INTRODUCTION

Chili pepper (Capsicum annuum) is one of the major vegetable commodities in Indonesia. One of the challenges in chili pepper cultivation is the infection of Meloidogyne incognita which causes gall formation in root systems. The purposes of this research were to determine 1. the response of four varieties of chili pepper against M. incognita infections; 2. damaged intensity caused by this pest in the root system; and; 3. development of M. incognita populations in root systems (rhizosphere). This research was conducted in Plant Pest Science Laboratory and Greenhouse of the Department of Crop Protection, Faculty of Agriculture, University of Gadjah Mada. This research included cultivation of three chili pepper varieties (Cabai Merah Besar, Pilar F1; Cabai Merah Keriting, Kastilo F1; and Paprika, Red Star 2060), rearing of M. incognita, inoculation, and observation. Chili pepper respond to M. incognita infections were evaluated based on agronomic aspects of the plants, such as root histopathology observation, disease intensity, and nematode populations. Results showed that each variety of chili pepper expressed various responses to infections. All varieties had different root weight and length, but had similar sensitivity based on the appearance of root damage and egg mass scoring of M. incognita. Although nematode population could develop on roots, observation from root tissues showed lignification after infection of J2 M. incognita. Chili pepper var. Red Star 2060 was more susceptible against M. incognita than chili pepper var. Pilar F1 and chili pepper var. Kastilo F1.

Keywords: chili pepper; host plant response; M. incognita

ABSTRACT

Chili Pepper (Capsicum annuum) is one of the major vegetable commodities in Indonesia. One of the challenges in chili pepper cultivation is the infection of Meloidogyne incognita which causes gall formation in root systems. The purposes of this research were to determine 1. the response of four varieties of chili pepper against M. incognita infections; 2. damaged intensity caused by this pest in the root system; and; 3. development of M. incognita populations in root systems (rhizosphere). This research was conducted in Plant Pest Science Laboratory and Greenhouse of the Department of Crop Protection, Faculty of Agriculture, University of Gadjah Mada. This research included cultivation of three chili pepper varieties (Cabai Merah Besar, Pilar F1; Cabai Merah Keriting, Kastilo F1; and Paprika, Red Star 2060), rearing of M. incognita, inoculation, and observation. Chili pepper respond to M. incognita infections were evaluated based on agronomic aspects of the plants, such as root histopathology observation, disease intensity, and nematode populations. Results showed that each variety of chili pepper expressed various responses to infections. All varieties had different root weight and length, but had similar sensitivity based on the appearance of root damage and egg mass scoring of M. incognita. Although nematode population could develop on roots, observation from root tissues showed lignification after infection of J2 M. incognita. Chili pepper var. Red Star 2060 was more susceptible against M. incognita than chili pepper var. Pilar F1 and chili pepper var. Kastilo F1.

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INTRODUCTION

Chili pepper is a major vegetable commodity in Indonesia, which is both consumed locally and exported to other countries. Chili pepper production averagely reaches 1,915,120 million ton each year (Sekretariat Jenderal Kementerian Pertanian, 2016).

Most chili pepper variety are susceptible to the root-gall nematode, Meloidogyne incognita (Oka et al., 2004). Chili pepper yield lost due to the infection of M. incognita is estimated to reach 12–90%, depending on population levels, and the biotic and abiotic factor of its surrounding environment (Sasser & Freckman, 1987). Oka et al. (2000) stated that economic loss due to the infection of root-gall nematodes may reach US $100 million each year globally.

M. incognita are well-known for its ability to reproduce rapidly at temperatures above 18°C and Secondary infection by plant pathogen, such like a fungus. In the the process of penetration and feeding, Infection of M. incognita on plant root become subject to infection by fungal pathogens. Its effects to plant host may even spread wider in tropic climates due to environmental conditions that affect nematodes reproduction, survival and dispersion (Luc et al., 2005). M. incognita have not been considered to be a serious pest in San Joaquin Valley on bell peppers, another chili variety. However, the nematode is a serious pest on bell peppers in Coachella Valley due to its desert condition (Aguiar et al., 2014). Environmental differences may indirectly affect M. incognita by alternating temperature, soil pore sizes, and water availability. Temperature may affect metabolic changes, movement, and activity. M. incognita in general requires higher temperature (such as in tropical climates) between 25–30°C while suitable soil type for nematode movement are soil types that are light and sandy (Mulyadi, 2009).

Infected host roots show galls and swollen root tissue, which contain nematodes. In addition, root veins of infected plants were plugged by sap-like substances. This inhibits water and nutrition trans-location from roots to other organs (Vovlasa et al., 2005).
Plants are able to recognize, react, and activate resistant response when they are infested by parasites or pathogens (Kosuge, 1969; Fitriyanti et al., 2009). Susceptible plants that are infested by root-gall nematodes will have enlarged cells followed by cell division that are not accompanied by cytokinesis. In contrast, resistant plants may respond by producing hypersensitive reaction when infested by root-gall nematodes by producing localize necrotic cells around feeding sites, which have darker colors compared to its surrounding cells (Kaplan & Keen, 1980). “Carolina Wonder” and “Charleston Belle” are example of chili varieties that are resistant against *M. incognita* based on the existence of the homogeneous gen-N (Thies & Fery, 1998; Aguiar et al., 2014). In order to manage *M. incognita*, research on the responses of four *Capsicum* spp., varieties when infested by nematodes is required. Selecting resistant varieties is essential in crop rotation to suppress nematode populations.

**MATERIALS AND METHODS**

*M. incognita* Rearing

Tomato seeds were planted is sterile growing medium placed in polybags to obtain 14-day-old plants. Each 14-day-old plant were then planted in a polybag with 45 cm in diameter. Seven days after being moved to larger polybags, tomato plants were infested with egg masses of *M. incognita*. After 60 days since infestation, tomato plants were taken and eggs of *M. incognita* were then harvested and hatched into L2 as an inoculum.

Preparation of Plant for Test

Growing medium was a mixture of sterilized soil and manure (ratio 1:1). Sterile growing medium were then placed into 45 cm diameter polybags, and filled up to ⅔ of the polybag volume. Chili seeds were placed in small polybags with diameter of ± 6 cm. After 3 weeks, test plants were transplanted to larger polybags with diameter of 30 cm. Chili pepper varieties used in this study were *Cabai Merah Besar* (Pilar F1), *Cabai Merah Keriting* (Kastilo F1), and *Paprika* (Red Star 2060). Inoculation treatments in this study were: (1) inoculated with *M. incognita* and (2) untreated, with 10 replications for each treatment.

Inoculation of *M. incognita* on Plants

*M. incognita* eggs were extracted from roots of tomatoes by using 1% NaOCl solution according to methods by Hussey and Baker (1973) to clean debris. Eggs of *M. incognita* were then extracted and left at room temperature for 3–4 days until they hatched to L2. The suspension consisting of 1000 L2 M. incognita was inoculated on the 30 day old test plants using a syringe to inject the suspension into the soil around the test plants.

**Observation and Data Analysis**

Observation were done on histopathology of test plant roots, damage intensity, which followed procedures from Zeck (1971), and nematode populations from root tissues and soil surrounding test plants were extracted using Whitehead Tray Technique with modification (Southey, 1986). Data were then compared using an ANOVA. Significant differences were then test using a DMRT post-hoc test at α=0.05.

**RESULTS AND DISCUSSION**

Root Damage Intensity

Result of root damage intensity scoring based on Zeck (1971) was shown in Figure 1 and Table 1. Response between chili pepper variety were not significantly different. However, data numerically showed that *Paprika* were more susceptible due to the many galls found on its root (Figure 1A and B) and the highest gall scoring 7.7. Gall scorings were then followed by *Cabai keriting* (7.14) and *Cabai besar* (6.85). These scores indicated that all varieties tested had similar root sensitivity against *M. incognita*. This is supported by the same egg mass scores that all chili pepper varieties had (c.a. 5) (Table 2). Root damage scores from tested varieties indicate nematode population in root system that may directly impact growth, especially their leaves. Lower leaf growth may result in lower production. These results were consistent with the findings of Anwar et al. (2013) regarding to crop suitability as nematode host based on galls found on roots. Host crops considered as susceptible against *M. incognita* had root gall score of 6–8.2.

**Scores of Root Damage by *M. incognita***

All three tested varieties were grouped based on the number egg masses were formed on roots based on Quesenberry et al. (1989). Scoring showed that all three tested varieties had similar scores implying that all plant varieties were susceptible to *M. incognita*. 

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Histopathology observation showed that there was absorption of safranin on inoculated chili pepper varieties. This observation used safranin (red) and fast green (green). Coloring using safranin was used to color lignin and lignified cells, while fast green was used to show cytoplasm and cellulose (Johansen, 1940; Sass, 1971).

Samples from 6-day-old test plants showed a variation of resistant response to nematodes (Figure 2). Paprika roots (Figure 2A) showed accumulation of red in transport veins. This indicates that lignification process by plants. This process results in cell becoming impermeable to water and nutrition from outside causing cells to die (Slusarenko et al., 2000). Response may occur at different times due the different sensitivity levels of plants to *M. incognita* infection (Thomas *et al.*, 1995). Lignification that is considered as a resistant response of Paprika roots was not able to counter *M. incognita* numbers causing Paprika to possess the highest root damage scores. Populations from Paprika were higher compared to other varieties.

**Table 1.** Root damage of four *Capsicum annuum* varieties inoculated with *Meloidogyne incognita*

| Variety          | Damage scores |
|------------------|---------------|
| Cabai Keriting   | 7.14 a        |
| Cabai Merah Besar| 6.85 a        |
| Paprika          | 7.71 a        |
| Control          | 0.00 b        |

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.

**Table 2.** Scoring of *Meloidogyne incognita* egg masses from test plant 60 days after inoculated (DAI)

| Treatment          | Egg mass scores |
|--------------------|-----------------|
| Cabai Keriting     | 5.00 a          |
| Cabai Merah Besar  | 5.00 a          |
| Paprika            | 5.00 a          |
| Control            | 0.00 b          |

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.
stages, such as L3, L4 or adult females (Figure 4), number of galls, number of egg masses produced and reproduction levels.

**Population of L3, L4, and Adult *M. incognita* in Root Tissue**

Coloring was done to precisely show *M. incognita* due to difficulties of microscopically differ between nematodes and root tissue. Results showed significantly different L3, L4, and adult populations between inoculated and controls (Table 5). *Paprika* was relatively susceptible against *M. incognita*. High populations collected from *Paprika* root system is caused by its soft root tissues compared to *Cabai besar* and *Cabai keriting*, which caused nematodes...
to easily locate feeding sites and develop to further stages. Root galls indicated *M. incognita* ability to form feeding sites. The higher the ability of nematodes to form feeding sites, the more severe the galls that are formed.

**CONCLUSION**

Histopathology observation showed that *Paprika* variety was more sensitive to *M. incognita* infection compared to *Cabai keriting* and *Cabai besar*. *Cabai keriting*, *Cabai besar*, and *Paprika* root systems were similarly susceptible to *M. incognita* infection based on their scores on root damage and egg masses. *M. incognita* developed well on *Paprika* variety based on the populations of nematodes in 100 ml of soil surrounding plant root and populations of L3, L4, and adult nematodes in root tissue. *Paprika* was a susceptible chili pepper variety against *M. incognita*.

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