J. Trop. Agric. and Fd. Sc. 26(2) (1998): 119–126

Root alterations and nutrient uptake of mangosteen (*Garcinia mangostana* L.) seedlings in response to arbuscular mycorrhizal inoculation

[Perubahan akar dan penyerapan nutrien anak benih manggis (*Garcinia mangostana* L.) hasil inokulasi mikoriza arbuskel]

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Key words: arbuscular mycorrhiza, root alteration, nutrient uptake, mangosteen

Abstrak

Kajian terhadap perubahan sifat sistem akar dan penyerapan nutrien oleh anak benih manggis (*Garcinia mangostana* L.) hasil inokulasi kulat mikoriza arbuskel (MA) telah dijalankan. Pengukuran berat kering sahaja tidak dapat mengesan perubahan kecil kepada akar akibat jangkitan kulat. Pertumbuhan akar tunjang, berat kering akar dan nisbah akar:pucuk didapati tidak dipengaruhi oleh inokulasi kulat MA. Walau bagaimanapun, inokulasi MA berjaya memanjangkan dan meningkatkan percabangan akar. Apabila dibandingkan dengan anak benih tanpa jangkitan, inokulasi kulat MA meningkatkan ketumpatan panjang akar sebanyak 58–87%, ketumpatan percabangan akar sebanyak 20–30%, bilangan hujung akar sebanyak 22–25% dan bilangan akar sisi 15–26%. Perubahan sistem akar secara positif meningkatkan penyerapan nutrien. Penyerapan P, Zn dan Cu masing-masing meningkat sebanyak 67–88, 50–93 dan 34–37%. Hasil daripada kajian ini menunjukkan bahawa peningkatan pertumbuhan dan penyerapan nutrien oleh anak benih manggis yang diinokulat dengan MA adalah disebabkan oleh perubahan positif pada sistem akarnya.

Abstract

Alterations to root system characteristics and nutrient uptake of mangosteen (*Garcinia mangostana* L.) seedlings in response to arbuscular mycorrhizal (AM) inoculation were studied. Weight-related parameters were unsuccessful in detecting small changes in infected roots. Tap-root penetration, root dry weight and root-to-shoot ratio were not influenced by AM inoculation. However, AM inoculation induced significant changes to length-related characteristics. In comparison with the uninoculated controls, AM inoculation increased root length density by 58–87%, root branching density by 20–30%, number of root tips by 22–25% and number of laterals by 15–26%. Positive alterations to root system were accompanied by tremendous increase in nutrient uptake. Uptakes of P, Zn and Cu were 67–88, 50–93 and 34–37% higher in inoculated seedlings. These results indicate that improved growth and nutrient uptake of the AM-inoculated seedlings were due to positive alterations of root system characteristics by the symbiosis.

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Introduction

Roots are major vegetative organs involve in the uptake of essential nutrients and other substances for plant growth and development. Leskovar and Stofella (1995) have indicated that size, morphology and architecture of the root system may control the relative size and growth of a plant. Therefore, proper functioning of roots can ensure optimum plant growth and yield.

A report by Masri et al. (1998b) showed that the slow growth of mangosteen seedlings was strongly related to its poorly developed root system. Masri et al. (1998a) also showed that infection by arbuscular mycorrhizal (AM) fungi had significantly enhanced the growth of mangosteen seedlings through improvements in the uptake of immobile nutrients particularly phosphorus. Since proper functioning of the root system can ensure optimum plant growth, it is important to study the growth and functions of roots in response to AM infection.

Earlier studies on other plants have indicated that mycorrhizal infection did not influence root morphology (Carling and Brown 1982; Harley and Smith 1983). Carling and Brown (1982) documented that macroscopic alterations of root morphology, which typically accompanies ectomycorrhizal development, were absent in AM infections. However, these studies are largely confined to measurements of root weight and such approaches may not be adequate to identify detailed changes in root morphology as a result of symbiosis with AM fungi (Buwalda et al. 1984; Smith et al. 1986).

More recent studies, however, have found that AM infection caused substantial alterations to root morphology of host plants. Berta et al. (1990) revealed root morphological changes as a result of AM infection which include an increase in number of adventitious roots, a decrease in mean root length and greater root branching. Hooker et al. (1992) also found significant alterations to root system morphology of poplar plants in response to AM infection.

Although mangosteen roots may be easily infected by mycorrhizal fungi due to their coarse and scanty characteristics, their capacity to absorb more nutrient and water depends largely on the positive alterations of the entire root system as a result of the infection. It is, therefore, highly desirable to examine the modifications of mangosteen root system as induced by AM infection. To date, no such reports have been documented for mangosteen.

Materials and methods

Inoculation and growth conditions

Treatments comprised two fungal inocula. The first inoculum was a pure strain of *Glomus mosseae* [WUM 9(6)] obtained from 9-month-old pot culture grown with *Setaria anceps* in sterilised sandy soil following the method of Azizah and Omar (1987). The second inoculum consisted of mixed cultures of *Scutellospora calospora* and *G. mosseae* in equal proportions (w/w). A hundred gram of each inoculum was placed as a thin layer, 5 cm below the soil surface in each polyethylene bag. These polyethylene bags, each measuring 30 cm x 36 cm, were previously filled with 10 kg of unsterilised potting mixture of sand, soil and cow dung in the ratio of 3:2:1 by volume. One-month-old germinated mangosteen seedlings were selected and individually transplanted to their respective bags. Treatments were later designated as GM for the pure strain of *G. mosseae*, MS for the mixed culture and –M for the uninoculated control plants. All seedlings were grown in a nursery of 50% shade for a period of 18 months.

Root growth measurements

Plants were harvested at 3-month intervals over the 18-month period. At each harvest, leaves, stems and roots were separated. Dry weights of leaves and stems were determined after oven drying at 80 ºC for 48 h. Soil plus root samples in each polyethylene bag were gently washed under slow running tap-water to separate roots from adhering soils. After exposition of all roots, tap-root length

was measured manually with a ruler. Number of laterals attached to the tap-root was also manually counted with the help of a magnifying glass. Root length, number of root tips and length of white roots were measured immediately after washing was completed.

Root length density was estimated using the method proposed by Tennant (1975). All live roots were collected and evenly distributed on a nylon mesh placed over a plastic sheet that had 1-cm square grid lines drawn on it. Number of times roots intersected with vertical and horizontal lines of the grid were counted. Root length was estimated by multiplying the total number of vertical and horizontal intersections by a conversion factor of 0.7857 (Tennant 1975). Root length density was calculated by dividing root length with a standard soil volume of the polyethylene bags (21 195 cm^3). The standard soil volume was calculated by using the formula $\pi r^2 h$, where $\pi = 3.14$, r (radius of polyethylene bag) = 15.0 cm and h (average height of soil in bag) = 30 cm.

The number of root tips which were used as an indicator of the quantity of absorbing roots (Weller 1971), was counted manually with the help of a magnifying glass. Only plump whitish root tips were counted as these root tips were assumed to have a living primary cortex at the time of sampling.

For root dry weight, all roots were assembled and dry weight was determined after oven drying at 80 °C for 24 h. Root-to-shoot ratio was expressed as the ratio of root and shoot dry weights. Root branching density was calculated as the number of laterals per unit length of tap-root (Fiel et al. 1988).

Nutrient concentration and uptake

Three to five fully matured leaves from each treatment replicate were collected at 18 months of age and oven dried at 80 °C for 48 h. Dried leaves were subsequently ground using a hammer mill. Plant P, Zn and Cu concentrations were measured by inductively coupled plasma (ICP) emission spectrometric analysis using wet-ashing procedures (Ahmad 1993). Nutrient uptake per plant was expressed as concentration multiplied by total plant dry weight (Kumaran and Azizah 1995).

Design and statistical analysis

Treatment x harvest combinations were arranged in a randomised complete block design (RCBD) with four replications. All data were subjected to Analysis of Variance performed by using procedures of Statistical Analysis System (SAS Institute Inc. 1985). For significant mycorrhiza x age interaction effects, treatment means were compared at each plant age using the Least Significant Difference (LSD) method.

Results

Tap-root elongation was not influenced by mycorrhizal inoculation *(Table 1)*. Tap-roots from all treatments were not significantly different in length at all growth stages. Maximum tap-root length of 37–39 cm in all treatments were observed at 18 months. Similarly, all treatments were also not significantly different in root dry weight until 15 months after inoculation *(Table 1)*. However, at 18 months of growth, root dry weight of mycorrhizal seedlings was significantly higher than the uninoculated plants. Mycorrhizal seedlings had 36–50% more root dry weight than the uninoculated plants at this age. Root-to-shoot ratio was also not significantly affected by AM infection *(Table 1)*. Instead, root-to-shoot ratio decreased with time indicating less dry matter being partitioned to roots as plant grows.

Root length

Contrastingly, root length estimates produced significant results. Root length density (RLD) of inoculated seedlings was consistently and significantly greater than the uninoculated controls as early as 6 months after inoculation *(Figure 1)*.

Table 1. Tap-root length, root dry weight and root-to-shoot ratio of mangosteen seedlings as affected by arbuscular mycorrhizal inoculation

Months after inoculation	Tap-root length (cm)			Root dry weight (g/plant)			Root-to-shoot ratio		
	GM	MS	–M	GM	MS	–M	GM	MS	–M
3	15.7	15.0	16.2	0.27	0.20	0.28	0.21	0.16	0.21
6	19.7	20.1	20.8	0.84	0.59	0.60	0.19	0.16	0.19
9	24.8	24.0	25.1	1.70	1.37	1.05	0.17	0.18	0.16
12	30.5	30.3	29.3	3.17	2.67	2.19	0.15	0.14	0.14
15	31.5	30.6	29.9	4.62	4.74	4.34	0.14	0.16	0.15
18	39.3	37.2	38.4	8.07a	7.35a	5.37b	0.15	0.15	0.16

Mean values with different letters in a row are significantly different at $p < 0.05$ according to LSD
GM and MS are inoculated with *Glomus mosseae* and a mixture of *G. mosseae* and *Scutellospora calospora* respectively. –M is the uninoculated treatment

Although there were fluctuations in RLD among the inoculated plants from 9 to 18 months, they consistently maintained their significant values compared with the controls. At 18 months, the RLD of mycorrhizal seedlings was about 58–87% higher than the uninoculated controls.

Root branching

Lateral root production was significantly induced by AM colonisation *(Figure 2).* Both inoculated seedlings produced significantly greater number of laterals than the uninoculated seedlings as early as 9 months after inoculation. At 18 months, the GM and MS-treated seedlings respectively had 26.0% and 14.9% more laterals than the uninoculated seedlings. Root branching density of the inoculated seedlings was significantly greater from 9 months onwards as compared with the uninoculated plants *(Figure 3).* Root branching density was estimated to be 20–30% higher in the mycorrhizal-treated seedlings compared with the uninoculated controls.

Quantity of absorbing roots

AM inoculation influenced the production of root tips per plant *(Figure 4).* AM-inoculated seedlings had significantly larger number of root tips as early as 6 months after inoculation. From 6 months onwards, the number of root tips was consistently and

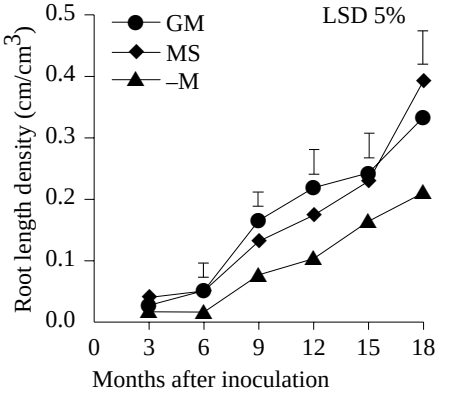

Figure 1. Effect of arbuscular mycorrhizal inoculation on root length density of mangosteen seedlings

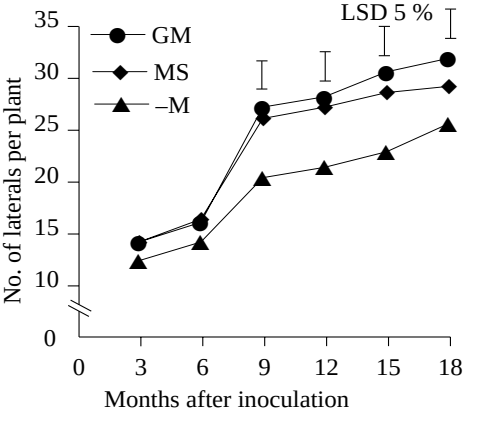

Figure 2. Effect of arbuscular mycorrhizal inoculation on lateral root production of mangosteen seedlings

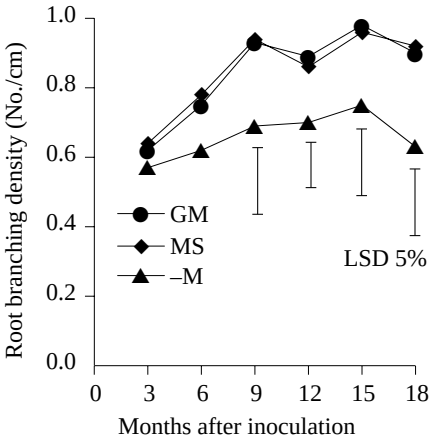

Figure 3. Effect of arbuscular mycorrhizal inoculation on root branching density of mangosteen seedlings

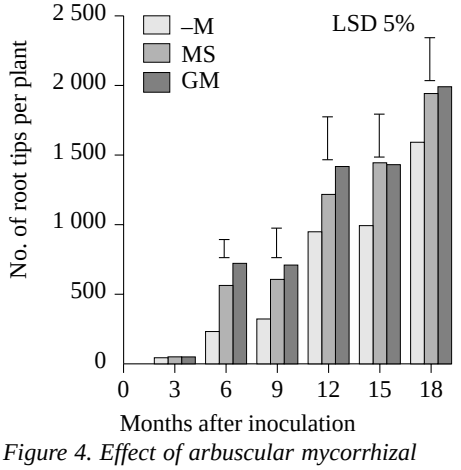

Figure 4. Effect of arbuscular mycorrhizal inoculation on the number of root tips produced by mangosteen seedlings

significantly larger in mycorrhizal plants compared with the uninoculated seedlings. At 18 months, the number of root tips of colonised seedlings was 22–25% larger than the uninoculated plants.

Nutrient uptake

The concentration and uptake of P, Zn and Cu by mangosteen plants are affected by mycorrhizal inoculation *(Table 2)*. Inoculated seedlings had significantly higher concentration of P in their leaves. Higher P concentration of mycorrhizal plants was accompanied by higher P uptake. At 18 months, the uptake of P by GM and MS seedlings was 88% and 67% more than the uninoculated seedlings respectively. Similarly, Zn and Cu concentrations were 7–17% and 20–33% higher while uptakes of Zn and Cu was 50–93% and 34–37% higher in mycorrhizal compared with the uninoculated seedlings.

Discussion

Length of tap-root was not affected by mycorrhizal infection. These results clearly indicate that AM symbiosis did not influence the ability of mangosteen tap-root to penetrate deeper into soil depths. Similarly, root-to-shoot ratio did not respond to AM infection. Instead, root-to-shoot ratio decreased with time suggesting less dry matter is being partitioned to roots as plant grows. Harley and Smith (1983) also reported occasional differences in root-to-shoot ratio of mycorrhizal and non-mycorrhizal plants.

Root dry weight took 18 months to respond to AM inoculation. At this stage, mycorrhizal mangosteen seedlings had 36–49% more root dry weights than the uninoculated seedlings. Several researchers have also reported poor response of root dry weight to AM inoculation (Harley and Smith 1983; Buwalda et al. 1984; Smith et al. 1986). However, root dry weight increment was higher in mycorrhizal plants. This indicates that root growth of mangosteen responded positively to AM infection though it might not be easily detected. Other workers also found increased root biomass of host plants as a result of AM infection (Mosse 1981; Manjunath and Habte 1991). This positive response may be closely related to the coarse characteristics of mangosteen roots (Rukayah and Zabedah 1992), which make them easily infected by AM fungi.

Mycorrhizal colonisation significantly induced large changes when length-related parameters of roots were measured. Significant differences in RLD between inoculated and uninoculated seedlings were

Table 2. Effect of arbuscular mycorrhizal inoculation on concentration and uptake of P, Zn and Cu by mangosteen seedlings at 18 months of growth

Treatment	P conc. (%)	P uptake (mg/plant)	Zn conc. (μg/g)	Zn uptake (μg/plant)	Cu conc. (μg/g)	Cu uptake (μg/plant)
GM	1.45a	95.0a	23.5a	1 547.8a	5.5b	354.3a
MS	1.51a	84.6a	21.5b	1 199.3b	6.1a	339.2b
–M	1.36b	50.6b	20.1c	800.2c	4.6c	222.5c

Mean values with the same letters in a column are not significantly different at $p < 0.05$ according to LSD
GM and MS are inoculated with *Glomus mosseae* and a mixture of *G. mosseae* and *Scutellospora calospora* respectively. –M is the uninoculated treatment

observed as early as 6 months after inoculation. At 18 months, the RLD of inoculated seedlings was 58–87% higher than the uninoculated seedlings. Hooker et al. (1992) similarly reported great increment in the length of secondary and tertiary roots of poplar seedlings due to AM infection. Mycorrhizal infection also altered dry mass partitioning to root system of neem tree (*Azadirachta indica*) resulting in greater root length (Phavaphutanon et al. 1996). These results clearly show that root length is a better parameter than root mass in detecting small changes to root system caused by AM infection. More importantly, these differences in root length could have significantly increased the uptake of mineral nutrients by mycorrhizal plants. This is so because acquisition of nutrients by plant roots is more related to root length or root surface area rather than root biomass (Baon et al. 1994).

Other profound effect of AM infection is root branching. Roots of mycorrhizal mangosteen were progressively more branched, while the uninoculated plants exhibited almost constant development. It was estimated that root branching density of mycorrhizal mangosteen was 20–30% higher than the uninoculated controls. Berta et al. (1990) also reported greater root branching of mycorrhizal onion (*Allium porrum* L.). Similarly, root branching of inoculated poplar plants was six times greater than the non-mycorrhizal plants (Hooker et al. 1992). Greater root branching of mycorrhizal seedlings was due to greater lateral root production. Lateral root production of mycorrhizal seedlings was estimated to be 15–26% higher than the uninoculated seedlings.

In this study, quantity of absorbing roots was estimated by the number of root tips. Number of root tips was larger in mycorrhizal plants as early as 6 months after inoculation. Mycorrhizal seedlings had an estimated 22–25% larger number of root tips than the uninoculated seedlings 18 months after inoculation. These results clearly show that AM infection significantly enhanced active root production. Root tips are usually considered most active in absorbing water and minerals from the soil (Kramer and Kozlowski 1960). Rapid regeneration of active roots could lead to successful field establishment and subsequent growth (Watson and Himelick 1982; Koffa and Cruz 1995).

Several researchers observed improved uptake of nutrients such as P, Zn and Cu that have narrow diffusion zones around roots by mycorrhizal symbiosis (Lambert et al. 1979; Marschner and Dell 1994). Results of this study showed higher concentration and uptake of P by mycorrhizal mangosteen. Azizah (1991) as well as Azizah and Martin (1992) found similar results with other crops. A more detailed discussion on P nutrition of mycorrhizal mangosteen was described by Masri et al. (1998a). It is concluded that improvement in mineral uptake by mycorrhizal mangosteen was mainly due to positive alterations of root characteristics by AM symbiosis.

Conclusion

Arbuscular mycorrhizal fungi was successful in inducing large changes to root system characteristics of mangosteen. The most profound alterations were greater root length, more profuse root branching and greater quantity of absorbing roots. Compared with the uninoculated seedlings, root length density was increased by 58–87%, root branching density by 20–30%, quantity of root tips by 22–25% and lateral root production by 15–26%. However, measurement of root dry weight alone was unsuccessful in detecting these changes. Instead, measuring length-related parameters as well as root branching successfully detected small changes in infected mangosteen roots. Positive changes to root characteristics were accompanied by a tremendous increase in uptake of nutrients, particularly P, Zn and Cu.

Acknowledgements

We thank the Ministry of Science, Technology and Environment of Malaysia and MARDI for research grant through the IRPA Project Number 01-03-03-0024. Special acknowledgements are due to Hamzah Rasheed, Zulkafli Ahmad and Ahmad Doon for their technical assistance.

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Accepted for publication on 17 April 1998