used to control exogenous silencing genes targeted at nematodes can be affected

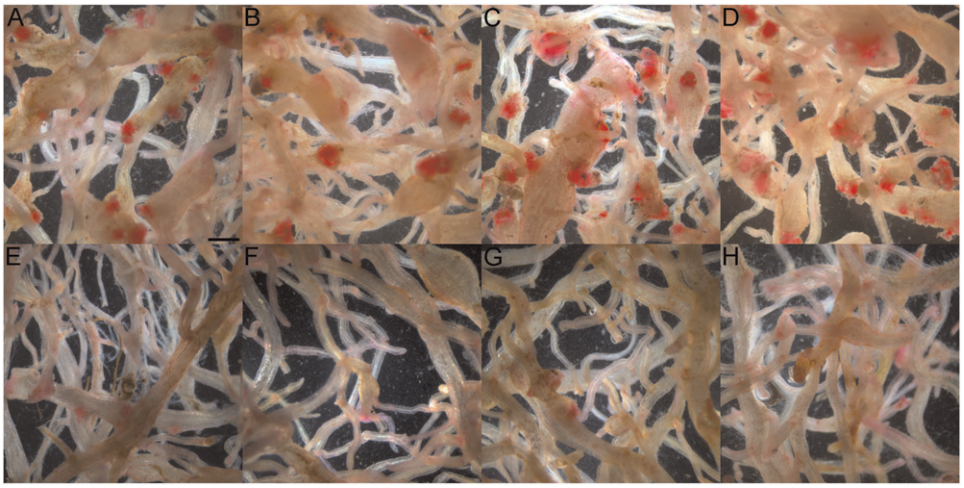

FIG. 5. Egg masses of *Meloidogyne* spp. in roots of potato lines with and without *16D10i-2* RNAi transgene. Potato plants were inoculated with nematode eggs and developing egg masses from mature females were stained 35 days after inoculation with phloxine B. A and B. Egg masses of *M. incognita and M. javanica*, respectively, in roots of 82-4, PA99N82-4 advanced breeding line. C and D. Egg masses of *M. incognita* in roots of RB, wild type 'Russet Burbank' and E34, empty vector control plants, respectively. E–H. Egg masses of *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*, respectively, in D21, *16D10i-2* transgenic line D21. Scale bar = 1 mm.

by epigenetic silencing in the plant. This phenomenon has recently been demonstrated for the Cauliflower mosaic virus 35S promoter (Kyndt et al., 2013), a ubiquitous promoter that also was used to control *16D10i-2* in this study.

Breeding for resistance against pathogens is a high priority in integrated pest management strategies. Unfortunately, the number of known root-knot nematode resistance genes in potato is extremely limited (Gebhardt and Valkonen, 2001; Williamson and Kumar, 2006). Previous studies indicated that moving root-knot nematode resistance genes across species barriers within the Solanaceae can lead to resistance in the recipient plant (Goggin et al., 2006). However, this approach is limited by the fact that the respective resistance gene might not be effective against root-knot nematodes that are relevant in the recipient plant species (Brown et al., 1997). As an alternative, this study demonstrates that targeting effector genes that are highly conserved between a wide range of

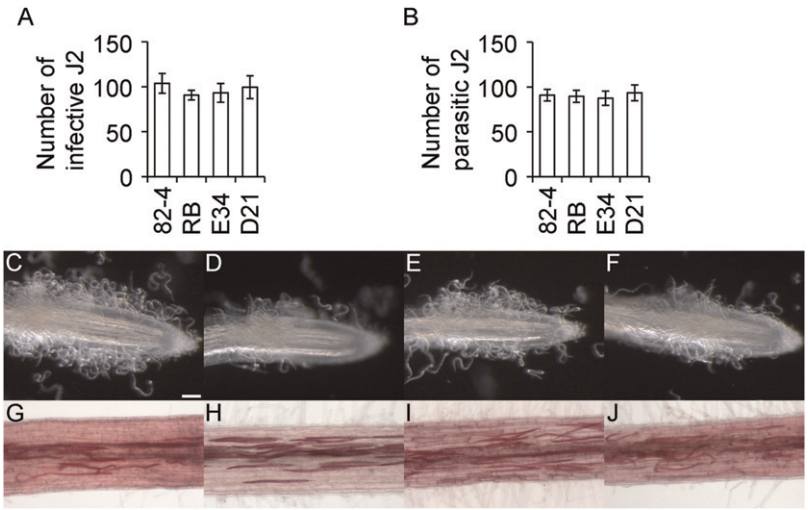

FIG. 6. Attraction and invasion of *Meloidogyne incognita* J2 to potato roots with and without *16D10i-2* RNAi transgene. A. Number of infective J2 touching the 10-mm terminal end of potato roots with and without *16D10i-2* RNAi transgene at 6 hr after inoculation. B. Number of parasitic J2 invading potato roots with and without *16D10i-2* RNAi transgene at 4 days after inoculation (DAI). Each bar represents the mean of 15 root tips per independent line with SE. Student's *t*-test did not indicate significant differences ($P > 0.05$). 82-4, PA99N82-4 advanced breeding line; RB, wild type 'Russet Burbank'; E34, empty vector control; D21, *16D10i-2* transgenic line D21. C–F. Attraction of *M. incognita* infective J2 to representative root tips at 6 hr after inoculation in potato lines 82-4, RB, E34, and D21, respectively. G–J. Invasion of *M. incognita* parasitic J2 in representative roots at 4 DAI after acid fuchsin staining in potato lines 82-4, RB, E34, and D21, respectively. Scale bar = 100 μm.

Meloidogyne spp. may be a promising control strategy and should lead to significant increases in nematode resistance.

Even though RNAi-based resistance is able to suppress the reproductive success of the nematode, this approach does not lend itself to shielding the plant from the primary infection. For a plant-mediated RNAi strategy to work, the nematode must take up plant cytoplasm containing small RNA or dsRNA complementary to an endogenous gene. Therefore, only feeding nematode life stages are vulnerable to acquiring the silencing trigger from the host. Nonfeeding life stages, such as the infective J2 are free to invade host roots, however. In *M. chitwoodi*, the *16D10* transcript is highly upregulated in infective J2, and in *M. incognita* antiserum confirmed the accumulation of 16D10 peptide in the subventral gland cells (Huang et al., 2006b; Dinh et al., 2014a). In this study, no difference was detected in the ability of *M. incognita* J2 to locate or invade roots of potato plants expressing or lacking the silencing gene *16D10i-2*. This observation suggests that *16D10i-2*-mediated resistance mechanisms occur after nematodes have invaded host roots and become sedentary. Importantly, the *16D10i-2* silencing effect can be transmitted epigenetically to nematode offspring and provide resistance even in non-RNAi plants (Dinh et al., 2014b).

In summary, this study demonstrates that RNAi-induced silencing of a nematode effector gene can lead to a dramatic reduction of a broad range of *Meloidogyne* spp. in stable transgenic lines of 'Russet Burbank' potato, thereby providing an attractive new tool for molecular breeding strategies against root-knot nematodes in this important crop.

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