DAMAGE POTENTIAL OF ROOT-KNOT NEMATODE (*Meloidogyne incognita* Chitwood) POPULATION DENSITY ON PLANT GROWTH PARAMETERS RELATED TO PLANT AGE OF MUNG BEAN (*Vigna radiata* (L.) Wilczek)

Kanokporn Siengchin[1], Pornthip Ruanpanun[1,*], Prakit Somta[2]
[1]Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand
[2]Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
*Corresponding author: fagrptr@ku.ac.th

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ABSTRACT

Mung bean (*Vigna radiata* (L.) Wilczek) is an economically important leguminous crop in Asia, where root knot disease caused by *Meloidogyne incognita* (RKN) is a major constraint to mung bean production. Despite its significance in mung bean production, the effects of RKN on the growth, yield, and rhizobial nodulation in mung bean have rarely been investigated. Moreover, the influence of time of RKN infection i.e. size of galls and giant cell formation is not known in relation to plant age of mung bean. This research investigated the effect of initial population density of RKN inoculated at different ages of the susceptible mung bean cultivar "Kamphaeng Saen 2" (KPS2) on the growth, yield, rhizobial nodulation, size of galls, and giant cell formation. The impact on growth, yield and rhizobial nodulation of different nematode densities and plant ages were determined compared to a control. The initial population density levels (100, 500, 1,000, 2,000 and 4,000 infective second-stage juveniles (J2s) of *M. incognita*) were negatively correlated with growth, yield (except root weight) and rhizobial nodule parameters. In contrast, plant age at inoculation was positively correlated with these parameters at each inoculum level. Additionally, the sizes of root galls and giant cells of young mung bean plants infected with RKN were larger than for older plants.

Key words: nematode inoculum density, yield loss, giant cell

INTRODUCTION

Mung bean (*Vigna radiata* (L.) Wilczek) is a socio-economically important leguminous crop of Asia, including India, Myanmar, China, Thailand, Pakistan, Bangladesh, Indonesia, and the Philippines. In Thailand, the cultivated area for mung bean is about 130,000 ha with a seed yield of about 90,000 tons. Mung bean seeds are good source of protein, high quality starch, and nutrients (Keatinge et al. 2011). Mungbean seeds are used for food as well as bean sprout and starch industries. Usually, Thai farmers grow mung bean after rice or corn or before corn, as a component in various cropping systems. Cultivation of mung bean can improve significantly soil fertility and productivity of subsequent crops because mung bean has the ability to fix atmospheric nitrogen in the soil by symbiotic association with *Rhizobium* species (Somta and Srinives 2007).

Root-knot nematodes (*Meloidogyne* spp.) are among the most devastating soil-borne crop pests worldwide. They have a diverse host range and can spread in agricultural fields under a wide range of climatic conditions, especially in tropical areas (Caillaud et al. 2008). These pests cause annual losses in global agriculture amounting to billions of dollars (Abad et al. 2008). *Meloidogyne*

spp. parasitize plant root systems and thus directly affect the uptake of water and nutrients needed for normal plant growth and reproduction (Perry et al. 2009). Their infection of plant roots can also be a component of disease complexes with other pathogens including vascular diseases caused by *Fusarium*, *Pythium*, *Rhizoctonia*, and *Sclerotium* (Johnson 1998; Roberts et al. 1995). *M. incognita* is the most important root-knot nematode species throughout agricultural areas in Thailand (Nimnoi et al. 2017; Ruanpanun and Chamswang 2016; Ruanpanun and Khun-In 2015; Ruanpanun et al. 2010) and is one of major reasons for the low annual yield in mung bean worldwide (Bridge et al. 2005; Khan et al. 2016). *M. inc ognita* is the most important species that cause severe yield loss, ranging from 18 to 65% under field conditions (Sharma et al. 2000).

The severity of mung bean infestation by *Meloidogyne* spp. is affected by the initial population density (Haider et al. 2003; Haseeb et al. 2005; Samathanam and Sethi 1996; Tiyagi and Alam 1988). The pathogenic potential of *M. arenaria*, *M. incognita*, and *M. javanica* on mung bean was 4,000, 2,000, and 1,000 J2s/ kg soil, respectively (Khan et al. 2012). In addition, *M. javanica* at an initial population density of 2,000 J2s can induce changes in the physiology of infected mung bean (Ahmed et al. 2009). Although *M. incognita* occurs in Thailand, the relationship between the initial density of this nematode, plant age at the time of nematode invasion and growth, yield and rhizobial nodulation on Thai mung bean cultivars is not known. Moreover, the effects of these factors on the root gall size and giant cell formation in mung bean have not been reported.

This research was undertaken to quantify the effects of five initial population densities (100, 500, 1,000, 2,000, and 4,000 J2s) of *M. incognita* on the growth, yield, rhizobial nodulation, root gall size and giant cell formation of mung bean. The relationship between inoculum levels and theses parameters at five plant ages (2, 3, 4, 5, and 6 weeks) was also determined. The results will serve as advisory service information regarding the establishment of RKN populations and the start of infection with respect to a particular variety of mung bean for efficient management of RKN in Thailand and other countries with similar climatic conditions.

MATERIALS AND METHODS

Nematode inoculum preparation. Freshly hatched second-stage juveniles of *M. incognita* were propagated by culturing in 14-day-old chili seedlings in the greenhouse for 45 days. The roots were washed under running tap water to remove adhering soil. Egg masses on root galls were collected from the root and surface-disinfected with 1% (v/v) sodium hypochlorite (NaOCl). The residual NaOCl was removed by washing with sterile distilled water three times (Sun et al. 2006). Egg masses were incubated in sterile distilled water for 48 h. J2s of *M. incognita* were carefully removed and acclimatized for greenhouse experiment.

Effect of nematode density and plant age on mung bean assay. The influence of the initial nematode density and plant age on the growth, yield, and rhizobial nodulation of mung bean were evaluated twice during May-August (wet season) and September-December (dry season) in 2017 at Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom province, Thailand. Mung bean cv. Kamphaeng Saen 2, released by Kasetsart University in 1986, was used in all experiments. KPS2 is a popular cultivar in Thailand because of its high seed yield, good stability, early maturity, and relative resistance to *Cercospora* leaf spot and powdery mildew diseases (Srinives 1994). Seeds of KPS2 were sown in pots (9.0 cm dia. x 7.5 cm height) filled with approximately 250 ml of peat moss. After germination, seedlings were thinned to one plant per pot, and then transferred to a new larger pot (15.0 cm dia. x 15.0 cm height) containing nematode-free soil (50.0% sand, 38.0% silt, 12.0% clay, pH 7.0, and organic matter, 1.1%). The plants were inoculated with 100, 500, 1,000, 2,000, and 4,000 J2s of *M. incognita* at 2, 3, 4, 5, and 6 weeks after planting. The pots were left for 24 h without watering to allow the *M. incognita* to settle their roots. Plants without nematode inoculation served as a control. In total, 260 pots/experiment [(5 nematode levels x 5 plant ages x 10 replications) + (10

controls)] were arranged in a completely randomized design (CRD), and plants were grown in greenhouse at 35 ± 5ºC and watered when required.

Data measurement. After 65 days of growth, the mung beans were uprooted and their roots were gently cleaned in running tap water to remove soil. The plants were cut at the margin of the root and shoot. The lengths and weights of the root and the shoot were measured. Then, rhizobial nodules were separated from mung bean roots, the number of nodules was counted and the weight of the total nodules per plant was measured. The mature brown pods of mung bean were harvested and the total yield of each plant was recorded.

Histopathological analysis of effect of plant age on giant cell formation in mung bean. Three root tips (50.0 mm in length) were harvested randomly from each KPS2 roots (2, 3, 4, 5, and 6 weeks old plants) which inoculated by varied RKN population densities (100, 500, 1,000, 2,000, and 4,000 J2s) as well as the non-infected mung bean root control. The presence of *M. incognita* in root tissue was first observed by staining with acid fuchsin (Bybd et al. 1983) at 3 weeks post-inoculation. Residual roots were provided for histopathological analysis by inclusion in the resin. The root sections were prepared following the method described by Silva et al. (2013). Briefly, the roots from each test plant were washed free of soil and fixed with triethanolamine formalin (TAF) at 70°C for 24 h and then inspected under a microscope. Selected root pieces were passed through an alcohol series and embedded in paraffin wax to obtain small blocks. Semi-thin transversal sections of the roots, measuring 0.5 to 1.5 µm in thickness, were obtained using a microtome and stained with 0.05% toluidine blue prepared in acetate buffer (pH 4.7) for observation under light microscope of any anatomical changes in the of plant tissue.

Statistical analysis. The relationship between the initial population density level of *M. incognita* and the growth, yield, and rhizobial nodulation were determined using Pearson's correlation analysis. The means of the percentage reduction in growth, yield and rhizobial nodulation of each treatment were compared to the control using two-way analysis of variance (ANOVA). Mean separation was tested using Tukey's test ($P < 0.05$). Combined ANOVA (wet and dry seasons) was performed to determine interactions between experiments. All data analysis was carried out using the R-program 3.4.1 statistical software (R Core Team 2017). Since the combined ANOVA revealed no significant interaction between the experiments (seasons), only results from the wet season experiment are presented.

RESULTS AND DISCUSSION

Effect of nematode density and plant age on mung bean (growth, yield, and rhizobial nodulation). Incidence of *Melodogyne* spp. has been reported from legume-growing regions worldwide (Ahmed et al. 2009; Davis and Mitchum 2005; Sharma et al. 2000). The effect of different initial inoculum levels on the growth and yield of diverse plants suffering from these pests has also been investigated (Haider et al. 2003; Hussain et al. 2011; Kayani et al. 2017) Also our study found that the interactions of the inoculum densities of RKN at the time of inoculation on the growth, yield and rhizobial nodulation of KPS2 and the interaction of plant age on these parameters were highly significant (Table 1). The percentage reductions in the growth, yield and rhizobial nodulation in KPS2 were directly proportional to the inoculum level of *M. incognita* J2s, while the percentage reductions of these parameters were directly proportional to the age of the plants at the time of inoculation. The maximum reduction in growth (root and shoot lengths, shoot weights; Table 2 and Fig. 1, Table 3 and 4, respectively), yield (Table 5 and Fig. 2) and rhizobial nodulation (number of nodules and nodule weight; Table 6 and 7, respectively) was recorded in the mung bean inoculated with 4,000 J2s at age 2 weeks, whereas, the minimum reduction in these parameters was recorded in 6-week-old mung bean inoculated with 100 J2s.

Table 1. Pearson's correlation analysis (2-tailed) between different growth, yield and, rhizobium parameters and inoculum densities and plant ages at time of inoculation.

Parameter	Correlation with	
	Inoculum level	Plant age
Reduction in root length	-0.669	0.590
Increase in root weight	0.579	-0.650
Reduction in shoot length	-0.673	0.652
Reduction in shoot weight	-0.617	0.582
Reduction in yield/plant	-0.458	0.773
Reduction in number of rhizobium	-0.592	0.561
Reduction in rhizobium weight	-0.361	0.566

Correlation of all parameters is significant at $P = 0.01$.

Table 2. Percent reduction in root length (cm) in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent reduction* in root length (cm) at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	57.4 ± 4.1Ac***	75.3 ± 1.2Ab	84.7 ± 2.4Aab	89.7 ± 2.2Aa	93.2 ± 0.8Aa	0.898
3	46.0 ± 4.1Bd	60.8 ± 5.3Bc	73.5 ± 2.8Bb	79.9 ± 3.1Bab	85.6 ± 2.87Ba	0.956
4	21.0 ± 4.1Ce	34.5 ± 5.4Cd	52.8 ± 4.8Cc	65.8 ± 4.5Cb	78.2 ± 2.5Ca	0.995
5	8.2 ± 2.3De	19.7 ± 1.6CDd	38.6 ± 2.8CDc	53.7 ± 2.8CDb	76.3 ± 3.3Ca	0.984
6	0 ± 2.8Dd	26.8 ± 2.6Dc	46.6 ± 4.4Db	59.8 ± 4.4Dab	69.7 ± 3.8Da	0.976

*Percentage of reduction = [(root length of control - root length of treatment) × 100)]/ root length of control
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

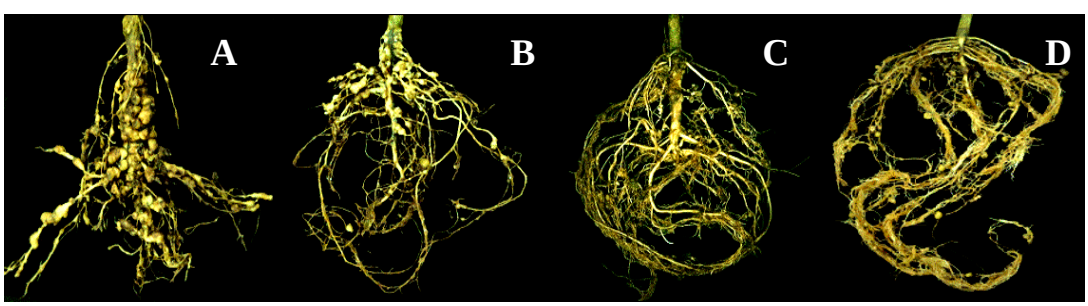

Fig. 1. Mung bean cv. Kamphang Saen 2 roots inoculated with J2s of *M. incognita* at age 2 (A), 4 (B), and 6 (C) weeks compared to non-inoculated control (D).

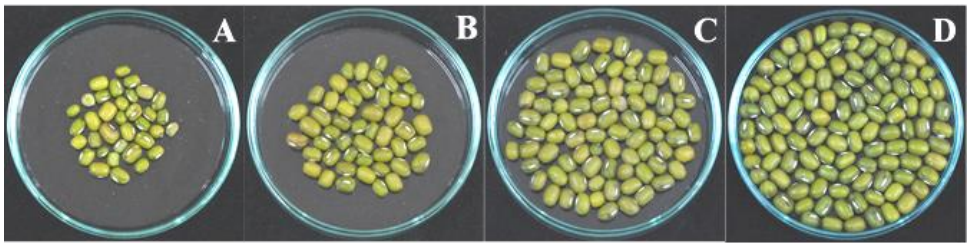

Fig. 2. Yields of mung bean cv. Kamphang Saen 2 inoculated with J2s of *M. incognita* at age 2 (A), 4 (B), and 6 (C) weeks compared to non-inoculated control (D).

These results indicate that the nematode population density in the soil at planting negatively affects plant growth and yield in general (Sharma Poudyal et al. 2005). Increasing the initial *Meloidogyne* J2s density reduced growth (measured by shoot and root length, and by shoot and root weight) and the yield of plants (Khan et al. 2012; Soomro and Hauge 1993). Recently, Kumar et al. (2018) reported that increasing levels of *M. incognita* inoculum caused the number of root galls of blackgram (*V. mango* L. cv. TU 98-14) and the final nematode population in the soil to increase significantly. Moreover, the inhibitory and damage potential of this pest on the growth of the legume were higher at a high initial population density. Increasing *M. incognita* inoculum density resulted in decreasing growth, leaf area, chlorophyll, seed protein, N activity, and leghaemoglobin of mung bean cv. PDM 139 (Abbasi and Hisamuddin 2014). In contrast, decreased shoot length, root length, and shoot weight of KPS2 were reported due to the increased inoculum levels, with the increase in root weight being greater at a higher inoculum density.

The nodulation on mung bean roots which had been infected by *Meloidogyne* spp. decreased significantly compared to non-infected plants. Root-knot nematode attacked leguminous plant roots and also affected rhizobial nodulation (Taha 1993). The intrusion of nodules by RKN caused the root nodules to dysfunction and be eliminated prematurely. The infective J2s of *Meloidogyne* spp. invaded the root nodules that led to severe damage to the vascular bundles, the bacteroid zone, and the cortex of the nodule (Taha and Kassab 1979). Consequently, the nodule invasion by nematodes decreased the bacteroid population and leghemoglobin contents of nodules and this affected rhizobial multiplication and the development of the nodule (Khan et al. 2016). Moreover, the specific root mass for invasion by the rhizobia decreased because of the attack on the young lateral roots and root hairs by the root-knot nematode in mung bean (Wilcox-Lee and Lorea 1987). An increase in the nematode inoculum levels resulted in adverse effects on the number of nodules, as there was a significant reduction in nodulation of mung bean (Khan et al. 2012). Consistent with previous research, the current results showed that the weight and number of rhizobial nodules significantly decreased at a higher nematode density.

Although the impact of the damage of the initial RKN inoculum density decreased as the plant age increased at the inoculation time, all inoculum levels caused a root weight increase. The maximum increment (1059.1%) in root weight was obtained in 2-week-old plants inoculated with 4,000 J2s. In contrast, the minimum values were recorded in 6-week-old plants inoculated with 100 J2s (Table 8). The initial inoculum level was positively and significantly correlated with the root weight, but plant age was negatively and significantly correlated with this character. Root weight of cucumber increased less when infected with a lower nematode inoculum density (Kayani et al. 2017). It was possible that infected roots at a higher inoculum density produced larger and more numerous galls than those infected at a lower inoculum density, resulting in a greater increase in the root weight as previous researches described in green poppy and potato (Niyaz and Hisamuddin 2008; Peters 1961).

Table 3. Percent reduction in shoot length (cm) in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent reduction* in shoot length (cm) at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	33.1 ± 3.3Ae***	38.7 ± 2.6Ad	44.3 ± 3.4Ac	51.7 ± 3.2Ab	57.5 ± 2.5Aa	0.998
3	21.3 ± 3.3Bd	28.5 ± 3.0Bc	34.6 ± 4.1Bb	38.8 ± 3.2Bb	51.8 ± 3.6Ba	0.965
4	16.1 ± 3.0Cd	22.7 ± 2.1Cc	32.1 ± 2.1Bb	34.3 ± 4.3BCb	45.5 ± 4.0Ca	0.973
5	6.3 ± 1.6Dc	10.9 ± 2.6Dc	25.5 ± 4.5Cb	31.3 ± 3.9CDab	37.9 ± 2.4Da	0.970
6	0.9 ± 2.5Ec	5.7 ± 2.8Ec	18.3 ± 2.1Db	26.7 ± 4.2Da	33.5 ± 3.6Ea	0.985

*Percent reduction = [(shoot length of control - shoot length of treatment) × 100]/ shoot length of control
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

Table 4. Percent reduction in shoot weight (g) in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent reduction* in fresh shoot weight (g) at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	57.3 ± 3.9Ac***	62.8 ± 5.8Abc	70.6 ± 4.6Aab	73.6 ± 3.4Aa	76.0 ± 5.7Aa	0.954
3	49.6 ± 4.2Ac	58.7 ± 3.8Abc	65.7 ± 4.0ABab	69.7 ± 3.8Aa	72.7 ± 5.4Aa	0.949
4	28.1 ± 5.3Bd	42.0 ± 5.0Bc	57.5 ± 3.0BCb	68.3 ± 3.7Aab	71.9 ± 1.9Aa	0.961
5	18.6 ± 5.6BCd	35.1 ± 3.6BCc	49.7 ± 4.3Cb	60.5 ± 5.0Bab	69.5 ± 5.0Aa	0.984
6	13.5 ± 5.0Cc	29.6 ± 3.4Cb	35.7 ± 5.4Db	48.9 ± 4.9Ca	59.5 ± 5.3Ba	0.987

*Percent reduction = [(shoot weight of control - shoot weight of treatment) × 100]/ shoot weight of control
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

Table 5. Percent reduction in yield (g) in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent reduction* in yield (g) at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	77.6 ± 3.3Ad***	82.0 ± 2.5Acd	85.4 ± 0.8Abc	88.8 ± 3.0Ab	95.3 ± 4.5Aa	0.983
3	68.2 ± 5.4ABc	73.0 ± 3.0ABbc	75.8 ± 2.4ABb	84.0 ± 3.0ABa	88.3 ± 2.2ABa	0.979
4	60.5 ± 5.0Bd	63.7 ± 5.1Bcd	72.1 ± 2.9Bbc	76.7 ± 3.5Bab	84.2 ± 1.7Ba	0.984
5	28.4 ± 3.1Cd	45.5 ± 3.9Cc	56.7 ± 4.7Cbc	64.7 ± 3.7Cab	75.7 ± 3.3Ca	0.981
6	8.5 ± 4.7Dd	27.5 ± 4.6Dc	39.5 ± 3.3Dbc	50.8 ± 3.4Dab	57.5 ± 3.7Da	0.969

*Percent reduction = [(yield of control - yield of treatment) × 100]/ yield of control
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

Table 6. Percent reduction in number of rhizobial nodule in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent reduction* in number of rhizobial nodules at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	67.5 ± 3.8Ac***	73.7 ± 3.5Abc	84.2 ± 2.8Aab	91.2 ± 3.9Aa	97.4 ± 3.7Aa	0.991
3	57.9 ± 4.2Ac	65.8 ± 4.2ABc	75.4 ± 3.5ABb	79.8 ± 3.7ABb	93.0 ± 4.7Aa	0.982
4	37.7 ± 4.0Bd	57.9 ± 3.7ABc	69.3 ± 3.6Bbc	82.5 ± 4.1ABab	88.6 ± 3.7Aa	0.967
5	24.6 ± 4.4Cc	48.3 ± 3.4BCb	63.2 ± 3.5BCab	68.4 ± 4.1BCab	78.1 ± 4.1Ba	0.930
6	4.8 ± 4.1Dc	36.0 ± 4.4Cb	52.6 ± 2.6Cab	57.9 ± 4.3Cab	68.4 ± 3.8Ca	0.903

*Percentage of reduction = [(No. of rhizobial nodule of control - No. of rhizobial nodule of treatment) × 100]/ No. of rhizobial nodule
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

Table 7. Percent reduction in rhizobial nodule weight (g) in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent reduction* in rhizobial nodule weight (g) at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	92.0 ± 5.1Ab***	98.7 ± 0.6Aa	97.7 ± 4.0Aa	99.5 ± 0.5Aa	99.3 ± 1.0Aa	0.910
3	84.0 ± 2.7Ac	87.6 ± 3.4Abc	89.1 ± 2.6Aabc	93.0 ± 2.6Aab	95.4 ± 3.6Aa	0.984
4	82.6 ± 5.0Ac	85.5 ± 2.2Ac	86.9 ± 1.9Abc	91.6 ± 2.4Aab	94.4 ± 4.5Aa	0.976
5	57.3 ± 3.8Bb	82.6 ± 2.6Aa	84.2 ± 2.6Aa	92.7 ± 3.1Aa	95.7 ± 3.2Aa	0.823
6	8.3 ± 3.7Cb	51.0 ± 2.8Ba	62.3 ± 3.4Ba	75.7 ± 2.4Ba	82.5 ± 3.7Ba	0.873

*Percentage of reduction = [(rhizobial nodule weight of control - rhizobial nodule weight of treatment) × 100]/ rhizobial nodule weight of control
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

Table 8. Percent increase in root weight (g) in mung bean cv. Kamphaeng Saen 2 at different plant ages with five inoculum densities of *Meloidogyne incognita*.

Plant age (weeks)	Mean percent increase* in root weight (g) at inoculum levels of RKN**					R^2
	100	500	1000	2000	4000	
2	124.8 ± 13.6Ae***	224.6 ± 49.9Ad	425.7 ± 60.7Ac	705.1 ± 48.1Ab	1059.1 ± 85.6Aa	0.957
3	116.1 ± 10.5Ad	160.1 ± 25.0Bd	284.3 ± 34.6Bc	443.0 ± 5.5Bb	663.6 ± 42.8Ba	0.946
4	12.5 ± 19.1Bd	146.3 ± 50.0Bc	229.5 ± 36.8Cbc	275.7 ± 30.3Cab	319.9 ± 40.0Ca	0.939
5	2.8 ± 16.0Bc	25.0 ± 36.0Cc	113.0 ± 23.4Db	168.1 ± 36.1Dab	229.1 ± 74.5Ca	0.979
6	0 ± 17.8Bb	15.3 ± 13.0Cb	16.0 ± 15.7Eb	64.2 ± 24.6Ea	82.8 ± 20.2Da	0.920

*Percentage of increase = [(root weight of treatment - root weight of control) × 100]/ root weight of control
**Average of 10 replicates.
*** Values in the table with the same letter are not significantly ($P < 0.05$) different according to Tukey's test; capital letters compare column values and small letters compare row values.

Effect of plant age on size of root gall and giant cells of mung bean. Roots of KPS2 stained with acid fuchsin were observed under the light microscope. The results showed that different ages of plant at inoculation affected the size of the root galls of plants inoculated with J2s of *M. incognita*, whereas the inoculum level did not affect this parameter at the same plant age of inoculation (data not shown). A reduction in root gall size was directly proportional to the age of the plant at inoculation. The largest root galls were found in plants inoculated at age 2 weeks, while the smallest root galls were found in plants inoculated at age 6 weeks after emergence (Fig. 3).

Fig. 3. Roots of mung bean cv. Kamphang Saen 2 inoculated with J2s of *M. incognita* at age 2 (A), 4 (B), and 6 (C) weeks compared to non-inoculated control (D). G: root gall, N: nematode.

Normally, *M. incognita* enters the plant roots and causes hypertrophy and hyperplasia in the plant root tissue. Three to five hypertrophied cells around the head of nematode were transformed into abnormally large cells called as giant cells. These giant cells act as specialized sinks support the nutrient to nematode (Azam et al. 2011). The size of giant cells in KPS2 was determined through micro-section of roots observed under microscope. The results indicated that different ages of plant at inoculation had an effect on the giant cell size of plants inoculated with J2s of *M. incognita*, whereas nematode inoculum levels had no effect on this parameter (data not shown). The decrease in giant cell size was directly proportional to the age of the plant at inoculation (2-6 weeks). The largest giant cells (45.1 µm in width) were recorded in plants inoculated at age 2 weeks. In contrast, the smallest giant cells (12.4 µm in width) were found in plants that had been inoculated 6 weeks after germination (Fig. 4). Moreover, number of giant cells per nematode found in plants inoculated at age 2 (3-6 giant cells) and 4 weeks (2-4 giant cells) was more than in plants inoculated at age 6 weeks (1-2 giant cells). These results suggested that younger mung bean was well suitable to satisfy the parasitic needs of *M. incognita.* Our results were consistent with findings that demonstrated the susceptibility of a plant to pathogens depending on the maturation and senescence of plant tissue (Bruehl 1987). Tenderness and succulence in young plant tissues support hyperplasia in the vascular cylinder and the cortical cells around the feeding site that seemed to be related to a large gall size (Vovlas et al. 2005). Moreover, these findings were consistent with previous studies in many different plants where an inversed relationship between increasing plant age and decreasing galling root size was reported (Eapen 1992; Griffin and Hunt 1972; Olthof 1983; Sivapalan 1972). It was determined that the inoculum level of *M. incognita* had no effect on the size of galling on roots and giant cells in mung bean. It is believed that size of the root gall may be related to the number of nematodes in the plant tissue (Dropkin 1954) and that a high number of nematodes may result in high root penetration or a high multiplication rate of nematodes (Eapen 1992). However, giant cell number and size may be related to host plant species (Vovlas et al. 2005).

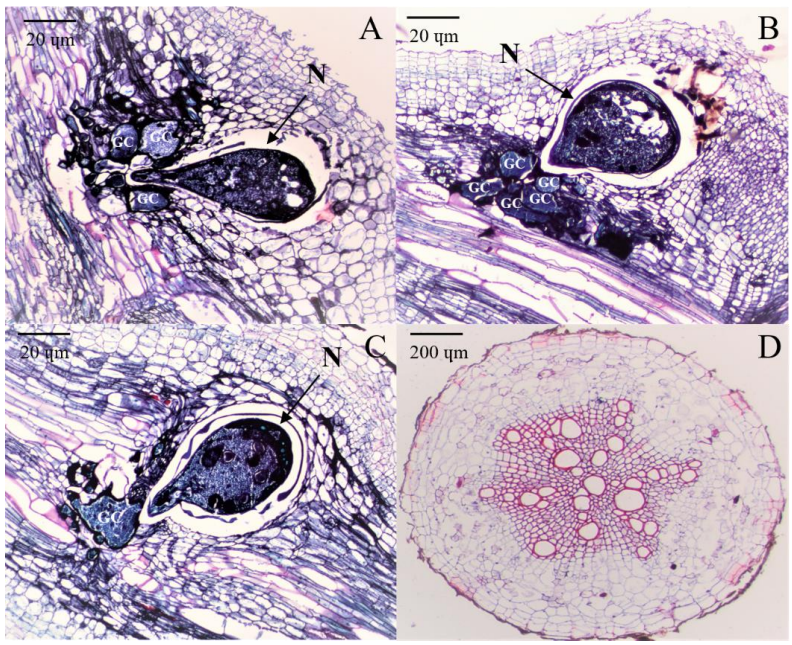

Fig. 4. Light micrographs of root tissues of mung bean cv. Kamphang Saen 2 showing the female *M. incognita* (N) and giant cell formation (GC) after inoculation of J2s at age 2 (A), 4 (B), and 6 (C) weeks, and root cell without nematode infection (D).

CONCLUSION

M. incognita deteriorated the growth and caused significant yield losses in mung bean cv. Kamphang Saen 2 at the initial population density of at least 100 J2s/plant. Greater damage was found when the initial population density of the nematode in the soil increased and younger mung bean were more susceptible to nematodes than older plants. Farmers should be aware of these factors to manage efficiently root-knot nematode.

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