Biological and Structural Mechanisms of Disease Development and Resistance in Chili Pepper Infected with the Root-knot Nematode

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Biological and structural mechanisms of the nematode disease development in chili pepper caused by the root-knot nematode, Meloidogyne incognita, were investigated. Out of 39 chili pepper cultivars/lines tested, six were found resistant, while 33 were susceptible to M. incognita, of which a susceptible cultivar Chilseongcho and three resistant cultivar/lines CM334, 02G132 and 03G53 with different resistance degrees were selected for microscopic studies on the disease development. Gall formation was greatly reduced in the resistant cultivars/lines. Nematode penetration occurred both in the susceptible and resistant chili pepper roots; however, the penetration rates were significantly lowered in the three resistant peppers compared to the susceptible pepper cv. Chilseongcho. In the susceptible pepper, giant cells were extensively formed with no discernible necrosis around the nematode feeding sites. In the highly resistant pepper cultivar CM334, no giant cell was formed, but extensive necrosis formation was observed around the penetrating nematodes. In the other two resistant pepper lines (02G132 and 03G53), both giant cells and prominent necroses were formed, and the necrotic responses appeared to inhibit the further development of giant cells or accelerate their early degeneration. Although the nematode penetration was retarded significantly in the resistant cultivars/lines, all of the above results suggest that the disease resistance of pepper may be related to post-infectional defense mechanisms (nematode growth and development) more than pre-infectional ones (penetration and establishment). Variations in structural modifications in the resistant cultivar/lines may reflect their genetic differences related to the nematode resistance.

Keywords: giant cell formation, Meloidogyne incognita, necrosis, penetration, resistance

Preparation of nematode inoculum. Four-week-old pepper plants (Capsicum annuum cv. Bugang) were inoculated with the second-stage juveniles (J2) of Meloidogyne incognita that have been used previously in root-knot nematode worldwide (Oka et al., 2000). They are important pathogens of several Solanaceous crops, especially red pepper, potato and tomato (Barker, 1998; Sasser, 1977). Other than chemical methods with fumigant and non-fumigant nematicides, use of resistant cultivars is considered one of the most effective and environmentally-safe alternatives (Hartman and Sasser, 1985).

The root-knot nematodes penetrate roots as second-stage juveniles (J2) to establish a feeding site usually within the pericycle and vascular tissues and form giant cells soon after their infection (Agrios, 2005). Galls are formed due to hyperplasia of root cells around giant cells. Intensive root galling seriously reduces root efficiency and often results in permanent wilting, premature defoliation, and eventually plant death. When susceptible plants are infected with RKN, cell enlargement accompanied by nuclear division without cytokinesis gives rise to large, multinucleate giant cells; in contrast, resistance plants to RKN are characterized by hypersensitive reaction (HR) (localized cell necrosis around the nematode head) (Kaplan and Keen, 1980). RKN juveniles surrounded by necrotic cells fail to develop, and die. These responses can occur at early infection stages, thereby preventing the nematode penetration and migration (Paulson and Webster, 1972) or at later stages, inhibiting the development of giant cells and suppressing the nematode development and multiplication (Pontier et al., 1999).

In chili pepper, however, little study has been conducted about the disease development of the root-knot nematode as yet. There fore, this study aims to examine mechanisms of resistance to the root-knot nematode (Meloidogyne incognita) in pepper cultivars and lines selected through resistance screening experiments in relation to the nematode disease cycle from penetration through development and reproduction with the aid of microscopic techniques.
experiments (Khan et al., 2008). Sixty days after inoculation, plants were carefully uprooted from pots and the root systems were gently washed with tap water to remove adhering soil. Egg masses of *M. incognita* were handpicked with the help of the forceps. The eggs were placed on Baermann funnel for three days to allow J2 to hatch out (Southey, 1986).

**Plant materials.** Thirty-nine cultivars/lines of chili pepper kindly provided by Dr. B. D. Kim, Department of Horticulture Science, Seoul National University, were used in the experiments. Seeds of the chili pepper cultivars/lines were germinated in Petri plates at 28°C for 2 days and sown in 4.5×4.5 cm plastic cell trays filled with potting soil sterilized at 121°C and 15 psi. Seeded trays were kept in growth chamber at temperature of 25°C for four weeks, and watered daily.

**Host status of chili pepper cultivars/lines for *M. incognita***. Four-week-old chili pepper plants (39 cultivars/lines) were transplanted individually into 6-cm-diameter plastic pots containing sterilized sand and potting soil mixture. Each plant was inoculated with 1,000 J2 of *M. incognita* dispensed in 10 ml of water around the root zone with a pipette, after which the pots were lightly watered. Each cultivar/line was replicated five times. Pots were arranged in randomized complete block design on greenhouse benches maintained at 25±2°C. Forty-five days after *M. incognita* inoculation, plants were carefully uprooted from pots, the root systems were gently washed with tap water to remove adhering soil, and the roots were examined with naked eyes for root galls formed on each rootlet. Root galls were observed with the help of the forceps. The eggs were placed on Baermann funnel for three days to allow J2 to hatch out (Southey, 1986).

For examining the nematode reproduction, plants were carefully uprooted from pots forty-five days after *M. incognita* inoculation, and the root systems were gently washed with tap water to remove adhering soil. Nematode egg masses formed on rootlets were examined with naked eyes.

**Structural changes of pepper root tissues infected with *M. incognita***. The four pepper cultivars/lines were planted and inoculated with J2 as described above. Plants were uprooted from pots at 2, 5 and 10 days after inoculation. Infected pepper root specimens were fixed with Karnovsky's fixative consisting of 2% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 0.05 M cacodylate buffer (pH 7.2) for 4 h. The root specimens were washed in 0.05 M cacodylate buffer (pH 7.2) for three times for 15 min each, and post-fixed in 1% osmium tetroxide in the same buffer for 2 h (kept at 4°C in a refrigerator). They were washed briefly with distilled water for 1-2 min, and en block stained in 0.5% uranyl acetate overnight at 4°C in a refrigerator. Then the specimens were dehydrated in an ethanol series of 30, 50, 70, 80, 90%, and finally three times in 100% ethanol for 10 min each. The specimens were further treated with two changes of propylene oxide each for 15 min, and embedded in Spurr's epoxy resin (Spurr, 1969), followed by polymerization at 70°C for 8 h. Sections were made 500-700 nm in thickness with a glass knife on an ultramicrotome (MT-X, RMC, Tucson, AZ, USA). The sections were stained with 1% toluidine blue O in 2% sodium tetraborate and observed under a light microscope (Axiophot, Carl Zeiss, Germany).

**Results**

**Host status of chili pepper cultivars/lines for *M. incognita***. Out of 39 chili pepper cultivars/lines screened for susceptibility and resistance to *M. incognita*, 33 cultivars/lines including cv. Chilseongcho and others (data not shown) were determined to be susceptible because numerous large root galls were formed with G1 ≥ 2.0 in all of these peppers. On the other hand, almost no gall was formed with G1 < 1.0 in 6 pepper cultivar/lines Ls. 02G132, 03G62, 04G8, and 99G198 determined to be resistant and line Ls. 03G53 and cv. CM334 to be highly resistant (Table 1).