愛媛大学農学部農場報告
(Bull. Exp. Farm Fac. Agr., Ehime Univ.) 24 : 1 − 7 (2002)

Effect of Inoculation with an Arbuscular Mycorrhizal (AM) Fungus on Phoshorus Nutrition in Loquat (*Eriobotrya japonica* Lindl.) Seedlings

Kipkoriony L. RUTTO, Fusao MIZUTANI, Yuki ASANO and Kazuomi KADOYA[1)]

Summary

A factorial experiment was carried out to study the effect of inoculation with an arbuscular mycorrhizal fungus on the phosphorus nutrition of loquat (*Eriobotrya japonica* Lindl.) seedlings growing under four P fertilizer regimes. As compared with the non P-fertilized control, growth and biomass production were high at all P levels in both mycorrhizal and non-mycorrhizal seedlings. Contents of major mineral elements in leaves were fairly uniform except for P. The non-mycorrhizal control plants had significantly lower P concentrations than all the other treatments. Shoot P content was significantly higher in both the mycorrhizal and non-mycorrhizal P-fertilized treatments than in the corresponding controls. Root samples from the inoculated treatments were colonized by *G. mosseae* though infection levels were quite low (<30%). The non P-fertilized controls showed marginally higher infection and limited arbuscular-type colonization.

Introduction

Loquat also called Japanese medlar is a fruit of wide appeal thought to be native to southeastern China and southern Japan. Loquats are adapted to sub-tropical and mild temperate climates and will do well in soils of moderate fertility and good drainage. Extreme summer heat is detrimental to loquat and dry hot winds can cause leaf scorch. In Japan, loquats are grown on hillsides where they benefit from good airflow[6)]. In order to examine the mycorrhizal relations of loquat, root samples were collected at random from mature loquat trees in and around the Ehime University Farm (33°57'N, 132°47'E, 20 m above sea level). Results from these preliminary analyses showed loquat as having very low mycorrhizal infection. Arbuscular mycorrhizal fungi form symbiotic associations with more than 85% of all plant species[1)]. The symbiotic relationship is characterized by a bi-directional flow of nutrients ; photosynthates from the host plant and soil derived mineral elements (particularly phosphorus) from the fungal partner[11)].

There are very few reports on the mineral nutrition of loquat and practically none on the

1) Professor Emeritus, Faculty of Agriculture, Ehime University.

relationship between loquat and arbuscular mycorrhizae. Based on the infection levels of field samples, this experiment was carried out to test the hypothesis that loquat does not derive substantial nutritional benefits from the symbiotic association with arbuscular mycorrhizae.

Materials and Methods

Seedling establishment and inoculation with G. mosseae

Loquat seeds were germinated in Kanuma soil in July 2000 and later transplanted into 5L pots containing an average of 5kg each of a low nutrient sandy soil. Mycorrhizal pots (+AM) were inoculated with 5g each of a soil based inoculum containing an average of 200 spores per gram of the AM fungus *G. mosseae* (Idemitsu Kosan, Tokyo). Non-mycorrhizal pots (−AM) received similar amounts of autoclaved inoculum. The plants were maintained under greenhouse conditions, watered periodically and fertilized once every four months with a combination of KH_2PO_4, KNO_3 and NH_4NO_3 calculated to give four different levels of P (Table 1). Fertilizer requirements were computed based on recommendations made by Morton[6] for the compound fertilizer NPK. Plant height was measured monthly from January to September 2001. The seedlings were harvested in October and both root and shoot fresh weights were recorded. Dry weights were measured after drying at 70°C for 72hrs.

Table 1 The fertilizer combinations used in the experiment to study phosphorus nutrition in mycorrhizal and non-mycorrhizal loquat seedlings.

P level	Weight of compound (g)		
	KH_2PO_4	KNO_3	NH_4NO_3
P_1	0.0	2.33	1.66
P_2	1.0	1.58	1.94
P_3	2.0	0.85	2.23
P_4	4.0	0.00	2.57

Estimation of root infection

Root pieces (1±0.2cm) were sampled at seedling harvest and cleared immediately by autoclaving in 10% KOH followed by staining with 0.03% Chlorazol Black E (CBE) solution[2]. Infection levels and properties were estimated under a compound microscope using the grid-intersect method[4].

Results

Seedling growth

There was no big difference in seedling growth between treatments during the cold months (January-April). However, there was a rapid increase in growth for all treatments receiving P fertilization (P2-P4) between April and September irrespective of *G. mosseae* inoculation (Fig.

1). Seedling growth in the control treatment (P1) was slow with the monthly increase in height being almost constant even in the spring and summer months.

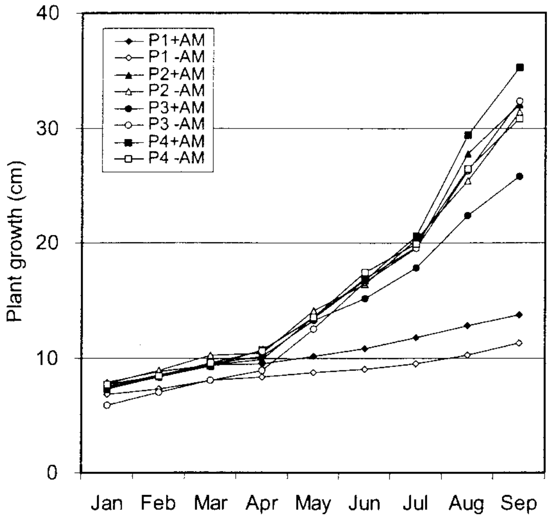

Fig. 1 Change in plant height with time of mycorrhizal (+AM) and non-mycorrhizal (−AM) loquat (*E. japonica*) seedlings maintained under greenhouse conditions at four different P (P1-P4) treatment levels.

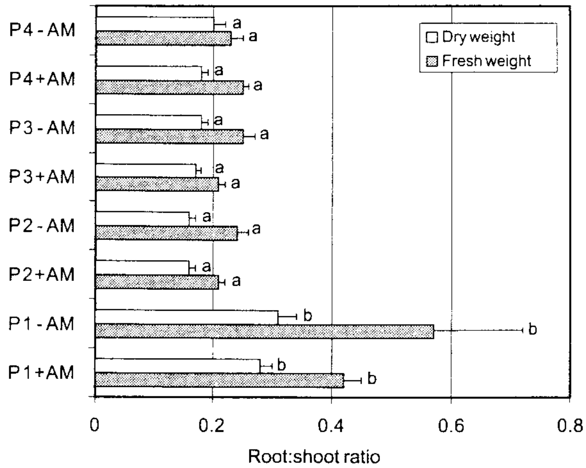

Fig. 2 Fresh and dry weight root : shoot ratios in mycorrhizal (+AM) and non-mycorrhizal (−AM) loquat (*E. japonica*) seedlings grown under greenhouse conditions at four different P (P1-P4) treatment levels. Bars represent SE and different letters denote significant differences within fresh and dry weight categories at $p<0.05$ (Newman Keul's post test, n=5).

Biomass yield

Fresh and dry weight root : shoot ratios were significantly higher in the non-fertilized mycorrhizal and non-mycorrhizal controls (Fig. 2) than in treatments receiving P fertilization. There was a significant difference between treatments receiving P fertilization and the controls in final shoot and root fresh and dry biomass yield. However, similar to the growth rate, no measurable differences between treatments could be attributed to the presence or absence of the fungal partner (Fig. 3).

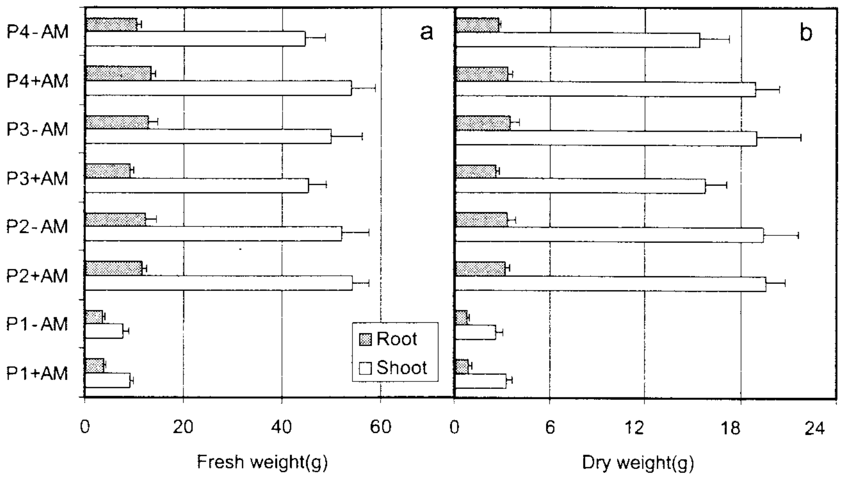

Fig. 3 Biomass yield from mycorrhizal (+AM) and non-mycorrhizal (−AM) loquat (*E. japonica*) seedlings fertilized with four different levels of P (P1-P4) and grown in the greenhouse. Bars represent SE (n=5).

Seedling nutrition

There was no difference between treatments in shoot N, K and Ca content. Shoot P was highest in samples from both the mycorrhizal and non-mycorrhizal treatments with the highest P fertilization (P4). There was a significant difference in shoot P content between P treated (P2-P4) and control samples and between mycorrhizal and non-mycorrhizal samples within the control treatment (Table 2). Shoot Mg content was lowest in the non-mycorrhizal control treatment and highest in the non-mycorrhizal P4 treatment but was generally uniform in all the other treatments.

Table 2 Shoot content of selected elements in samples collected from mycorrhizal (+AM) and non-mycorrhizal (−AM) loquat (*E. japonica*) seedlings maintained under greenhouse conditions at four different P (P1-P4) treatment levels.

Treatment	Mineral content (%)				
	N	P	K	Ca	Mg
P1+AM	0.18±0.03^{z}a	0.09±0.020b^y	0.26±0.04a	4.23±0.13a	0.23±0.08ab
P1−AM	0.17±0.01a	0.05±0.006a	0.22±0.02a	3.57±0.44a	0.13±0.02a
P2+AM	0.22±0.02a	0.13±0.002c	0.22±0.03a	4.27±0.03a	0.25±0.09ab
P2−AM	0.17±0.01a	0.13±0.002c	0.21±0.03a	3.93±0.48a	0.22±0.07ab
P3+AM	0.18±0.01a	0.14±0.002c	0.20±0.02a	4.00±0.25a	0.31±0.09ab
P3−AM	0.20±0.04a	0.15±0.004c	0.21±0.01a	4.10±0.38a	0.28±0.05ab
P4+AM	0.19±0.03a	0.17±0.007c	0.24±0.05a	4.07±0.24a	0.23±0.05ab
P4−AM	0.21±0.02a	0.17±0.003c	0.27±0.04a	4.00±0.20a	0.46±0.05b

^{z}Mean±SE (n=5)
^{y}Means within columns followed by different letters are significantly different at $p<0.05$ (Newman-Keul's post test).

Mycorrhizal infection

An examination of the degree of root infection by *G. mosseae* showed that all inoculated treatments established the symbiotic relationship with the fungus at all P levels. Infection was highest in the control treatment and lowest in the P4 treatment. Arbuscular infection was present only in root samples collected from the control treatment (Fig. 4). There was a negative correlation between the level of infection and the amount of P fertilizer applied per treatment (Fig. 5).

Discussion

Most members of the Rosaceae family have been shown to form beneficial relationships with AM fungi. For example, colonization with AM fungi is reported to stimulate apple growth in unsterilized soil under field conditions[7], to increase total fresh and dry shoot weight, leaf number and stolon production in strawberry[5] and to suppress nematode infestation in peach rootstocks transplanted into replant soils heavily infested with the nematode *Meloidogyne javanica*[3].

Loquat does not seem to benefit from the symbiotic relationship with *G. mosseae* except in

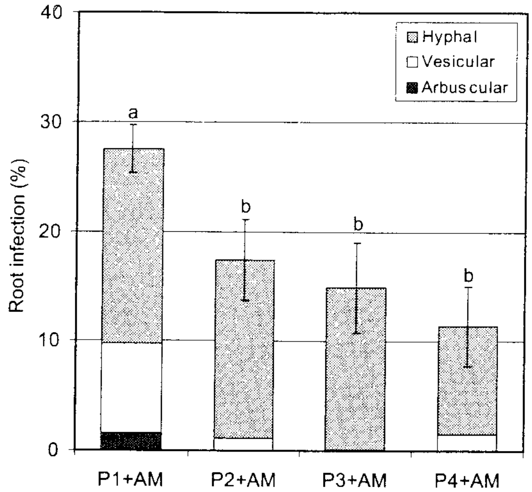

Fig. 4 Root infection by the AM fungus *G. mosseae* of loquat (*E. japonica*) seedling roots. Samples were collected from seedlings maintained under greenhouse conditions at four different P (P1-P4) treatment levels. Bars represent SE and letters denote significant differences in total infection at $p<0.05$ (Newman Keul's test, n=5).

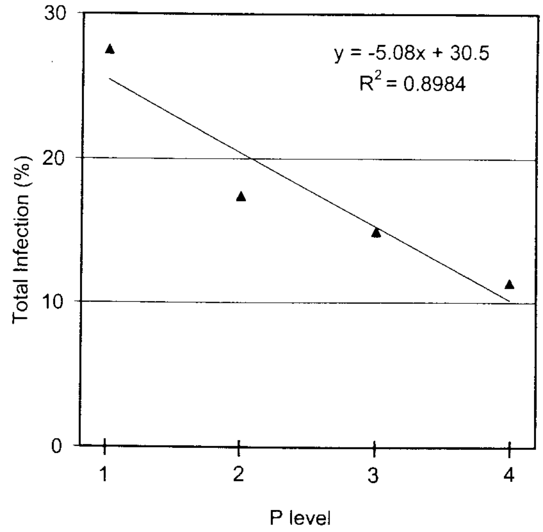

Fig. 5 The correlation between total mycorrhizal infection and the level of P fertilization in an experiment to study the effect of colonization by *G. mosseae* on the phosphorus nutrition of loquat (*E. japonica*) seedlings.

cases of extreme P deprivation. This is because the arbuscular component of infection was observed only in the control treatment without any P fertilization. It is has been shown that the exchange point for the transfer of mineral elements from the fungal partner to the plant host takes place at the arbuscule/cell interface. Polyphosphate is hydrolyzed in the arbuscules and transported as inorganic phosphate (P_i) across the plasma membrane of the host root cell[10]. There is a possible relationship between infection and the fine texture of loquat roots. Plant species with coarse roots seem to benefit more from the mycorrhizal relationship[8] and it is highly likely that mineral acquisition by loquat is more efficient than in coarse rooted species like citrus. This would imply that the threshold level of P availability that exerts a negative impact on the relationship between loquat and AM fungi is much lower than for most other agricultural plants. This is because a prolonged compatible relationship between plants and AM fungi is based primarily upon the transfer of P from the symbiont to a host with a net P deficit. Where available soil P is not limiting to plant growth, roots will exclude or limit the mycorrhizal infection. Another possible explanation is the likely presence of inhibitors within loquat roots that hinder the rapid formation of a functional relationship.

Sinkai[9] reported that loquat trees grow better in high moisture conditions and that low soil moisture conditions accelerate physiological leaf drop. We observed that under greenhouse conditions, high summer temperatures cause leaf scorching and rapid media dehydration; factors that are likely to have impacted negatively on seedling growth. Field studies employing a more diverse AM species range coupled with a variety of management strategies should form a basis for future research.

Literature Cited

(1) Brundrett, M.C. 1991. Mycorrhizas in natural ecosystems. Adv. Ecol. Res. 21 : 171-313.

(2) Brundrett, M.C. 1994. Clearing and staining mycorrhizal roots. pp. 42-46. In : M. Brundrett, L. Melville, and L. Peterson, (eds.). Practical methods in mycorrhiza research. Mycologue Publications, Sidney, BC.

(3) Calvet, C., J. Pinochet, A. Hernández-Dorrego, V. Estaún and A. Camprubí. 2001. Field microplot performance of the peach-almond hybrid GF-677 after inoculation with arbuscular mycorrhizal fungi in a replant soil infested with root-knot nematodes. Mycorrhiza 10 : 295-300.

(4) Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84 : 489-500.

(5) Khanizadeh, S., C. Hamel, H. Kinamehr, D. Buszard and D.L. Smith. 1995. Effect of three vesicular-arbuscular mycorrhizae species and phosphorus on reproductive and vegetative growth of three strawberry cultivars. J. Plant Nutr. 18 : 1073-1079.

(6) Morton, J.F. 1987. Loquat. In : Fruits of warm climates. Miami, Fl. pp. 103-108.

(7) Plenchette, C., V. Furlan and J.A. Fortin. 1981. Growth stimulation of apple trees in unsterilized soil field conditions with VAM mycorrhizal inoculation. Can. J. Bot. 59 : 2003-2008.

(8) Reinhardt, D.R. and R.M. Miller. 1990. Size classes of root diameter and mycorrhizal colonization in two temperate grassland communities. New Phytol. 116 : 129-136.

(9) Sinkai, K., M. Sakakibara and M. Fukuya. 1982. Effects of watering and organic fertilizer application on the growth of loquat trees growing in shale soil. Res. Bull. Aichi Agric. Res. Cent. 14 : 267-270.

(10) Smith, S.E. and V. Gianninazzi-Pearson. 1998. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Ann. Rev. Plant Physiol. Plant Biol. 39 : 221-244.

(11) Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel and D.A. Zuberer. 1998. Principles and applications of soil microbiology. pp. 314-432. Prentice Hall Inc. Upper Saddle River, NJ.

菌根菌接種の有無とリンの施用量違いがビワ実生のリン吸収に及ぼす栄養

キプコリオニ ラバン ルット・水 谷 房 雄
浅 野 裕 城・門 屋 一 臣[1)]

摘　　要

ＡＭ菌根菌の接種の有無がビワ苗のリン吸収に及ぼす影響について調べた。試験区として菌根菌を接種した区としなかった区、リン酸の施用量は４段階とした。菌根菌の接種の有無に関わらず、リンの施用量が増えると苗の生体重は増加した。リンを施用した区に比べて、施用しなかった区はリンの含量が低かった。また、施用しなかった区においては、菌根菌を接種した区では、接種しなかった区よりもリンの含量が高かった。菌根菌を接種した区では菌根菌の感染が見られたが、その感染率は30％以下であった。また、リンを施用した区に比べて、しなかった区で菌根菌の感染率は高かった。

1）現在：愛媛大学農学部名誉教授