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Vermicompost increases defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants

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ABSTRACT

Sustainable agriculture aims to manage soil and plant health while relying less on chemical inputs. The individual effect of organic amendments or resistant crop cultivars on the suppression of root pests through modulating soil and plant performance is being well documented. However, the interactions between organic amendments and crop cultivars are less well studied. A pot experiment was conducted across two tomato cultivars of distinct resistance to root-knot nematodes (RKNs, *Meloidogyne incognita*) with three amendments including inorganic fertilizer (IF), conventional compost (CC) and vermicompost (VC). All treatments were inoculated with second-stage juveniles of *M. incognita* to simulate the root-knot nematode disease in field condition and to focus on the comparison among different soil amendment effects. Plant growth (shoot height, shoot biomass, root biomass and root C:N ratio), root defense metabolites (phenolics) and their related genes expression, and soil properties including pH, electrical conductivity, available nutrients, 3-indoleacetic acid (IAA), microbial biomass and activity were analyzed at 14 and 30 days post inoculation (dpi). Compared with inorganic fertilizer, vermicompost significantly decreased the numbers of nematode-induced galls on susceptible (Sus) and resistant (Res) cultivar roots by 77% and 42% respectively at 14 dpi, and by 59% and 46% respectively at 30 dpi. Vermicompost also significantly increased root defense metabolite concentrations, defense related gene expression, and improved soil properties ($p < 0.05$) except for mineral nitrogen. Multivariate analyses further indicated that soil properties particularly pH, root primary and secondary defense metabolites were negatively associated with root gall. Moreover, soil microbial activity, pH and IAA concentration were the main soil properties positively associated with plant defense metabolites production and biomass for both susceptible and resistant cultivars. Overall, vermicompost could significantly suppress root pests via modulating soil properties as well as plant defenses, particularly for the susceptible plant.

1. Introduction

Root-knot nematodes (RKNs) *Meloidogyne* spp., are sedentary endoparasites of many plant species and *Meloidogyne incognita* in particular is widespread and considered as very economically important pest (Hussain et al., 2011). During parasitism, RKNs establish and maintain an intimate relationship with their host. The eggs hatch to first-stage juveniles (J1s) and then to second-stage juveniles (J2s) which invade the root in the zone of elongation. They migrate intercellularly, first to the root apex and then to the vascular cylinder, where they are able to hijack the vascular cylinder cells and induce the formation of specialized giant cells (Abad et al., 2003; Caillaud et al., 2008). It is well known that RKNs retard plant growth and increase plant susceptibility to pathogen attack (Williamson, 1998). Nowadays, in agricultural production, tomato (*Solanum lycopersicum* L.) is subject to the infections of RKNs. Although the application of chemical nematicides could effectively control RKNs, there is increasing concern over the toxic residual effects on the environment and non-target organisms (Damalas and Eleftherohorinos, 2011). Therefore, there is an increasing demand to employ environment-friendly strategies to combat RKNs for sustainable agriculture all over the world (Collange et al., 2011).

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Organic amendment has been suggested as a promising alternative practice to improve soil quality and plant health such as suppressing plant-parasitic nematodes (Collange et al., 2011; Oka, 2010; Thoden et al., 2011) and develop sustainable crop production in small and large scale experiments (Bowles et al., 2014; Franco-Otero et al., 2012; Liu et al., 2009; Ninh et al., 2015). Currently, more evidence has shown that vermicompost, which is composted in the presence of earthworm, was superior to conventional compost in terms of soil and plant health (Doan et al., 2013; Edwards et al., 2010; Pathma and Sakthivel 2012; Song et al., 2015; Yang et al., 2015). For example, it has been suggested that vermicompost could enhance crop growth and resistance against various belowground and aboveground pests (Cardoza and Buhler, 2012; Edwards et al., 2010). Moreover, numerous findings also suggested that the abundant humic acids substances and hormones such as IAA, cytokinins and gibberellins in vermicompost could suppress nematode infestation (Arancon et al., 2006; Oka, 2010).

Among many ecological practices to minimize the losses caused by RKNs, another effective strategy is breeding resistant tomato cultivars, which offers a practical way of reducing nematicide applications (Molinari, 2011; Talavera et al., 2009). Currently, most commercial tomato cultivars with resistance to RKNs are based on the single *Mi1.2* gene (Williamson, 1998). The *Mi* gene present in some tomato cultivars has been reported to mediate resistance to RKNs by inducing localized necrosis of nematode feeding tissue very early after nematode infection (Molinari, 2011). However, it has been reported that the effectiveness of the *Mi* gene depends on soil properties (Williamson, 1998). Furthermore, the emergence of tolerant nematodes overcoming the tomato resistance gene may constitute a severe limitation to this control strategy (Jacquet et al., 2005). Besides, resistant cultivars sometimes can be deficient in beneficial crop traits such as the tolerance trait (i.e. compensatory growth) due to the tradeoff between growth and resistance. When plants are under insect attack, they will cost more resource or energy in resistance traits thus reducing the resources available to growth (Lind et al., 2013). In agricultural systems, susceptible cultivars are still widely grown worldwide particularly in developing countries. Thus, Integrative pest management (IPM) which aims to reduce pesticide usage by combining pest-resistant crop cultivars with other biological practices should be considered (Birch et al., 2011).

However, until now, few studies have investigated the effects of integrated management practices on protecting tomatoes from the RKNs (Collange et al., 2011; Mukhtar et al., 2014). Particularly few studies have compared the effects of different organic amendments in light of distinct cultivars on RKNs. Thus, it is meaningful to explore the interactive effects of cultivar traits and organic amendments to augment the efficiency of integrated management on RKNs suppression. Such work will facilitate the development of environmentally justified management which aims to reduce chemical fertilizer and pesticide usage (Birch et al., 2011; Singh et al., 2007).

To our knowledge, no studies have managed to elucidate the mechanisms underlying RKNs suppression imposed by vermicompost via plant- and soil-mediated process. We hypothesized that, compared to inorganic fertilizer, vermicompost would promote the accumulation of root defense to RKNs by accumulating defense compounds via up-regulating the expression of genes involved in secondary metabolism or modifying soil properties against RKNs. Specifically, it is further hypothesized that stimulatory effects of vermicompost on root defense compounds was much stronger for the susceptible cultivar than the resistant one. Therefore, in order to simulate the effects of organic amendments on the occurrence of root-knot nematodes in the field, a factorial experiment with different soil amendments and tomato cultivars was set up to systematically investigate the plant growth, biochemical and molecular responses to RKNs as well as the modifications of soil properties.

2. Materials and methods

2.1. Experimental conditions

The experiment was carried out in a climate chamber at Nanjing Agricultural University, China (N 32°03′, E 118°46′) under conditions of 16:8 h (L:D) photoperiod with 15,000 Lx of active radiation, 26 ± 1 °C day and 15 ± 1 °C night temperatures. The experimental soil was collected from the plough layer (0–18 cm) of a vegetable field converted from rice paddy field 3 years earlier (E 120°57′ and N 32°12′, Suzhou, China), which was cropped with tomato and spinach rotations under a conventional inorganic fertilizer regime. The soil was sieved through a 5 mm diameter mesh, adjusted to 60% water holding capacity and incubated at 25 °C in darkness for 1 month prior to the pot experiment. The soil characteristics were as follows: pH (H_2O) 5.35, electrical conductivity 0.17 dS m^{-1}, total organic carbon 19.3 mg g^{-1}, total nitrogen 1.75 mg g^{-1}, mineral nitrogen (nitrate and ammonium) 18.6 $\mu g\, g^{-1}$, available phosphorus 89.5 $\mu g\, g^{-1}$ and available potassium 114.3 $\mu g\, g^{-1}$. Analysis of the soil nematode assemblage (Byrd et al., 1972; Hooper et al., 2005) revealed no eggs or juveniles of RKNs in this soil (Table S1).

2.2. Experimental design

We conducted a fully 2×3 factorial experiment, in which tomato cultivars (resistant and susceptible to RKNs) and fertilizer amendments (inorganic fertilizer, conventional compost and vermicompost) were factors. The two cultivars were the susceptible Jinpeng-1 (Sus) and the resistant Jinpeng- M6 (Res). Compared with the susceptible cultivar, the resistant cultivar contained the *Mi1.2* gene expressing resistance against root-knot nematodes (see Fig. S2), otherwise both susceptible and resistant cultivars have similar physiological and growth characteristics. The three fertilizer amendments were inorganic fertilizer (IF), conventional compost (CC) and vermicompost (VC). The fertilizer 'IF' consisted of chemically pure $CO(NH_2)_2$, $NH_4H_2PO_4$ and KCl. The fertilizers 'CC' and 'VC' were organic composts produced without and with earthworm treatment (specie: *Eisenia fetida*; size: 120–150 mg fresh mass per individual; initial inoculation density: 1.5 kg m^{-2}) from the same cow manure, respectively, for about three months in an adjacent site, Suzhou Wofeng Waste Treatment Co., Ltd. In total, they were total six treatments, with eight replicated pots for each treatment to provide two destructive samplings.

Experimental units were plastic pots holding 1.5 kg dry equivalent soil. In the IF treatment, 0.46 g urea, 0.38 g $NH_4H_2PO_4$ and 0.24 g KCl were added into each experimental pot, according to the local NPK application rate in the field (256:100:110 kg ha^{-1}). The contents of NPK in the conventional compost and vermicompost were analyzed (Table 1). Based on the local application rate and an earlier study (Doan et al., 2013), 7.0 g dry equivalent conventional compost or vermicompost was added to each pot, corresponding to the field application rate (3 t ha^{-1}). Additional inorganic fertilizer was supplemented to the compost treatments to equalize the initial total fertilizer levels across all treatments by adding an extra 120 mg N, and 10 mg P to each pot of conventional compost treatment and 110 mg N, and 20 mg P to each pot of vermicompost treatment.

Tomato seeds were surface sterilized in 10% H_2O_2 (v/v) for 30 min, washed and then soaked in sterile distilled water for 12 h before being germinated on damp gauze at 25 °C for 3 days. One equal sized tomato seedling with three leaves was transplanted