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Study on use Endophytes for Enhancement of Tomato Toward Nematode *Meloidogyne incognita* in Vietnam

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ABSTRACT: Six endophytic fungal isolates (*Fusarium* spp.) were chosen from our collection and investigated for their ability to enhance of tomato toward activity of root-knot nematode *Meloidogyne incognita* in greenhouse experiments in Vietnam. When the root system of tomatoes were pre-inoculated with the four endophytes, the *Meloidogyne incognita* was reduced significantly 29-56% the number of root galls and 34-56% the number of egg masses six weeks after nematode inoculation, respectively. Induction of induced systemic resistance of *M. incognita* in tomato roots by the same endophytic fungi was tested. Depending on the isolate, the number of galls decreased between 29-44% and the number of egg masses reduced 37-42% in treated tomatoes with endophytic isolates when compared to those un-treated without fungi. This is the first time that systemic resistance induced by a fungal endophyte has been investigated in tomato in Vietnam.

***Keywords*:** non-pathogenic endophytes, tomato, root-knot *Meloidogyne incognita*, biological control, Vietnam.

INTRODUCTION

Root-knot nematodes are (*Meloidogyne* spp.) an economically important polyphagous group of highly adapted obligate plant parasites. There is worldwide distribution of these parasites (Moens *et al*., 2009). *M. incognita* is the most prominent and most widely distributed representative of genus *Meloidogyne*. It has a broad host range, reproducing on more than 2000 plant species. The presence of galls on the root system is a primary symptom associated with *Meloidogyne* in infection. When the plants are severely infected by *M. incognita*, the root system is reduced in size and the vascular system completely disorganized and secondary roots are almost absent. When seedlings are infected numerous plants die and those plants that survive are greatly reduced in flowering and yield potential. The nematode damaged root system also facilitates invasion by plant pathogenic bacteria and fungi.

Non-pathogenic *F. oxysporum* endophytes, which colonized the inner tissue of the plant, are considered to be potential biological control agents. Some non-pathogenic *F. oxysporum* isolates were documented for their ability as biological control agents for *M. incognita* on tomato (Halmann & Sikora, 1994; Dababat & Sikora, 2007; Vu & Nguyen, 2007). Halmann and Sikora (1994) suggested that the fungi might produce toxins or compete for space with endo-parasitic nematodes and thereby significantly reduced nematode population.

Induced systemic resistance is a phenomenon whereby resistance to infectious disease is systemically induced by localized infection or treatment with microbial components or by a diverse group of structurally unrelated organic and inorganic compounds (Kue, 2001). Induced systemic resistance has been suggested as a mechanism that could be responsible for disease control by non-pathogenic *F. oxysporum* (Mandeel & Baker, 1991; Fuchs *et al*., 1997; Alabouvette *et al*., 1998). Induced systemic resistance against plant parasitic nematodes was first found in potato and tomato (Hasky-Guenther *et al*., 1998; Sikora & Shaukat, 2002; Hauschild *et al*., 2004) and recently described by Vu *et al*. (2006) and Dababat & Sikora (2007).

In Vietnam, studies on use non-pathogenic endophytic *Fusarium* spp. as biological control agents toward root-knot nematode *M. incognita* were conducted some years ago and get some initial results (Vu & Nguyen, 2007, Vu & Nguyen, 2009). The present study is the first recorded in induced systemic resistance of non-pathogenic endophytes toward root-knot nematode *M. incognita* on tomato in Vietnam.

MATERIALS AND METHODS

A. General techniques

Six non-pathogenic fungi strains were chosen from our collection for test of their capacity as biological agents for the root-knot nematode *Meloidogyne incognita* on tomato. Their strains were isolated originally from the cortical tissue of surface sterilized tomato roots.

Fungi isolates were cultured on potato dextrose agar plates (PDA) and placed in incubator at 24ºC for 7 days. Spores were collected into a micro-bank tube and store at -20ºC. Fungal propagation for all experiments was initiated with spores from this stock. Initial fungal inoculums that were then grown in PDA disks for several days in an incubator at 24ºC. 100 ml of distilled water was added for each PDA disk and fungal mycelia were separated from the broth by passing it through cheese cloth. Spore solution were collected and re-suspended by distilled water to the concentration required for experimental use.

Root-knot nematode *Meloidogyne incognita* was isolated from infected tomatoes which were collected from the field and maintained on tomatoes race HT42 in greenhouse at 28ºC for all experiments. Nematode inoculums was extracted from 2-3 months-old-tomato plants. Eggs and juveniles were extracted from root galls using 1.5% NaOCl solution as described by Hussey and Barker (1973). The infected plant was uprooted and washed with tap water to remove adhering soil particles. The roots were then cut into 1-2 cm pieces and macerated 2x10 seconds in a small amount of water. The macerate was filled to 500 ml into a flask containing of a 1.5% NaOCl solution and shaken for 3 minutes to free of eggs from gelatinous matrix. The eggs and juvenile suspension was poured over a sieve combination of 250-100-45-20 µm aperture and gently rinsed with sterilized water to remove the NaOCl. Eggs and juveniles were collected on the 20 µm sieve and used directly for inoculation.

Tomato race HT42 was used on our experiments. The tomato seeds were sown in sterilized sand for germination at the greenhouse conditions. Four weeks after germination, tomato was transferred to individual pots containing 500g sterilized soil placed in the greenhouse. The plants were placed in the greenhouse conditions (at Plant Genetic Resources of Vietnam, VAAS), watered and fertilized to insure proper plant growth.

B. Experiments design

Experiment 1: Influence of non-pathogenic endophytic fungi on M. incognitareproduction

Six-week-old tomatoes also were inoculated with *Fusarium* endophytes at a rate of 10^7 spores/plant. Six weeks after endophytic *Fusarium* inoculation, 2000 active juveniles and 600 eggs in 5 ml were injected for each plantin several holes around the base of tomato. The experiment was determined six weeks after nematode inoculation. The roots were removed and washed free from soil and stained in 0.015% Phloxine B for 20 minutes to facilitate egg mass counting. The number of galls and egg masses per plant was then determined. Plant treated by tap water served as controls. Experiment was conducted in the greenhouse from 10/2011 to 6/2012 at VAAS. Experiment was repeated twice with 10 replications for each treatment.

Experiment 2: Induced systemic resistance of non-pathogenic endophytic fungi toward M. incognita

The same method as above was applied in this experiment. Six-week-old tomatoes were inoculated with *Fusarium* endophytes with amount 10^7 spores/plant. Two weeks after fungi inoculation, the tomato was uprooted and removed root system and transplanted again to individual pot contained 500 g sterilized soil. Four weeks after transplanting, 2000 active and 600 eggs in 5 ml were injected for each plant in several holes around the base of tomato. The root galls and egg masses were determined 6 weeks after nematode inoculation. Plant treated by tap water served as controls. Experiment was conducted in the greenhouse from 10/2012 to 6/2013 at VAAS. Experiment was repeated twice with 10 replications for each treatment.

Statistical analysis was done using SPSS 16.0 software. One –way ANOVA, followed by Ducan´s Multiple Range Tests was used to test for significant differences ($P \leq 0.05$) among means.

RESULTS AND DISSCUSION

Experiment 1: Influence of non-pathogenic endophytic fungi on nematode reproduction

The effect of non-pathogenic *Fusarium* endophytes on the reproduction of root-knot nematode *M. incognita* on tomatoes was showed in Fig. 1 and Fig. 2. The number of root galls on root system caused by *M. incognita* decreased significantly by 29-56% six weeks after nematode inoculation in tomatoes treated with *Fusarium* spp. isolates BN2.1, BN2.2, LHL2.10, LHL1.2, NH2.7 and LMLC3.3 compared to non-treated tomatoes (Fig.1). However, the number of root galls on the tomatoes treated by *Fusarium* isolates BN2.1, BN2.2, LHL2.10 was significantly lower when compared to with isolates LHL1.2 and NH 2.7 (Fig. 1).

The same tendency was observed in the number of egg masses on root system of tomatoes. The number of egg masses on root system was reduced by 34-56% in tomatoes treated with *Fusarium* endophytic isolates compared to non-treated tomatoes served as controls. There were no significant differences in the number of egg masses on root system between the different *Fusarium* isolates (Fig. 2).

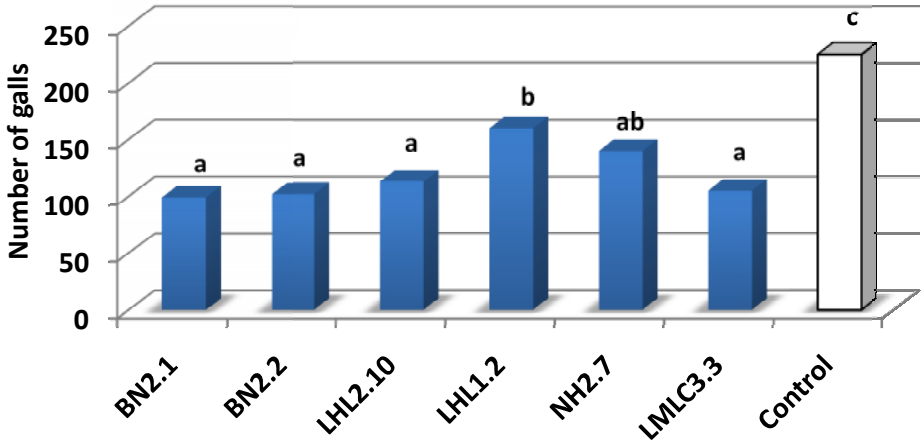

Fig. 1. Effect of endophytic *Fusarium* isolates BN2.1, BN2.2, LHL2.10, LHL1.2, NH2.7 and LMLC3.3 on the number of root galls caused by *Meloidogyne incognita* on tomato. Means with the same letter are not significantly different based on Ducan´s Multiple Range Test (P ≤ 0.05, n = 10).

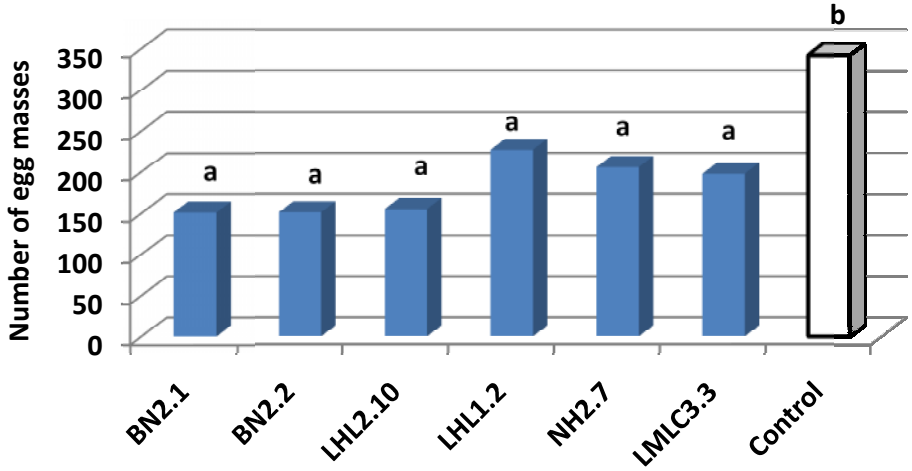

Fig. 2. Effect of endophytic *Fusarium* isolates BN2.1, BN2.2, LHL2.10, LHL1.1, NH2.7 and LMLC3.3 on the number of egg masses caused by *Meloidogyne incognita* on tomato. Means with the same letter are not significantly different based on Ducan´s Multiple Range Test (P ≤ 0.05, n = 10).

Experiment 2: Induced systemic resistance of non-pathogenic endophytic fungi toward M. incognita

The effect of non-pathogenic endophytic *Fusarium* on the reproduction of root-knot nematode *M. incognita*in the experiment of induced systemic resistance was shown in Fig. 3 and Fig. 4.

The application of all six non-pathogenic endophytic *Fusarium* strains into system of re-grown roots in the experiment of induced systemic resistance resulted significant reduction of number of root galls compared to controls. The reduction of root galls on the re-grown roots ranged 29-44% in tomatoes treated with endophytic fungi isolates when compared to with untreated plants as controls. There were no significant differences in the number of root galls between different endophytic fungi isolates (Fig. 3). The same tendency was observed in the number of egg masses on re-grown root system of tomatoes. The number of egg masses on re-grown root system was reduced by 37-42% in tomatoes treated with *Fusarium* endophytic isolates compared to non-treated tomatoes served as controls. There were no significant differences in the number of egg masses on re-grown root system between the different *Fusarium* isolates (Fig. 4).

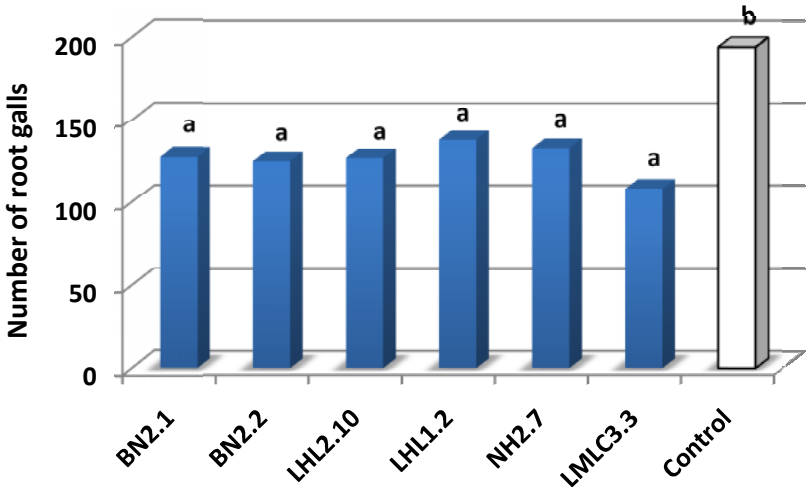

Fig. 3. Effect of endophytic *Fusarium* isolates BN2.1, BN2.2, LHL2.10, LHL1.2, NH2.7 and LMLC3.3 on the number of root galls on root system caused by *Meloidogyne incognita* on tomato in the experiment of induced systemic resistance. Means with the same letter are not significantly different based on Ducan´s Multiple Range Test ($P \leq 0.05$, n = 10).

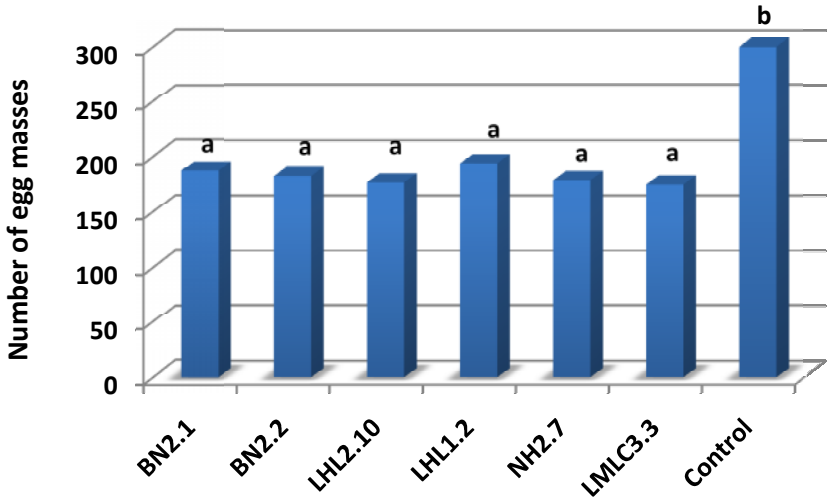

Fig. 4. Effect of endophytic *Fusarium* isolates BN2.1, BN2.2, LHL2.10, LHL1.2, NH2.7 and LMLC3.3 on the number of egg masses caused by *Meloidogyne incognita* on tomato in the experiment of induced systemic resistance. Means with the same letter are not significantly different based on Ducan´s Multiple Range Test ($P \leq 0.05$, n = 10).

The results of the present studies clearly showed the biological activity of non-pathogenic endophytes isolated from Vietnam against the ability reproduction of root-knot nematode *Meloidogyne incognita* in the number of root galls and egg masses. It was demonstrated that pre-treated with the non-pathogenic *Fusarium* isolates caused significant decreases in the number of root galls and egg masses in greenhouse experiments. Similar results were reported earlier for the other non-pathogenic endophytes on tomatoes (Dababat & Sikora, 2007; Dababat & Sikora, 2007; Vu & Nguyen, 2007; Vu & Nguyen, 2009).

Previous studies demonstrated that several hydrolytic enzymes were increased in tomato plants grown in sterilized soil and artificially infested with mutualistic *F. oxysporum*, that reduced disease symptoms of *Fusarium* wilt (Tamietti *et al.*, 1993); Alabouvette, 1998). Nevertheless, the physiological changes in plants treated with other *F. oxysporum* isolates are still largely unknown and more research in require.

Induction of systemic resistance by non-pathogenic micro-organisms is well known (Kue, 1995; Van Loon, 1997). Induced systemic resistance was considered the mode of action of mutualistic organisms in plants against fugal *Fusarium* wilt pathogens (Kroon, 1991; Fuchs *et al*., 1997; Alabouvette, 1998). In the present studies, *Fusarium* induced systemic resistance in tomato plant and resulted in a reduction of the number root galls as well as egg masses of root-knot nematode *Meloidogyne incognita* in root system of tomatoes. The application of the non-pathogenic endophytic *Fusarium* isolates to the re-grown root system resulted in a significant reduction of the number of root galls and egg masses of root-knot nematode *M. incognita* in the experiment of induced systemic resistance. Induced systemic resistance was clearly demonstrated to be the main component of the overall mode of action of non-pathogenic endophytic *Fusarium* on tomato plants or the key factor affecting nematode behaviour (Dababat & Sikora, 2007; Sikora *et al*., 2008).

The present study is the first time that induced systemic resistance of non-pathogenic endophytic *Fusarium* has been detected against any pathogen or nematode in tomato plants in Vietnam.

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