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Soybean cultivars affect nodulation competition of *Bradyrhizobium japonicum* strains

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Summary

The influence of five Thai soybean cultivars on nodulation competitiveness of four *Bradyrhizobium japonicum* strains was investigated. Cultures of *B. japonicum* strains THA5, THA6, USDA110 and SEMIA5019 were mixed with each other prior to inoculating germinated soybean seeds growing in Leonard jars with nitrogen-free nutrient solution. At harvest, nodule occupancy by each strain was determined by a fluorescent antibody technique. The term 'general competitive ability' was introduced to describe the average competitive nodule occupancy of a strain in paired co-inoculation with a number of strains on soybean. The nodule occupancies by an individual strain were directly correlated with the proportions of that strain in the inoculum mixtures. USDA110 showed higher nodulation competitiveness than the other strains on three of the five cultivars. The Thai strain THA6 appeared to be more competitive than USDA110 on cultivar SJ5. Thus, nodulation competitiveness of the *B. japonicum* strains was affected by the cultivars of soybean used.

Introduction

Bradyrhizobium japonicum is a slow growing root nodule symbiont, which is widely used as an inoculant in soybean fields through the world. Generally, soybean inoculated with *B. japonicum* forms highly effective nodules and frequently increases soybean yields, especially in fields where soybeans are cultivated for the first time (Caldwell & Vest 1970). The major problem of soybean inoculation is that the existing indigenous strains in the field may often suppress the introduced inoculant strains applied to soybeans subsequently. Therefore, it is necessary that the highly effective introduced strain has also the capacity to compete with the resident ineffective rhizobia in the soil (Dowling & Broughton 1986). The competitive mechanism is a complex interplay between each strain and the host plant. Numerous abiotic and biotic factors are known to influence the competitiveness of specific rhizobial inoculants (Dowling & Broughton 1986; Bottomley 1992; Turco & Sadowsky 1995).

Soybean cultivars are also known to influence nodulation competition among *B. japonicum* strains (Triplett & Sadowsky 1992). In Thailand, many soybean cultivars have been developed with characteristics appropriate for different geographical areas. These Thai soybean cultivars may have different selective influences on the soil bradyrhizobia and therefore the nodulation competition of introduced *B. japonicum* strains may be affected by the identify of these cultivars. To develop a successful strategy for inoculation of soybean fields in Thailand, it is necessary to determine how Thai soybean cultivars interact with different *B. japonicum* strains. The usual method for the assessment of competitive ability for nodulation involves the inoculation of a cultivar with equal mixtures of two inoculant strains. The results of such studies may provide the relative nodule occupancies of the two competing strains. However, nodule occupancies from a paired competition assay involving only two strains cannot be used to predict the competitive abilities of these strains in relation to other strains. To predict the competitive ability of a strain with other strains in general, it would be necessary to conduct competition experiment with a number of strains. We describe 'general competitive ability (GCA)' of a strain for nodulation as the average nodule occupancy of the strain in paired co-inoculation assays with a number of strains. This is in contrast to the 'specific competitive ability', which is the average nodule occupancy of a strain in paired co-inoculation assay with another strain. The objectives of this investigation are to assess the GCAs of four *Bradyrhizobium* strains for nodulation of soybean using all possible paired combinations of these strains in co-inoculation experiments, and to determine the effect of five Thai soybean cultivars on the general nodulation competitive abilities for these strains.

Materials and methods

B. japonicum strains

Cultures of *B. japonicum* strains USDA110 and SEMIA5019 were obtained from M.J. Sadowsky, University of Minnesota, St. Paul, MN. USA. *B. japonicum* strains THA5 and THA6 were obtained from the Thailand Department of Agriculture, Bangkok, Thailand.

Inoculum preparation

B. japonicum strains were cultured with YEM (Vincent 1970) and grown aerobically (agitated at 250 rev/min on a rotary shaker) at 28 °C until the cell concentration reached 10^8–10^9 cells/ml. Cultures were collected by centrifugation at 5000 rev/min for 10 min. The precipitate was further washed twice with sterile water. Cells were diluted with water to provide 10^8 cells/ml by direct determination in Petroff-Hauser counting chamber. For paired co-inoculation experiments, the two competing strains were mixed at the ratios of 1:1, 1:9 and 9:1 (v/v).

Soybean cultivars

The soybean cultivars used in this experiment, CM2, CM60, SJ2, SJ4 and SJ5, were obtained from the Department of Agriculture, Bangkok, Thailand. Seeds were rinsed in 95% ethanol for 10 s to remove waxy material and trapped air, further washed twice with sterile water. Seeds were then surface sterilized by immersion in 6% sodium hypochlorate for 3 min, followed by rinsing six changes with sterile water. Seeds were placed in petri dishes containing water agar until germinated.

Nodulation competition assay

Influence of soybean cultivars on competition
The germinated seeds of cultivars CM60, CM2, SJ2, SJ4 and SJ5 were grown in Leonard jars with three seeds per jar. Seeds were then inoculated with 1 ml of mixed inoculum containing two strains with a 1:1 ratio of 10^8 cells. Nodulation tests were performed in triplicate. Plants were supplemented with N-free medium and kept at 25 °C under light with a flux density of about 450/ μEs^{-1} m^{-2} and a 12–12 h light-dark regime. Plants were harvested at 4 weeks after inoculation. Nodule occupancy was determined through a fluorescent antibody technique applied to nodule contents. (Payakapong *et al.* 2003).

Influence of cell number on nodule occupancy
Germinated seeds of cultivars CM2 and SJ5 were inoculated with a mixture of two strains at ratios of 1:1, 1:9 and 9:1. All paired combinations of the four strains were applied and the experiment was three replicated. Nodule occupancies were grown and determined as described above.

Statistical methods

Mean and standard deviations for nodule occupancies were calculated from data obtained from three replications. GCA for nodulation was calculated as follows:

$$\mathrm{GCA} = \frac{\mathrm{P1} + \mathrm{P2} + \ldots \mathrm{P}n}{n}$$

where P1, P2, Pn are the proportions of nodule occupied by a strain in paired co-inoculation with strains P1, P2,…,Pn, respectively; and n is the total number of test isolates.

Results

Nodulation competitiveness of four B. japonicum *strains*

The nodulation competitiveness of four strains was assessed on five cultivars (Table 1). USDA110 showed higher nodule occupancies than all strains on most of the soybean cultivars. THA5 occupied the least number of nodules in paired competition with most other strains. SEMIA5019 and THA6 also showed relatively higher nodule occupancies in a majority of the paired inoculation tests with other strains. For nodulation competition involving SEMIA5019 and THA6, the former appeared to be more competitive on three of the five cultivars. The GCAs of these strains for nodulation were calculated from 15 paired competition assays involving three co-inoculating strains and five cultivars (Table 1). USDA110 had the highest GCA for nodule invasion, followed by THA6, SEMIA5019 and THA5 (Figure 1). For double strains occupancies, USDA110 showed the highest percentage, followed by SEMIA5019, THA5 and THA6. Interestingly, nodule occupancy of all strains showed higher percentages of double occupancies than single occupancies.

Influence of relative proportions of co-inoculating inoculum on nodule occupancy

To determine how the proportion of the two strains in the inoculum mixtures might affect soybean nodule occupancies by these strains, the three possible paired combinations involving four *B. japonicum* strains were used to co-inoculate soybean in three different ratios, 1:9, 1:1 and 9:1. The average nodule occupancies of each strain in paired combination with the other three strains were determined for the three inoculum proportions, 10, 50 and 90% (Figure 2a, b). The nodule occupancies by an individual strain were directly correlated with the proportions of that strain in the inoculum mixtures. The relative nodule occupancy values obtained from com-

Table 1. Nodule occupancy by *B. japonicum strains* THA5, THA6, USDA110, SEMIA5019 on five soybean cultivars.

Co-inoculated strains		Soybean cultivar	[a]Nodules (%) formed by		
A	B		A	B	Double occupancy
THA5	THA6	ST2	3.3 ± 3.5	66.1 ± 6.5	30.6 ± 3.4
		SJ4	25.0 ± 3.5	40.0 ± 4.2	35.1 ± 7.7
		SJ5	1.7 ± 2.9	94.4 ± 5.3	4.0 ± 2.5
		CM2	4.4 ± 0.8	59.9 ± 7.3	34.0 ± 7.4
		CM60	28.0 ± 5.0	35.5 ± 8.3	36.5 ± 4.4
THA5	USDA110	ST2	0.0 ± 0.0	75.4 ± 4.0	24.6 ± 4.0
		SJ4	2.2 ± 3.8	36.9 ± 2.3	60.9 ± 4.3
		SJ5	0.0 ± 0.0	64.2 ± 0.7	35.8 ± 0.7
		CM2	1.4 ± 2.5	59.0 ± 10.3	39.6 ± 9.1
		CM60	1.7 ± 2.9	37.4 ± 7.0	60.9 ± 4.6
THA5	SEMIA5019	ST2	1.7 ± 2.9	73.4 ± 5.2	24.9 ± 4.3
		SJ4	9.6 ± 9.7	35.8 ± 6.2	54.6 ± 6.5
		SJ5	18.9 ± 5.9	43.4 ± 7.3	37.7 ± 11.6
		CM2	4.4 ± 0.7	43.1 ± 11.0	49.8 ± 9.6
		CM60	4.3 ± 3.7	65.5 ± 4.0	30.2 ± 6.4
THA6	USDA110	ST2	3.3 ± 3.3	72.8 ± 4.8	23.9 ± 2.5
		SJ4	0.0 ± 0.0	80.3 ± 6.3	19.7 ± 6.3
		SJ5	15.0 ± 5.3	13.4 ± 3.5	71.6 ± 5.3
		CM2	10.2 ± 5.7	61.4 ± 9.5	28.4 ± 7.6
		CM60	1.9 ± 3.2	17.8 ± 1.9	80.4 ± 2.8
THA6	SEMIA5019	ST2	16.5 ± 2.1	43.9 ± 8.5	39.6 ± 6.9
		SJ4	31.4 ± 8.0	47.6 ± 7.1	21.1 ± 1.1
		SJ5	58.3 ± 5.9	20.4 ± 5.7	21.3 ± 5.0
		CM2	46.0 ± 11.3	4.2 ± 7.2	49.8 ± 4.2
		CM60	45.3 ± 6.8	22.2 ± 2.7	32.5 ± 7.9
SEMIA5019	USDA110	ST2	15.8 ± 3.2	14.9 ± 4.5	69.4 ± 4.5
		SJ4	20.0 ± 3.4	30.2 ± 3.7	49.8 ± 6.8
		SJ5	34.0 ± 4.1	7.9 ± 6.9	58.1 ± 10.2
		CM2	25.0 ± 2.9	20.3 ± 2.7	54.7 ± 5.4
		CM60	5.0 ± 4.6	36.9 ± 8.3	58.2 ± 5.4

[a] Means ± SD of three replications.

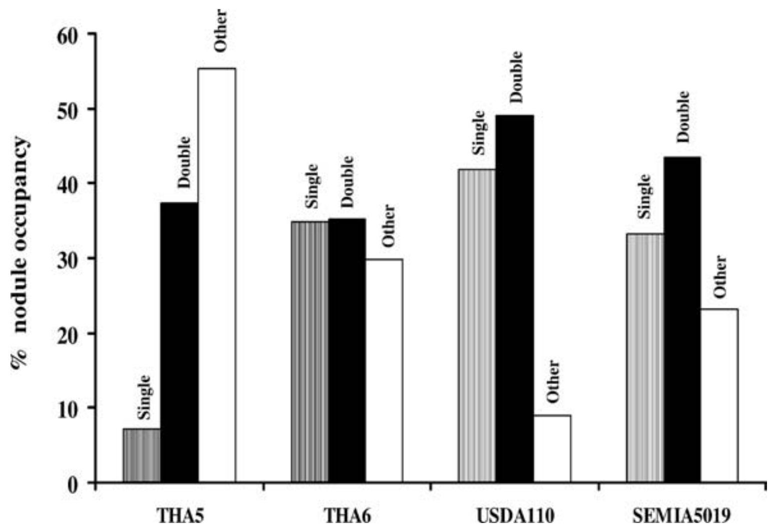

Figure 1. Average percentage of nodule occupancies by individual *B. japonicum* strains in experiments using all possible paired co-inoculations involving four strains. For each strain, the single (▥) and double (■) nodule occupancies are indicated. The average nodule occupancy percentages of the other strains (□) in the paired co-inoculations are also indicated.

petition experiments on two soybean cultivars, CM2 and SJ5, were not similar for the strains at the three inoculum proportions, indicating effects of cultivars on nodule occupancy.

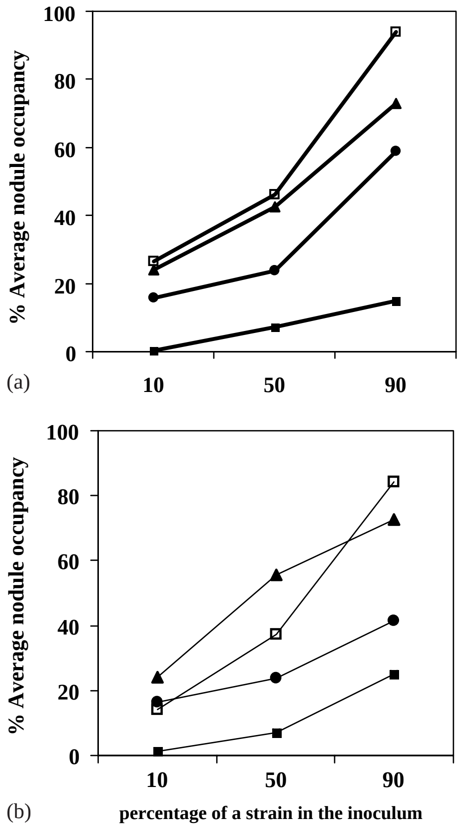

Figure 2. Average percentage of soybean nodule occupancies by *B. japonicum* strains USDA110 (□), THA6 (▲), SEMIA5019 (●) and THA5 (■) in paired co-inoculation experiments, where different proportions of a particular strain were used in combination with a competing strain on cultivars CM2 (a) and SJ5 (b).

Influence of soybean cultivars on nodulation competition

From Table 1, it appears that the competitive nodule occupancies by the strains were not consistent on all five cultivars. Both general and specific competitive abilities for nodulation were influenced by soybean cultivars. USDA110 showed higher average nodule occupancies than SEMIA5019 and THA6 on cultivars ST2, SJ4 and CM2, but not on SJ5 and CM60 (Figure 3). THA6 appeared to be more competitive than USDA110 and SEMIA5019 on cultivar SJ5. On CM60, THA6 showed lower average nodule occupancies than both USDA110 and SEMIA5019.

Discussion

We have introduced the concepts of general and specific competitive abilities for nodule occupancy to describe nodulation competitiveness of *B. japonicum* strains. A strain with a high GCA is expected to out-compete a large number of competing strains in the soil for occupying nodules. Identification of such a strain would require testing many strains in paired competition against a set of 'test isolates' of *B. japonicum*. The test isolates are strains with known physiological and symbiotic characteristics. In this study, all paired combinations of four strains were tested for nodulation competition, thus, for measuring the GCA of any one strain, the remaining three strains served as the test isolates. Among these four strains, USDA110 and SEMIA5019 were well characterized for symbiosis and other characteristics (Jordan 1982; Boddey & Hungria 1997). The results of this study show that the GCA for nodule occupancy of a strain can be influenced by host cultivars. Therefore, it would be appropriate to test *B. japonicum* strains on several cultivars for selecting the most competitive strains. However, competition experiments are very intensive and it may not be practical to test *B. japonicum* strains on a number of cultivars. Therefore, a well-adapted and widely grown soybean variety of a region may be selected as the host cultivar of choice for conducting competition experiments with *B.*

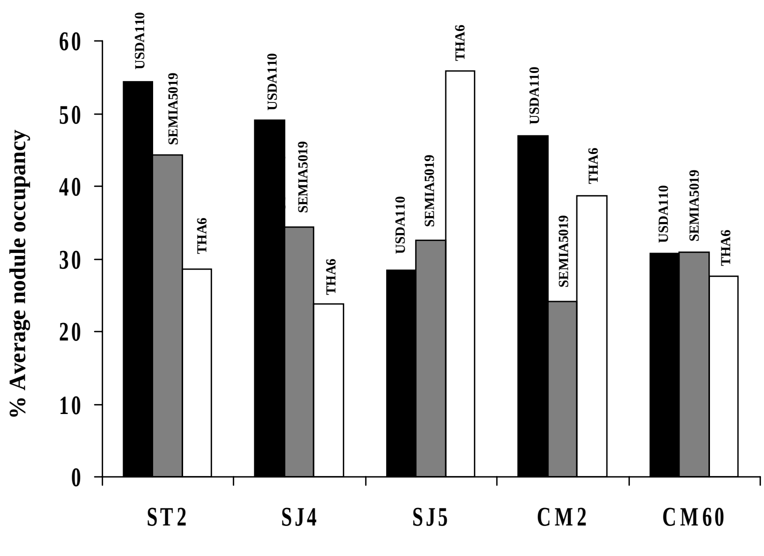

Figure 3. Average percentages of nodule occupancies by *B. japonicum* strains USDA110 (■), SEMIA 5019 (▥) and THA6 (□) in paired co-inoculation experiments on different soybean cultivars.

japonicum strains. In the present study, the five soybean cultivars used are well adapted to different regions of Thailand.

From this study, we have identified THA6 as a highly competitive *B. japonicum* strain for the soybean cultivar SJ5. SJ5 has been developed at the Chieng Mai Crops Research Center, Thailand. This cultivar was developed by crossing between SJ2 and Tainuang 4, followed by extensive selection. The major characteristics of this cultivar are high yield, short duration, tolerance to waterlogging, and contain in soil and the resistance to several diseases; leaf spot, rust and antractnose. (Department of Agriculture, Thailand website). This cultivar was selected for appropriated growing in all part of Thailand. Strain THA6 appeared to be more competitive than USDA110, occupying 27% higher nodule occupancy on SJ5. THA6 also appeared to be competitive on cultivar CM2, which is mainly grown in the North and Northeastern region of Thailand.

Generally, nodulation competition experiments involve testing paired inoculation of two strains in several proportions. The complexity and amount of data increase with the number of proportions in the paired inoculations. We have tested three proportions of the paired inoculants to determine nodule occupancies by the strains. We found that the nodule occupancy by a strain was directly co-related with the percentage of the strain in paired competition with another strain. The R^2 values for this relationship ranged from 0.8854 to 0.9987. Therefore, conducting competition experiments with several ratios of the paired inoculants may not be necessary. Our results show that inoculation ratio of only 1:1 can provide reasonable estimates of nodule occupancy by the competing strains of *B. japonicum*.

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