EVALUATION OF MEDICINAL HERBS FOR RESISTANCE TO ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, IN KOREA

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ABSTRACT

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Thirty-two species of medicinal herbs were tested in pots for their host suitability to *Meloidogyne incognita* under greenhouse conditions. Each plant was inoculated with 5000 eggs + second-stage juveniles. Host suitability was assessed 60-days post-inoculation on the basis of root gall index (GI) and reproduction factor (RF). Eighteen species, *Angelica acutiloba*, *A. gigas*, *A. tenuissima*, *Astragalus sinicus*, *Dolichos lalab*, *Dystaenia takesimana*, *Foeniculum vulgare*, *Glehnia littoralis*, *Hepatica asiatica*, *Lonicera japonica*, *Mentha canadensis*, *Osmunda japonica*, *Paeonia albiflora*, *P. mountan*, *P. suffruticosa*, *Potentilla discolor*, *Rehmannia glutinosa* and *Saururus chinensis* were recorded as susceptible to *M. incognita* with a GI of 2.7-5.0 and RF values between 1.5 and 13.1. Nine species, *Achyranthes bidentata*, *Acorus graminens*, *Adenophora triphylla*, *A. remotiflora*, *Atractylodes chinensis*, *Dicentra spectabilis*, *Hibiscus mutabilis*, *Pulsatilla koreana*, *Rubus coreanus* were considered as resistant with RF values of 0.3-0.8. Five species, *Allium tuberosum*, *Artemisia capillaris*, *Chrysanthemum frutescens*, *C. zawadskii* and *Rubia akane* were non-host with no galls or nematodes found on the roots.

Key words: host status, medicinal herbs, *Meloidogyne incognita*.

RESUMEN

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Se evaluó la reproducción de *Meloidogyne incognita* en 32 especies de hierbas medicinales en condiciones de invernadero. Se inoculó cada planta con 5000 huevos + juveniles de segundo estadio. La susceptibilidad de las plantas se evaluó 60 días después de la inoculación, con base en el índice de agallamiento (GI: root gall index) y el factor reproductivo (RF: reproduction factor). Dieciocho especies, *Angelica acutiloba*, *A. gigas*, *A. tenuissima*, *Astragalus sinicus*, *Dolichos lalab*, *Dystaenia takesimana*, *Foeniculum vulgare*, *Glehnia littoralis*, *Hepatica asiatica*, *Lonicera japonica*, *Mentha canadensis*, *Osmunda japonica*, *Paeonia albiflora*, *P. mountan*, *P. suffruticosa*, *Potentilla discolor*, *Rehmannia glutinosa* y *Saururus chinensis* fueron susceptibles a *M. incognita* con GI de 2.7 a 5.0 y valores de RF entre 1.5 y 13.1. Nueve especies, *Achyranthes bidentata*, *Acorus graminens*, *Adenophora triphylla*, *A. remotiflora*, *Atractylodes chinensis*, *Dicentra spectabilis*, *Hibiscus mutabilis*, *Pulsatilla koreana* y *Rubus coreanus* se consideraron resistentes, con valores de RF de 0.3 a 0.8. Cinco especies, *Allium tuberosum*, *Artemisia capillaris*, *Chrysanthemum frutescens*, *C. zawadskii* y *Rubia akane* no mostraron agallas o reproducción alguna y se consideraron inmunes.

Palabaras clave: hierbas medicinales, *Meloidogyne incognita*, susceptibilidad.

INTRODUCTION

Medicinal herbs are an important cash crop world-wide (Simon, 1986). Korea is one of the leading producers of medicinal herbs with approximately 10,000 hectares produced annually (Ministry of Agriculture and Forestry, Korea, 2003). However, due to limited arable land in Korea, the same fields have been used continuously to cultivate the same herb for several years. In such a monoculture system, soil-borne pests, especially plant-parasitic, nematodes, often become an important constraint in the production of medicinal herbs. Root-knot nematodes (*Meloidogyne* spp.) are one of the most widespread and damaging agricultural pests in the world causing an estimated US $100 billion loss/year worldwide (Oka *et al.*, 2000). *Meloidogyne incognita* (Kofoid and White) Chitwood is widely found parasitizing medicinal and ornamental plants growing in Korea and is considered economically important to several species of medicinal herbs and ornamental crops (Kim *et al.*, 1987; Park *et al.*, 1993 and 1998). However, the host status of several species has remained unexplored.

Resistant cultivars may provide an effective, economical and environmentally safe method for managing nematodes. The current availability and/or use of resistant cultivars and root stocks for nematode management reflects the success of research efforts in identifying and evaluating resistance sources, incorporating them into commercially acceptable crop selections, and implementing them into management programs (Ferris *et al.*, 1992). The knowledge of host suitability of medicinal herbs to root-knot nematodes is necessary to predict the potential effect on herb production and also the influence of each herb on nematode populations with regard to increasing or decreasing the risk of root-knot nematode on susceptible crops to be cultivated after herbs. The objective of the present work was to evaluate species of medicinal herbs for resistance to *M. incognita*, with the goal of identifying resistance in plants that might be used in crop rotation to reduce nematode damage.

MATERIALS AND METHODS

A population of *Meloidogyne incognita* race-1 was increased from a single egg mass on "Rutgers" tomato (*Lycopersicon esculentum* Mill) grown in pots containing 1000 cm^3 of sterilized field soil in the greenhouse at 25 ± 2°C to obtain inoculum for screening experiments. Eggs were extracted from galled tomato roots by agitating in 1% NaOCl (Hussey and Baker, 1973).

Thirty-two species (Table 1) of medicinal herbs were obtained from Uisong Medicinal Plant Experiment Station, Gyeongbuk, Korea. The seeds were sown in 4 × 4 cm plastic cell trays filled with vermiculite, kept in growth chamber at 25 ± 2°C for four weeks, and watered daily. Seedlings were transplanted into 6-cm diameter plastic pots containing 500-cm^3 of a steam-sterilized sandy loam (2.5% organic matter, 670 mg/kg phosphorus, 8.0 mg/kg calcium, 0.12 g/kg total nitrogen and pH 7.6). One week after transplanting, plants were individually inoculated with 5,000 eggs + second stage juveniles (J2) of *M. incognita* race-1 dispensed in 10 ml of water around the root zone with a pipette, after which the pots were lightly watered. Control pots received only water. Each medicinal plant was replicated five times. Pots were arranged in a randomized complete block design on greenhouse benches maintained at 25 ± 2°C. Three-week-old Rutgers tomato plants (nematode susceptible) were inoculated simultaneously to verify the viability of the inoculum. Plants were watered as needed to maintain the soil at field capacity and fertilized once with urea (0.10 g/pot) 30 days after nematode inoculation.

Sixty days after inoculation, plants were carefully uprooted from pots, the root systems were gently washed with tap water to remove adhering soil, and the roots were stained with Phloxine B (0.15 g/l tap

Table 1. Host suitability of 32 medicinal herbs to root-knot nematode, *Meloidogyne incognita*, assayed 60 days post-inoculation with 5000 eggs + juveniles (J_2) per plant.

Scientific name	Common name	Family	GI[x]	RF[y]	HS[z]
Achyranthes bidentata Blume	Speed well	Amaranthaceae	1.6 ± 0.5	0.5 ± 0.1	R
Dystaenia takesimana Kitagawa	Dystaenia	Apiaceae	4.0 ± 0.0	5.0 ± 0.6	S
Acorus graminens Soland	Sweet flag	Araceae	1.8 ± 0.4	0.8 ± 0.2	R
Adenophora triphylla var. Japonica Hara	Japanese lady bell	Campanulaceae	1.5 ± 0.5	0.7 ± 0.1	R
A. remotiflora Miq.	Remotiflorate lady bell	Campanulaceae	1.2 ± 0.4	0.7 ± 0.1	R
Lonicera japonica Thunb.	Japanese honeysuckle	Caprifoliaceae	4.0 ± 0.0	5.2 ± 0.6	S
Artemisia capillaris Thunb	Artemisia	Compositae	0.0	0.0	I
Atractylodes chinensis Koidz.	Atractylodes	Compositae	1.2 ± 0.4	0.3 ± 0.1	R
Chrysanthemum frutescens L.	Mgrit	Compositae	0.0	0.0	I
C. zawadskii Herb.	Mountain chrysanthemum	Compositae	0.0	0.0	I
Mentha Canadensis L.	Mint	Labiatae	5.0 ± 0.0	10.7 ± 0.8	S
Astragalus sinicus L.	Astragalus	Leguminosae	3.1 ± 0.5	1.5 ± 0.2	S
Allium sacculiferum Max.	Leek	Liliaceae	0.0	0.0	I
Hibiscus mutabilis L.	Sunset hibiscus	Malvaceae	1.2 ± 0.4	0.4 ± 0.1	R
Osmunda japonica Thunb.	Osmunda	Osmundaceae	3.4 ± 0.5	2.2 ± 0.2	S
Dicentra spectabilis L.	Bleeding heart	Papaveraceae	1.8 ± 0.4	0.8 ± 0.2	R
Dolichos lablab L.	Dolichos	Papilionaceae	3.8 ± 0.4	4.2 ± 0.9	S
Hepatica asiatica Nakai	Asian liver leaf	Ranunculaceae	2.7 ± 0.4	2.8 ± 0.6	S
Paeonia albiflora Pall	Peony	Ranunculaceae	5.0 ± 0.0	12.5 ± 1.0	S
P. mountan Andr.	Peony	Ranunculaceae	5.0 ± 0.0	11.3 ± 1.0	S
P. suffruticosa Andr.	Peony	Ranunculaceae	5.0 ± 0.0	13.1 ± 1.0	S
Pulsatilla koreana Nakai	Korean pasque-flower	Ranunculaceae	1.8 ± 0.4	0.7 ± 0.1	R
Potentilla discolor Bunge	Potentilla	Rosaceae	3.5 ± 0.5	4.2 ± 0.8	S
Rubus coreanus Miq.	Brambles	Rosaceae	1.6 ± 0.5	0.5 ± 0.2	R
Rubia akane Nakai	Rubia	Rubiaceae	0.0	0.0	I
Angelica acutiloba Kitagawa	Japanese angelica	Umbelliferae	5.0 ± 0.0	11.4 ± 1.2	S
A. gigas Nakai	Korean angelica	Umbelliferae	5.0 ± 0.0	10.8 ± 1.6	S
A. tenuissima Nakai	Chinese lovage	Umbelliferae	5.0 ± 0.0	10.5 ± 1.1	S
Foeniculum vulgare Gaert	Fennel	Umbelliferae	3.8 ± 0.4	3.2 ± 0.5	S
Glehnia littoralis Fr. Schm.	Siler divaricata	Umbelliferae	5.0 ± 0.0	8.5 ± 0.7	S
Saururus chinensis Decais	Saururus	Saururaceae	3.6 ± 0.5	3.8 ± 0.6	S
Rehmannia glutinosa Libosch.	Rehmanniae	Scrophulariaceae	3.8 ± 0.4	4.1 ± 0.7	S
Lycopersicon esculentum L.	Tomato	Solanaceae	5.0 ± 0.0	20.1 ± 2.0	S

[x]GI = gall index.
[y]RF = reproduction factor.
[z]HS = host status.

water) for 15 min. Host status was assessed by counting the number of galls per plant and rating the roots on a scale of 0-5 (gall index, GI); 0 = no galls, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >100 galls per root system (Taylor and Sasser, 1978). Nematode eggs were extracted from each root system by agitating roots in 1% NaOCL for four minutes. An estimate of nematode reproduction was based on reproduction factor (RF = Pf/Pi), calculated as the average eggs counted among replicates (Pf) divided by 5000 eggs (Pi) as the initial inoculum density.

Based on the GI and RF, the host suitability was assessed, using the following criteria: RF ≤ 1 and GI ≤ 2 resistant; RF > 1 and GI > 2 susceptible; and RF = 0 and GI = 0 immune. The experiment was repeated with the same plant materials and methods six months later. Similarity among experiments was tested by analyses of variance using experimental runs as factor. This allowed combining data from both experiments to determine the host suitability of tested species of medicinal herbs.

RESULTS AND DISCUSSION

The thirty-two species of medicinal herbs belonging to fifteen families varied in their *M. incognita* host status (Table 1). Eighteen species were determined susceptible, nine resistant and five immune. Among the susceptible species, *Angelica acutiloba*, *A. gigas*, *A. tenuissima*, *Glehnia littoralis*, *Mentha canadensis*, *Paeonia albiflora*, *P. moutan* and *P. suffruticosa* had severe root galling (GI = 5) and high reproduction factors (RF = 8.5-13.1). Lower GI (2.7-4.0) and RF (1.5-5.0) were observed on other susceptible species including *Astragalus sinicus*, *Dolichos lablab*, *Dystaenia takesimana*, *Foeniculum vulgare*, *Hepatica asiatica*, *Lonicera japonica*, *Osmunda japonica*, *Potentilla discolor*, *Rehmannia glutinosa*, and *Saururus chinensis*.

Based on GI and RF-values, *Achyranthes bidentata*, *Acorus graminens*, *Adenophora triphylla* var. Japonica, *A. remotiflora*, *Atractylodes chinensis*, *Dicentra spectabilis*, *Hibiscus mutabilis*, *Pulsatilla koreana*, *Rubus coreanus* were resistant with a GI range between 1.2 and 1.8, and RF between 0.3 and 0.8. Five species including, *Allium tuberosum*, *Artemisia capillaris*, *Chrysanthemum frutescens*, *C. zawadskii* and *Rubia akane* were observed to be immune to *M. incognita*, because no galls, eggs, or nematodes were isolated from the roots. The susceptible tomato cultivar Rutgers was severely galled (GI = 5) and the nematode population increased (Rf > 20) at the same inoculum density as used for medicinal herbs, indicating that the inoculum was viable and in sufficient quantity to initiate infection. Likewise, environmental conditions were conducive for critical evaluation of the host status of these medicinal plants.

In Korea, *M. incognita* has been commonly found in fields cultivated with medicinal herbs and ornamental crops (Kim *et al.*, 1987; Park *et al.*, 1993, 1998). The ability of the nematode to attack several of the tested medicinal herbs makes it a potential threat to susceptible crops. However, several species of medicinal herbs that were found immune or resistant to *M. incognita* race-1 could be used to reduce the nematode populations in infested fields. With adequate rotation, *M. incognita* race-1 populations could be reduced below an economic threshold level to allow planting of a susceptible crop. Nevertheless, there is need for medicinal herbs to be evaluated for resistance to additional races of *M. incognita*. The resistant species of medicinal herbs may also be important as source of resistance to *M. incognita* plant breeding programs. Continued evaluation of crop rotation for nematode management in medicinal herbs should be explored further in addition to evaluation of plant resistance.

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