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Occurrence of Arbuscular Mycorrhizal Fungi in Vegetables Grown in Nakornpathom Province, Thailand

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ABSTRACT

Arbuscular mycorrhizal colonization in the roots and spore numbers in the rhizosphere of vegetables grown in Nakornpathom province of Thailand were studied. A percentage of fungal infection in the roots was analyzed by a staining method of Phillip and Hayman. A quantity of mycorrhizal spores was determined by a modification of a wet-sieving and decanting technique, and centrifugation method. Spore numbers (mean±SD) of garlic, Chinese kale and broccoli plants were 64.59±28.23, 64.09±36.62 and 45.70±33.01 spores per 100 grams of dry soil, respectively. Three genera of spores were identified from these plants, including *Acaulospora*, *Gigaspora* and *Glomus* with occurrence of individuals ranging from 48.05% to 100%. Internal vesicules and arbuscules of mycorrhizal fungi were observed only in garlic roots for all samples with the infection rates of 28.95±26.75%. There was no relationship between spore numbers and mycorrhizal colonization levels in the garlic plants. An occurrence of mycorrhizal spores in soils for cultivation of broccoli and Chinese kale did not affect the fungal colonization in the roots.

Key words: mycorrhiza, vegetable, AM

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi form symbiotic and mutualistic association with fine roots of most terrestrial plant families. AM fungi are beneficial microorganisms and act as an extension of the root system. The plant receives many benefits from this relation, including enhanced uptake of poorly mobile soil nutrients and reduced susceptibility of roots to soil-borne pathogens (Quilambo, 2003). The fungus also receives carbohydrate and growth factors from the plant. Vesicles function as reserve organs and also for fungal multiplication. Arbuscles are the major site for nutrient exchange between the two symbiotic partners. Most agricultural plant species contain at least one type of mycorrhizae. These include corn, carrots, leek, potatoes, tomatoes, beans, onions and garlic. Only a few plant families are non-mycorrhizal colonization, such as Brassicaceae (e.g. cabbage) and Chenopodiaceae (e.g. spinach). AM fungi are obligate symbionts and can not yet be cultured in the absence of plant roots or a root organ culture.

The diversity of AM fungal community may have influence on plant communities and various fungal species preferentially associate with different plant species (Pringle and Bever, 2002). The host-specificity in mycorrhizal fungal response may promote the coexistence of AM

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fungi and the distinct fungal communities are associated with different hosts (Vandenkoornhuyse *et al.*, 2002). However, plants are typically colonized by a mixture of AM fungal species since host specificity of AM fungi appears to be very low (Smith and Read, 1997). The objective of this study was to survey an occurrence of AM fungi among garlic, Chinese kale and broccoli in commercial agricultural fields, in terms of mycorrhizal spore numbers in the soils and the ability of fungal colonization in the roots.

MATERIALS AND METHODS

Study site and sampling

Root and soil samples were collected from garlic, Chinese kale and broccoli plots where grown as commercial crops in Nakornchaisri district, Nakornpathom province between March and May, 2003. Three samples from each plot were randomly collected at both ends and in the middle of the plot area. Totally 27, 27 and 11 samples of garlic, broccoli and Chinese kale, respectively, were investigated. The rhizosphere soils were collected by shaking the soil loose from roots, and the soil adjacent to roots was collected.

Spore determination

Spores of AM fungi were isolated from the soil samples by a modification of a wet-sieving and decanting technique of Gerdemann and Nicolson and a centrifugation method of Ohms, according to Muthukumar and Udaiyan (2002). Spore numbers were enumerated under microscopic examination and expressed as spores per 100 grams of dry soil. Only intact spores were counted and aggregating spores were considered as one unit. AM fungal spores were identified according to Schenck and Perez (1991).

Assessment of fungal colonization

Fine roots from each sample were processed for clearing and staining by a method of Phillips and Hayman (1970). An amount of AM colonization was determined by the magnified intersection method of McGonigle *et al.*, (1990) with a slight modification and expressed as a percentage of mycorrhizal infection. Sixty intersections were observed for each sample; intersections between ten root segments (each 10 cm length) and seven vertical lines (1 cm width). The presence of vesicles or arbuscules in 1-cm sections of roots was recorded as a positive section. Total colonization or % infection of the roots was calculated as the numbers of positive section × 100/60.

RESULTS AND DISCUSSION

Out of the 27 samples of garlic plants examined, spore numbers in the soil samples ranged from 30 spores to 148 spores per 100 grams of dry soil, with an average of 64.59±28.23 (mean± standard deviation) spores. *Acaulospora* spores, *Gigaspora* spores and *Glomus* spores were observed with isolation rates of 66.67, 88.88 and 100% of soil samples from the garlic fields (Table 1). All of 27 samples collected from broccoli plants comprised *Acaulospora* spores and *Glomus* spores while *Gigaspora* spores had an isolation rate of 48.05% (Table 2). A quantity of mycorrhizal spores in the soils was between 10 spores and 135 spores per 100 grams of dry soil and the average of their spore numbers was 45.70±33.01 spores. From a total of 11 soil samples collected from Chinese kale plants, a number of fungal spores ranged between 11 spores and 120 spores per 100 grams of dry soil (Table 3). A mean spore number was 64.09±36.62 spores. *Acaulospora* spores were found in all soil samples of Chinese kale plants whereas both *Gigaspora* spores and *Glomus* spores were identified in 72.73% of the soil samples. Figure 1 compared types of mycorrhizal spores found in soil surrounding the roots of all kinds of vegetables in this study. High prevalence of *Glomus* spores was found in garlic and broccoli while high prevalence of *Acaulospora* spores was found in broccoli and Chinese kale.

Overall, AM fungal colonization in plant root samples was observed only in the garlic roots and all root samples contained internal vesicles and/or arbuscules of AM fungi. The percentages of mycorrhizal infection within the roots ranged from 3.33 to 93.33% with an average of 28.95±26.75%. The arbuscule formation was predominantly observed in the garlic roots. In addition, no correlation was found between spore numbers and levels of AM fungal colonization in the garlic plant (Figure 2). Coexistences of AM fungi; *Acaulospora*, *Gigaspora* and *Glomus* in soil samples of all vegetable plots of broccoli, Chinese kale and garlic was observed in the study. However, only garlic roots revealed AM fungal colonization. These results agreed with the report that some plant families including the Brassicaceae, Chenopodiaceae, Cyperaceae, Juncaceae and Caryophyllaceae, were never or rarely mycorrhzal (Gerdemann, 1968). Broccoli

Table 1 Fungal spore numbers in the root zone and mycorrhizal colonization in garlic.

Sample no.	Plot no.	Spore no.per 100 g soil	Mycorrhiza species*	Root colonization (%)
1	1	36	Ac, Gi, Gl	85.00
2	1	91	Ac, Gi, Gl	56.67
3	1	58	Gi, Gl	41.67
4	2	50	Ac, Gi, Gl	50.00
5	2	56	Gi, Gl	55.00
6	2	50	Gi, Gl	31.67
7	3	70	Ac, Gl	81.67
8	3	55	Ac, Gi, Gl	31.67
9	3	65	Gi, Gl	21.67
10	4	66	Ac, Gi, Gl	35.00
11	4	85	Ac, Gi, Gl	93.33
12	4	38	Gi, Gl	18.33
13	5	60	Ac, Gi, Gl	48.33
14	5	78	Ac, Gi, Gl	6.67
15	5	148	Gi, Gl	5.00
16	6	72	Gi, Gl	3.33
17	6	30	Ac, Gi, Gl	5.00
18	6	37	Gi, Gl	5.00
19	7	80	Ac, Gi, Gl	3.33
20	7	76	Ac, Gi, Gl	11.67
21	7	30	Gi, Gl	8.33
22	8	86	Ac, Gl	16.67
23	8	33	Ac, Gl	13.33
24	8	35	Ac, Gi, Gl	13.33
25	9	75	Ac, Gi, Gl	8.33
26	9	55	Ac, Gi, Gl	26.67
27	9	129	Ac, Gi, Gl	5.00

* Ac = *Acaulospora*, Gi = *Gigaspora*, Gl = *Glomus*

Table 2 Fungal spore numbers in the root zone in broccoli.

Sample no.	Plot no.	Spore no.per 100 g soil	Mycorrhiza species*
1	1	10	Ac, Gl
2	1	20	Ac, Gl
3	1	18	Ac, Gl
4	2	30	Ac, Gl
5	2	15	Ac, Gl
6	2	28	Ac, Gi, Gl
7	3	83	Ac, Gi, Gl
8	3	18	Ac, Gl
9	3	12	Ac, Gl
10	4	28	Ac, Gi, Gl
11	4	50	Ac, Gl
12	4	53	Ac, Gl
13	5	21	Ac, Gi, Gl
14	5	65	Ac, Gl
15	5	30	Ac, Gi, Gl
16	6	36	Ac, Gl
17	6	56	Ac, Gi, Gl
18	6	23	Ac, Gi, Gl
19	7	44	Ac, Gl
20	7	53	Ac, Gi, Gl
21	7	18	Ac, Gl
22	8	70	Ac, Gi, Gl
23	8	49	Ac, Gl
24	8	81	Ac, Gi, Gl
25	9	54	Ac, Gi, Gl
26	9	134	Ac, Gi, Gl
27	9	135	Ac, Gi, Gl

* Ac = *Acaulospora*, Gi = *Gigaspora*, Gl = *Glomus*

Table 3 Fungal spore numbers in the root zone in Chinese kale.

Sample no.	Plot no.	Spore no.per 100 g soil	Mycorrhiza species*
1	1	111	Ac, Gi
2	1	60	Ac, Gi, Gl
3	1	59	Ac, Gi
4	1	48	Ac, Gi, Gl
5	1	110	Ac, Gi
6	1	58	Ac, Gi, Gl
7	1	120	Ac, Gi, Gl
8	1	74	Ac, Gi, Gl
9	2	26	Ac, Gi
10	2	12	Ac, Gi
11	2	27	Ac, Gi

* Ac = *Acaulospora*, Gi = *Gigaspora*, Gl = *Glomus*

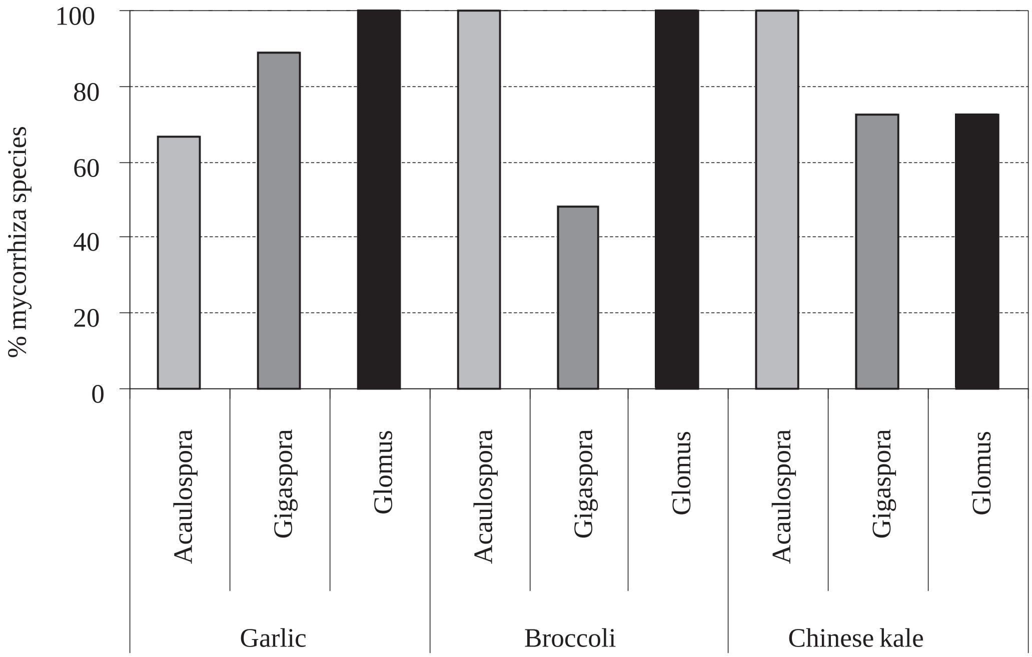

Figure 1 A comparison of mycorrhza species in soil surrounding the roots of garlic, broccoli and Chinese kale plants.

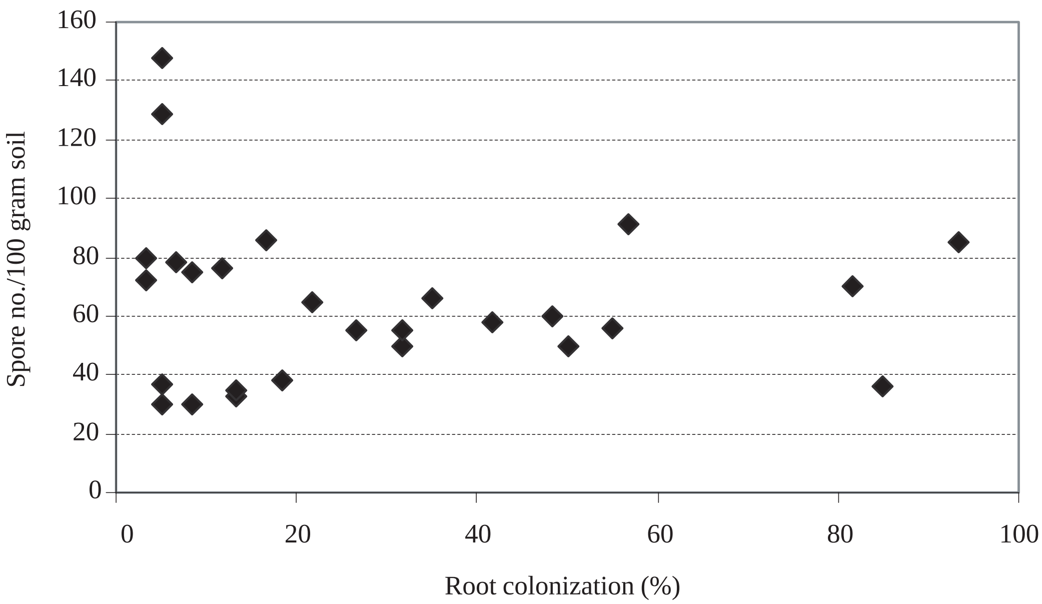

Figure 2 Relationship between a quantity of mycorrhizal spores and levels of fungal colonization in the garlic plant.

(*Brassica oleracea italica*) and Chinese kale (*Brassica oleracea alboglabra*) are both in a family of Brassicaceae, whereas garlic (*Allium sativum*) is classified in a family of Alliaceae. AM fungal colonization has been reported in some commonly grown crops, including onion (*Allium capa*), carrot (*Daucus carota*), garden pea (*Pisum sativum*), leek (*Allium porrum*), kidney bean (*Phaseolus vulgaris*), currant (*Ribes nigrum*), sweet corn (*Zea mays*), pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), and apple (*Malus domestica*) (Plenchette *et al*., 1983).

In this study, there was great variation of spore numbers in the soil samples collected from the same plot of all vegetables. In contrast, mycorrhizal colonization levels in garlic roots, collected from the same plot, showed similar % fungal infection. No relationship was observed between spore numbers in root zone and rates of fungal colonization in the garlic plant. Such result was comparable with several authors who indicated no correlation between spore numbers found in the soil and mycorrhization of adjacent plants (López-Sá nchez and Honrubia 1992; Michel-Rosales and Valdés 1996, and Bever 2002). However, Frioni et al., (1999) reported a direct correlation of colonization percentage and spore density in soil in native legumes tree with dominant spore types of *Acaulospora*, *Glomus*, and *Sclerocystis*. The response of a plant to mycorrhizal colonization might depend on soil moisture, inorganic nutrient, pH, mycorrhizal or plant species, type of root system, age of host plant, tillage practices, cropping rotations, and pesticide applications (Bethlenfalvay 1992; Johnson and Pfleger 1992; Kurle and Pfleger 1994; and Safir 1994). Additionally, spore numbers and mycorrhizal colonization could vary between years and seasons (Khan 1974, Allen *et al*., 1989, and Lugo and Cabello 2002).

In this study, garlic, broccoli and Chinese kale samples were collected at a period of vegetation of 80 days, 75 days and 45 days, respectively. Generally, harvesting schedules are about 180 days for garlic plant, 75 days for broccoli plant and 45 days for Chinese kale plant. Arbuscule formation has been reported that it might occurr in the agricultural plant roots after approximate 60 days of vegetation and vesicules might be formed about 90 days after cultivation. The process of AM fungal colonization of host plant roots composts of spore germination, hyphal differentiation, appressorium formation, root penetration, intracellular growth, arbuscule formation and nutrient transport. Following a formation of arbuscules, some mycorrhizal species, including *Glomus*, *Sclerocystis*, *Acaulospora* and *Entrophospora*, also form vesicules within the roots (Harrison, 1999). Arbuscular mycorrhizae influence plant root development relating to root size and architecture. (Berta *et al*., 1993 and Berta *et al*., 1990). Leek plants infected with the fungus *Glomus* species had more numerous, shorter and more branched, adventitious roots. Mycorrhizal roots including mycorrhizae spores and mycelia, were the main propagates left in the soil that colonized plant roots of the succeeding crop in a rotation system (Sylvia 1992).

CONCLUSION

The commercial agricultural areas of this study, AM fungal colonization were absent on both Chinese kale and broccoli roots while the soils adjacent to their roots composted of the fungi. In addition, colonization capacity of the mycorrhizal fungi were demonstrated within garlic root systems and coexistence of *Acaulospora* spores, *Gigaspora* spores and *Glomus* spores was observed in the soil samples.

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