A Nondestructive Technique for Screening Bean Germ Plasm for Resistance to *Meloidogyne incognita*

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ABSTRACT

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A technique to screen beans for resistance to root-knot nematodes based on egg mass counts was developed. Plants grown in seedling growth pouches were inoculated with 2,000 second-stage juveniles of *Meloidogyne incognita* race 1. Twenty-one days after inoculation, the roots were watered with erioglaucine dye (50 mg/ml) for 7 consecutive days. This dye stained the gelatinous matrix of the egg mass but not the female nematodes or the eggs. Stained egg masses were counted 28 days after initial inoculation. Egg mass counts were more highly correlated ($r = 0.85$) to egg counts than to either gall index ($r = 0.45$) or egg mass index ($r = 0.56$). Plants in growth pouches were then transplanted to pots containing UC mix soil and grown to maturity to test viability of the plants and to obtain seed from selected plants.

Additional keywords: *Phaseolus* spp.

Identifying and selecting *Phaseolus* spp. germ plasm resistant to root-knot nematodes (*Meloidogyne* spp.) for breeding purposes requires a simple, rapid, and reliable screening technique.

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Field evaluation requires major land and labor resources and may not be reliable due to escapes that occur when the field is not infested uniformly with nematodes. Therefore, initial screening often is carried out in pot experiments in a greenhouse. However, such experiments require much space, which limits the amount of material that can be screened at one time.

Several techniques have been employed to overcome these problems. Raised greenhouse benches filled with soil (1), compartmented trays and waxed paper cups (2), sections of plastic pipe filled with loamy sand, and resting on continually moistened bricks (8) all have been used for this purpose. The advantage of the last-mentioned technique was the elimination of normal greenhouse watering and easy recovery of the root system at the end of the exposure period. However, these methods require a destructive evaluation process. A desirable attribute of a resistance-screening protocol for breeding purposes is the ability to propagate resistant plants after their identification. Beans are particularly sensitive to replanting once their roots are washed free from soil.

Another method involved growing plants in peat pots with holes in the bottoms through which bean roots grew into nematode-infested soil in a greenhouse bench (10). The test plants were uprooted from the bench soil and were

evaluated for resistance at the end of the screening experiment. Desirable plants were then grown to maturity in the peat pots to facilitate crossing and seed collection. Problems with this technique include escapes and deterioration of peat pots. Seedling growth pouches have also been used to screen plants for root-knot nematode resistance based on direct observation of galling of the roots under the surface of the transparent pouch (3). In beans, root galling is not a completely satisfactory indicator of root-knot resistance (4), so it is necessary to use nematode reproduction based on egg mass counts or egg counts as the major criterion for evaluating resistance.

The objective of this study was to develop a simple screening technique, modified from that of Fassuliotis and Corley (3), that is quantitative, uses little laboratory or greenhouse space, and is nondestructive so that superior plants could be saved and grown out to maturity.

MATERIALS AND METHODS

Inoculum. *Meloidogyne incognita* (Kofoid & White) Chitwood race 1 (North Carolina State University No. 54) was multiplied on tomato (*Lycopersicon esculentum* Mill.) cv. Tropic in the greenhouse. Second-stage juveniles (J2) hatched from tomato roots incubated in distilled water at 25 C were used as the inoculum in all experiments. Seven days after transfer to seedling growth pouches, root systems of bean plants were inoculated with 2,000 J2 suspended in 4 ml of water.

Host material and seedling growth pouches. Common bean, *Phaseolus vulgaris* L., and tepary bean, *P. acutifolius* L., seeds were germinated on moist filter paper in petri dishes at 25 C and transferred, one seedling to each seedling growth pouch. The seedling growth pouch is made of a 15.5 × 12.5 cm paper wick folded at the top to make a 2-cm-deep trough in which the seedling is placed. The wick paper is placed inside a transparent plastic pouch.

After inoculation, the pouches were placed in a manila folder, two per folder, and the folders were then arranged side by side on trays. The seedlings were arranged in a completely randomized design. Twenty-one days after inoculation, plants were irrigated daily with an erioglaucine dye (Aldrich Chemical Co., Milwaukee, WI) solution (50 mg/ml) until the experiment was taken down 7 days later.

Egg mass staining. In a preliminary experiment using the growth pouches, roots of *M. incognita*-susceptible common bean cv. Black Valentine, inoculated with 2,000 J2 per plant, were irrigated with a solution containing 1, 10, 50, or 100 mg/ml of erioglaucine dye. Twelve plants treated with each dye concentration were divided into three groups of four plants. Three dye application regimes were compared as follows. Dye was applied daily for 7, 14, or 21 days before evaluation. Egg masses were stained at all dye concentrations and application regimes. However, the best egg mass staining intensity without staining the roots was obtained with 50 mg/ml erioglaucine applied for 7 consecutive days before harvest. This staining procedure was used in subsequent experiments.

Experiment one. Twenty-four bean cultivars and accessions were screened for resistance using the growth pouch technique. Four plants from each bean entry were inoculated with 2,000 J2 per plant and evaluated for resistance 28 days later. The root system of each plant grown in pouches was placed on a slide sorter with diffuse bottom light, and stained egg masses were counted by direct observation. Evaluations were made using an egg mass index (EI) (0 = no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 egg masses), and a root galling index (GI) (0 = no galling, 1 = 1–24%, 2 = 25–49%, 3 = 50–74%, and 4 = 75–100% galling of roots). After evaluation, the plastic pouch was removed from one plant from each entry and the plant was transplanted together with the paper wick into a 15-cm-deep fibre pot filled with UC mix soil. Plants were grown to maturity in the greenhouse. Eggs were extracted with NaOCl (7) from the remaining three plants to assess numbers of eggs per root system.

Experiment two. Three common bean cultivars representing different levels of resistance/susceptibility to *M. incognita* race 1 were used to compare the screening efficiency of the growth pouch technique to that of the standard pot test. Fifteen plants each of cultivars Black Valentine (susceptible), Nemasnap (moderately resistant), and A252 (resistant) were grown in seedling growth pouches. A suspension of 2,000 J2 in 4 ml of water was pipetted onto the roots of each seedling below the pouch trough. Twenty-one days later, the 7-day egg mass staining procedure was followed as previously described. Another 15 plants of each cultivar were germinated in petri dishes and grown in 10-cm-square fibre pots filled with loamy sand (93% sand, 4% silt, and 3% clay) and each pot was inoculated with 2,000 J2. Both groups of plants were maintained in a walk-in growth chamber at 25 C and 12 hr photoperiod throughout the duration of the experiment. Plants in pots were completely randomized within the chamber and the plants in the pouches were put in manila folders that were then completely randomized in their holding trays. Twenty-eight days after inoculation, the root systems were evaluated for egg mass numbers. They were then removed from the pouches and evaluated for galling, weighed, and eggs were extracted with NaOCl (7). Root systems of plants grown in pots were washed free of soil, blot-dried, and evaluated for galling. Roots were then weighed and eggs were extracted with NaOCl (7) for counting.

RESULTS

Erioglaucine dye at 50 mg/ml applied in irrigation water for 7 consecutive days before harvest resulted in excellent staining of gelatinous matrix with little or no staining of the roots. The female nematode or the eggs were not stained at any concentration of the dye. The best results were obtained when the application of the dye was started at or after the onset of egg matrix production (about 18 days after inoculation under our experimental conditions). Erioglaucine dye concentration was not found to be critical. However, when application of the dye is started earlier than 18–21 days after inoculation, the roots became stained, making some egg masses less visible. The health of the root system and survival or viability of the nematode was not affected by the dye.

The egg mass index scores agree closely with numbers of eggs per root system, as indicated by similar rankings for both parameters (Table 1). However, the gall index for many cultivars and accessions, such as Alabama No 1., A443, and G12727, did not correlate well with numbers of eggs per root system (Table 1).

Plants that were transplanted into UC mix soil immediately after evaluation for resistance survived and produced seed. However, in other tests we found that some plants died when transplanting was delayed until 5 or more weeks after inoculation (i.e., when plants were 6 or more weeks old).

When three cultivars with different levels of resistance were used to compare the two screening techniques, the eggs/plant counts from plants grown in growth pouches and those grown in the pots had similar rankings for resistance. Black Valentine was the most susceptible cultivar and A252 the most resistant. Egg mass counts were different ($P = 0.01$) for the three cultivars tested in the growth pouches (Table 2). However, the egg mass index did not distinguish Nemasnap from Black Valentine using the growth pouch technique, and the gall index failed to distinguish Nemasnap from A252. Final egg counts were higher on plants grown in growth pouches than those grown in pots. This is due in part to the 2–3 C higher temperature of plants grown in growth pouches compared with those grown in pots, and loss of some egg masses when roots of potted plants are washed free of soil.

Correlation coefficients of gall index, egg index, and egg mass counts with nematode reproduction (eggs/root system or eggs/g root fresh weight) were calculated. Egg mass counts were highly correlated with eggs/plant ($r = 0.85$) and with eggs/g root fresh weight ($r = 0.87$) in

Table 1. Screening of 24 *Phaseolus* spp. cultivars and accessions for resistance to *Meloidogyne incognita* race 1 using growth pouches

Cultivar	Eggs/plant[v]	Egg index[w,x]	Gall index[w,y]
G8108	79,300 a	4 abcd	2.5 b
310800[y]	77,466 ab	4 abcd	1.5 bcd
Alabama No. 1	64,666 abc	4.25 abc	0.25 d
A114	53,533 abc	4.75 a	2.5 b
Black Valentine	46,366 abc	4 abcd	4 a
A439	43,000 abc	4.75 a	2 bc
A21	35,633 abc	4 abcd	2 bc
A315	31,733 abc	3.25 abcde	0.5 cd
A443	23,366 abc	1.5 ef	0 d
IPA7419	21,666 abc	4.5 ab	1 bcd
Nemasnap	19,966 abc	2 ef	0.5 cd
G12727	16,966 abc	3 bcdef	0 d
PI462026	15,933 abc	2.75 cdef	2.25 b
NB86	14,866 abc	4.5 ab	0 d
A322	14,266 bc	2.25 ef	1.5 bcd
PI165426	13,866 bc	2.5 ef	0 d
PI477034[z]	13,133 bc	2.5 def	0.75 cd
A328	12,433 c	3 bcdef	1.5 bcd
A56	11,533 c	2.75 cdef	0.25 d
A55	11,366 c	2.75 cdef	1.25 bcd
Riotibagi	9,933 c	2.5 def	0 d
PI310606[z]	9,733 c	2 ef	1.5 bcd
A252	2,033 c	1 f	0 d
A445	1,100 c	0 f	0 d

[v] Values are means of three plants.
[w] Values are means of four plants. Means followed by same letter within the same column are not statistically different according to DMRT ($P = 0.01$).
[x] Egg index: 0 = no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = more than 100 egg masses.
[y] Gall index: 0 = no galling, 1 = 1–24%, 2 = 25–49%, 3 = 50–74%, 4 = 75–100% galling of roots.
[z] Cultivars are tepary beans (*P. acutifolius*), and the rest are common beans (*P. vulgaris*).

Table 2. Comparative screening of three common bean (*Phaseolus vulgaris*) cultivars for resistance to *Meloidogyne incognita* race 1 using seedling growth pouches and pots[x]

	Growth pouch				Pot
Cultivar	Egg mass numbers	Egg mass index[y]	Gall index[z]	Egg/plant	Egg/plant
Black Valentine	301 a	5 a	3.8 a	68,373 a	42,950 a
Nemasnap	131 b	4.4 a	0.1 b	23,966 b	3,066 b
A252	22 c	2.7 b	0.0 b	5,800 b	1,108 b

[x] Values are means of 15 plants. Means within a column followed by the same letter are not statistically different ($P = 0.01$) according to DMRT.
[y] Egg mass index (EI): 0 = no egg masses, 1 = 1–2, 2 = 3–10, 4 = 31–100, 5 = more than 100 egg masses.
[z] Gall index (GI): 0 = no galling, 1 = 1–24%, 2 = 25–49%, 3 = 50–74%, 4 = 75–100% galling of roots.

growth pouches, while the egg mass index ($r = 0.56$) was less correlated with the eggs/plant assay. In pot tests, gall index was better correlated ($r = 0.75$) to eggs/plant. The latter correlation is based on the three cultivars (Table 2) Black Valentine, Nemasnap, and A252, where galling corresponds well with nematode reproduction. When 24 cultivars representing a wide range of resistance and susceptibility levels were considered, correlation between gall index and nematode egg production was lower ($r = 0.45$). The correlation ($r = 0.54$) between egg mass numbers on plants grown in the growth pouches and eggs/plant on plants grown in pots was also lower, but significant.

DISCUSSION

Egg mass counts for evaluating bean plants for resistance to root-knot nematodes using growth pouches correlates very well ($r = 0.87$) to egg counts. Current methods for assaying plants for resistance using an egg mass index involves removing roots from soil and staining them with phloxine B solution (5,6). This procedure of uprooting plants for evaluation is destructive, and therefore desirable plants cannot be easily grown to maturity. To overcome this problem, growth pouches have been used to evaluate plants for resistance based primarily on galling assays (3). In beans and many other plants, galling is not a reliable indicator of resistance or susceptibility (4,6,9). For such plants, evaluation for resistance has been done by uprooting infected plants and extracting eggs from the roots or by examining the root system for egg masses. The problem of destructive evaluation of plants has hindered progress in breeding beans for resistance to root-knot nematodes (4) because, after identification, desirable plants cannot be grown for crossing or seed collection. Our technique overcomes these problems and takes only 5 weeks to identify desirable plants. The plants being evaluated take very little space, hence resistance screening work can be carried out in small growth chambers under a controlled environment. This very simple technique, done easily by a novice, combines nondestructive, but quick and quantitative, methods of evaluating plants for nematode reproduction and host suitability.

Evaluating root systems of plants 28 days after inoculation and immediately transplanting into pots allowed for excellent survival after planting. This technique will also be suitable for preliminary screening of germ plasm for resistance to root-knot nematodes, using limited space and also avoiding escapes.

Erioglaucine dye is reported for the first time as an egg mass stain that can be used in nematode resistance screening work. It may be useful in studying host-nematode interactions involving root-knot nematodes and possibly other egg mass producing nematodes, where egg masses are near or on the surface of roots. This dye also holds potential for staining egg masses in plants rooted in soil.

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