

The Bioassay of Vesicular Arbuscular Mycorrhizal Inoculums on Cassava Plant in Greenhouse

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ABSTRACT

The bioassay was carried out in greenhouse with Pakchong and Yangtalard soil. The experimental design was CRD with 4 replications and the host plant was KU-50 cassava. Stalks were grown in plastic pots containing 2 l of sterilized soil, nonsterilized soil, nonsterilized soil with *Glomus* sp. T6 and nonsterilized soil with *Glomus* sp. D13, depending upon each treatment. The bioassay was checked 2 times, 6 and 12 weeks. The results revealed that roots of cassavas were proliferately colonized by 2 isolates of *Glomus* sp. and the amount of root infections were higher than those of indigenous species from nonsterilized soil. The root infection of *Glomus* sp. T6 was higher than that of *Glomus* sp. D13 in both soils. P concentrations in the leaves of most VAM inoculated plants at both soils were higher than those of noninoculated plants. Height of plant in Yangtalard soil was higher than that in Pakchong soil. Growth of almost VAM inoculated plants were lower than plants grown in sterilized soil at 6 weeks, while the positive effect of VAM on growth of tested plants appeared at 12 weeks.

Key words: bioassay, vesicular arbuscular mycorrhizal fungi, cassava

INTRODUCTION

Vesicular arbuscular mycorrhizae (VAM) are ubiquitous and it is difficult to locate natural soil which is free of them. Therefore, introduction of nonindigenous VAM to be established in new environment seems to be difficult. However, many researchers suggested that, a large amounts of introduced species of VAM acquired the succession of establishment in the new soil and new plant (Jakobsen, 1994; Smith and Giovanetti-Pearson, 1988). Although the overall results of plant-VAM interactions are usually measured in term of plant growth, determining the VAM colonization and efficacy of introduced species on a specific plant in the controlled condition before utilization is

necessary (Dodd and Thomson, 1994). Many researchers concluded that root colonization of VAM, P concentration and growth of the host plant are postulated to be the important devices for determination of VAM efficacy (Smith and Giovanetti-Pearson, 1988).

Although there are different VAM species in the soil, their colonizations or infections on the host root are initiated from hyphal growing from soil-borne propagules or neighboring infected roots. Harley and Smith (1983) suggested that the first visible sign of colonization being the formation of appressoria on the root surface. Then, the infection units are developed within the root cortex. Hyphae grow longitudinally between the cells and intracellular development of arbuscules appears.

Vesicles, containing large amounts of lipid, are formed later in the maturation of an infection unit. At the same time, extraradical hyphae grow out into the soil.

This experiment was aimed to determine the viabilities of 2 VAM inoculums, *Glomus* sp. T6 and *Glomus* sp. D13 in Pakchong and Yangtalar soil, and to compare growth enhancement of VAM on cassava in greenhouse.

MATERIALS AND METHODS

CRD with 4 replications were carried out in greenhouse at Department of Soil Science, Faculty of Agriculture, Kasetsart University at Kamphaengsaen campus. Two soils from Kanchanaburi province, Yangtalar soil from Thamuang district and Pakchong soil from Saiyok district, were selected for plant growth medium. Each soil was prepared in 4 treatments, sterilized soil (SS), nonsterilized soil (NS), nonsterilized soil with *Glomus* sp. T6 (T6) and nonsterilized soil with *Glomus* sp. D13 (D13).

The soil in every pot was incorporated with 2 g N of NH_4NO_3 , 1 g K of K_2SO_4 , 300 mg Mg of MgSO_4 and 300 mg of rock phosphate. The plastic pots were used for experiment. All pots were surface sterilized by 70 % ethyl alcohol. In the pot, soil had 3 layers after preparation. The 1,400 ml of soil with the above treatments was filled to the bottom of the pot as the lowest layer. The mixture of 200 ml of VAM inoculum (in form of infected expanded clay) and 200 ml of nonsterilized soil was prepared and poured into the pot as inoculum or middle layer. Then, 200 ml of nonsterilized soil was placed as top layer. The soil was pressed firmly by hand. In other treatments, sterilized soil (treated in hot air oven at 180 degree Celcius for 3 hours) and also nonsterilized soil, 2 l of soil was placed directly to the surface-sterilized pot.

The healthy stalks of KU-50 cassava were

cut into 6 inches and grown vertically in the middle of the pot. The bottom site of each stalk must be buried in the inoculum layer. Distilled water was applied during growth of cassava. The plants were harvested at 6 and 12 weeks. After harvested, VAM root infection percentage, leaf P concentration, plant height, shoot dry weight and root dry weight were determined. Four young fully expanded leaves of cassava were collected for P determination. P of the plants were determined by Vanado-molybdate method described by Tassanee *et al.* (1989) and the VA mycorrhizal root infection percentages were evaluated by Gridline Intersect Method (Giovanetti and Mosse, 1980).

The variance of every parameter was analyzed. Then, the relevants average means were compared by Duncan's new multiple range test at 95 % confidence.

RESULTS

VA mycorrhizal root infection

All plants in bioassay appeared in common health of plant growth condition. The pathogenic effect from the inoculum did not occur during growth period.

There was no sign of VAM root infection in all plants grown in sterilized soil 6 and 12 weeks after planting. However, The percentages of VAM root infection among treatments of both soils and both periods of plant growth were highly significant. The quantities of root infection percentage of VAM fungi on bioassay plants are illustrated in Figure 1.

In Pakchong soil, maximim percentage of 14.65 % of VAM root infection at 6 weeks, appeared when inoculated with *Glomus* sp. D13 inoculum. *Glomus* sp. T6 was the second with 12.45 % of root infection on bioassay plants, that of indigenuos VAM in nonsterilized soil was 8.68 %. At 12 weeks, the percentage of VAM root infection were higher than those at 6 weeks, and the trend of the

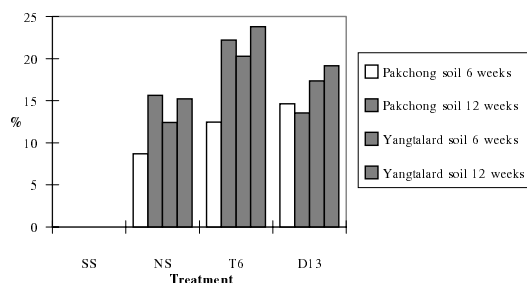


Figure 1 VA mycorrhizal root infection of host plant as inoculated with VAM fungi in Pakchong and Yangtaland soil.

abilities of 2 inoculums changed. The maximum root infection of 22.20 % was found in plant inoculated with *Glomus* sp. T6 which increased 9.75 % from that in 6 weeks. Meanwhile the percentages of root infection caused by indigenous VAM (15.65 %) also increased 6.97 %, where as that of plant inoculated with *Glomus* sp. D13 (13.53 %) in this period did not increase.

In Yangtaland soil, the abilities of *Glomus* sp. T6 and *Glomus* sp. D13 in root infection of bioassay plants were better than that of indigenous VAM fungi in nonsterilized soil. The maximum value of root infection, 20.28 %, was found in the host plant inoculated with *Glomus* sp. T6. Meanwhile the root infection of *Glomus* sp. D13 and indigenous VAM fungi were 17.35 and 12.43 % respectively. Consideration of the ability of VAM fungi at 12 weeks in Yangtaland soil, the sequency of root infection abilities on host plant of various VAM fungi were similar to those in 6 weeks. The maximum quantity of root infection of 23.80 % inoculated with *Glomus* sp. T6 inoculum was found. The root infection of *Glomus* sp. D13 and indigenous species were lower in the order of 19.15 and 15.23 % respectively.

Leaf P concentration

The quantities of leaf P concentrations of host plants are illustrated in Figure 2. The result

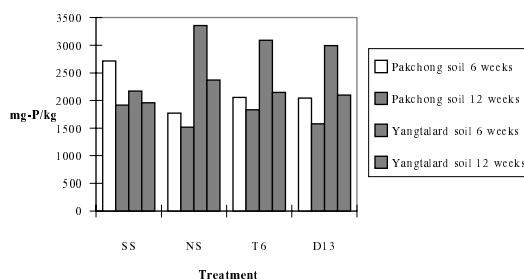


Figure 2 Leaf P concentration of bioassay plant as inoculated with VAM inoculum in Pakchong and Yangtaland soil.

revealed that, the concentration of phosphorus of plant at 6 weeks in Pakchong soil was significant, while that in Yangtaland soil was highly significant. Still, at 12 weeks, those values were nonsignificant in both 2 soils.

In Pakchong soil, phosphorus in the leaves of plant grown in sterilized soil was the highest concentration of 2,713.5 mg P/kg, at 6 weeks. The minimum value of phosphorus concentration, 1,768.3 mg P/kg, was found in plant grown in nonsterilized soil. Meanwhile phosphorus concentrations in the leaves of plants inoculated with *Glomus* sp. T6 and *Glomus* sp. D13 inoculums were in the vicinity. Their concentrations decreased at 12 weeks and all values were lower than 2,000 mg P/kg. However, that value in the plant grown in sterilized soil, 1,916.0 mg P/kg, was higher than the others. And, that of plant grown in nonsterilized soil still appeared in a minimum quantity of 1,514.5 mg P/kg compared to the other.

Concentrations of phosphorus in the leaves of plant in Yangtaland soil at 6 weeks grown in nonsterilized soil, inoculated with *Glomus* sp. T6 and *Glomus* sp. D13 inoculums were in the vicinity level of 3,358.8, 3,090.0 and 2,994.8 mg P/kg respectively. All these quantities were higher than those in sterilized soil (2,170.0 mg P/kg). This trend was clearly distinguished from that in Pakchong soil. Similarly, all concentrations of

phosphorus in the leaves of plants at 12 weeks decreased compared to those in 6 weeks. Though phosphorus concentrations in the leaves of plants among treatments were nonsignificant, its minimum quantity in this period was also found in the plant grown in sterilized soil (1,958.5 mg P/kg). Meanwhile their values in nonsterilized soil, *Glomus* sp. T6 and *Glomus* sp. D13 were 2,370.0, 2,148.3 and 2,095.8 mg P/kg, respectively.

Height of host plant

The enhancement of various VAM inoculums on height of plants at 6 weeks in Pakchong and Yangtalar soil were nonsignificant. While the plant height at 12 weeks in Pakchong soil was nonsignificant, that of plants in Yangtalar soil was significant (Figure 3).

In the bioassay at 6 weeks, all plants in Yangtalar soil were taller than those in Pakchong soil. The maximum height at this period was 18.35 and 37.50 cm in Pakchong and Yangtalar soil inoculated with *Glomus* sp. T6 inoculum respectively. At 12 weeks, height of host plant in Pakchong inoculated with *Glomus* sp. T6 and *Glomus* sp. D13 inoculum (36.55 and 36.98 cm) and also that of the plant grown in nonsterilized soil (36.58 cm) were in the same level, but higher than that of host plant which grew in sterilized soil

(33.30 cm). On the other hand, the host plant inoculated with *Glomus* sp. T6 and *Glomus* sp. D13 at 12 weeks in Yangtalar soil were shorter than both plants grown in sterilized and nonsterilized soils. The height of plant grown in sterilized and nonsterilized soils were 44.15 and 39.43 cm. However, at 6 weeks of this experiment, all plants in Yangtalar soil were taller than those plants in Pakchong soil.

Shoot dry weight

Growth enhancement of VAM inoculums in terms of shoot dry weight are illustrated in Figure 4. In Pakchong soil, shoot dry weight of host plant at both periods were nonsignificant. Although shoot dry weight of plant in Yangtalar soil at 6 weeks was nonsignificant, significance appeared at 12 weeks.

In the early stage of plant growth, in Pakchong soil, shoot dry weight of plant in sterilized soil was at the highest of 3.06 g/plant compared to the others. The plant inoculated with *Glomus* sp. T6 had less shoot dry weight of 1.92 g/plant. Later in 12 weeks, shoot dry weight of plants in all treatments were in the same level from 5.48 g/plant in nonsterilized soil to 5.93 g/plant in the plant inoculated with *Glomus* sp. D13

At 6 weeks in Yangtalar soil, the plant

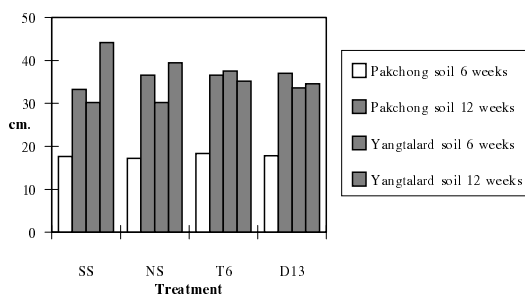


Figure 3 Height of host plant as inoculated by VAM inoculums in Pakchong and Yangtalar soil.

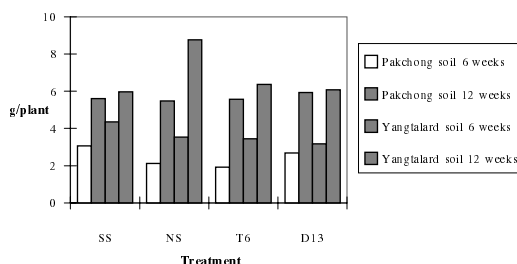


Figure 4 Shoot dry weight of host plant as inoculated with VAM inoculums in Pakchong and Yangtalar soil.

grown in sterilized soil showed maximum shoot dry weight of 4.35 g/plant, while that of plant inoculated with *Glomus* sp. D13 was at the minimum of 3.18 g/plant. Six weeks later, shoot dry weight of plant stimulated by indigenous VAM was 8.76 g/plant which was higher than those of the plants inoculated with *Glomus* sp. T6 and *Glomus* sp. D13 (6.36 and 6.07 g/plant). The shoot dry weight of plant in sterilized soil, 5.96 g/plant, was lower than the above quantities.

Root dry weight

The effects of VAM inoculums on growth of plant root were presented as root dry weight. The results are illustrated in Figure 5. Root dry weight of host plant in Pakchong soil at both periods and in Yangtalar soil at 6 weeks were nonsignificant. But, its quantity in Yangtalar soil at 12 weeks was significant.

The host plant inoculated with *Glomus* sp. D13 inoculum in Pakchong soil at 6 weeks had maximum root dry weight (1.46 g/plant) while that of the plant in sterilized soil had 0.71 g/plant which was the lowest among treatments. At 12 weeks, root growth of bioassay plant of all treatments were in the vicinity levels of 2.00, 1.97, 1.93 and 1.88 g/plant of plants inoculated with *Glomus* sp. T6, *Glomus* sp. D13, grown in nonsterilized and sterilized soils respectively. All root growth

quantities at 12 weeks were higher than those in 6 weeks.

On the other hand, the trend of root growth of plant in Yangtalar soil at 6 weeks appeared in opposite to that of plant in Pakchong soil. Plants grown in sterilized soil had 1.73 g/plant of root dry weight which were higher than the others. This result indicated that inoculation with VAM had no positive effect on root growth. However, the root of VAM inoculated plant at 12 weeks developed slowly and their root dry weight were 2.36 and 2.00 g/plant of plants inoculated with *Glomus* sp. T6 and *Glomus* sp. D13 inoculums. While the root in sterilized and nonsterilized soils developed quickly, root dry weight in those treatments were 3.05 and 2.61 g/plant which were higher than those quantities in VAM inoculated plants.

DISCUSSIONS

All parameters studied in this experiment such as root infection percentages, leaf P concentration, plant height, shoot dry weight and root dry weight of host plants in Yangtalar soil were interestingly higher than those in Pakchong soil at both periods of determination. The results indicated that Yangtalar soil had more suitable properties for bioassay than the other. The reasons may be due to the difference in its property. Texture of Yangtalar soil is loamy sand, while that of Pakchong soil is clay; therefore the aeration of the first soil is better than the other, and root plant can grow and penetrate into the soil easily. In addition, the available phosphate of Yangtalar soil was 16.113 mg P/kg which was higher than that of Pakchong soil (4.753 mg P/kg). Thus, difference in both soil properties may be the important reason to enhance more plant growth in Yangtalar soil than Pakchong soil.

VAM root infection on bioassay plant indicated the difference among their abilities of

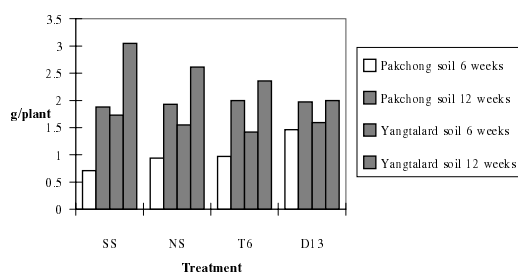


Figure 5 Root dry weight of host plant as inoculated with VAM inoculums in Pakchong and Yangtalar soil.

Glomus sp. T6, *Glomus* sp. D13 and indigenous VAM species in the soil on infection to the cassava root. *Glomus* sp. D13 showed very fast ability on plant root infection in early stage of plant growth, while *Glomus* sp. T6 showed rather slow level of root infection at the same time. This manner coincided with the suggestion of Dodd and Thompson (1994) that different VAM species had different abilities on root infection in some plants. Moreover, the lag phase of VAM infection process of different VAM species consumed different quantities of times (Sutton, 1973; Miranda and Harris, 1994). In this case, the lag phase of *Glomus* sp. T6 on root infection consumed more times than that of *Glomus* sp. D13. Therefore, root infection of plant inoculated with *Glomus* sp. T6 was low at 6 weeks in Pakchong soil. However, the rate of root infection at 12 weeks increased highly due to the high ability of *Glomus* sp. T6 on cassava root infection after a certain time.

Leaf P concentration of plant in Pakchong soil at both 6 and 12 weeks grown in sterilized soil were higher than those of indigenous VAM in natural soil and of *Glomus* sp. T6 and *Glomus* sp. D13. Similarly, Mala (1995) concluded that P concentration of maize without VAM inoculation particularly in early stage of plant growth was higher than that in the VAM inoculated plant. The reason may be due to the parasitic effect of VAM in the lag phase of root infection. Fortunately, the beneficial effect appeared later when the special structures of VAM infected and a certain amount of extraradical hyphae developed and extended to the nearby soil in the log phase (Sutton, 1973). However, the abilities of *Glomus* sp. T6 and *Glomus* sp. D13 inoculums on accumulation of P in the leaves of cassava were higher than that of indigenous VAM.

Generally, yield and growth of plants enhanced by VAM fungi were higher than those in noninoculated plants (Ortas *et al.*, 1996). However, growth of host plant in this experiment particularly

in terms of height, shoot dry weight and root dry weight grown in sterilized soil without inoculation were higher than those of VAM inoculated plants. The causing enhancement was not due to the effect of VAM inoculum, but may be caused by soil sterilization before growing the plant. This process partially dissolved plant nutrients from their components in microorganisms, organic materials and others in soil. Thus, available nutrients were released into the soil where plants can take up more easily. This phenomenon in some plants was reported (Heijne *et al.*, 1996) that, dry matter of noninoculated plant was higher than VAM inoculated plant. In Pakchong soil, particularly, containing 1.16 % of organic matter, the certain amount of readily soluble form of nutrient may be released from organic matter during soil sterilization. Thus plant took up nutrient easily and grew quickly. Moreover, Heijne *et al.* (1996) emphasized that soil sterilization may affect plant growth stimulation. He concluded that not only nitrogen content but also phosphorus content of noninoculated plant grown in sterilized soil were higher than those of inoculated plant. However, this effect decreased as plant age increased due to the depletion of nutrient by plant uptake. In this situation, there was no enhancing feature on solubilization and absorption on plant nutrient by VAM in sterilized soil. Thus, the available nutrient in soil decreased and plant had lower growth than the other treatments. In Yangtalar soil whose quantity of microorganism and organic matter were low, therefore, the amount of readily soluble plant nutrient from soil sterilization was lower and their effect on plant growth were also very little. Therefore, plant growth enhancement in term of shoot dry weight and root dry weight were in the vicinity levels but lower than VAM inoculated plants and then the stimulating on phosphorus uptake of inoculated plants was higher.

CONCLUSION

A large amount of VAM root colonization of *Glomus* sp. T6 and *Glomus* sp. D13 were found in this bioassay. The colonizations in both soil were higher than those of the indigenous VAM of the relevant soil. P concentrations in the leaves of most VAM inoculated plants in both soils were higher than those of noninoculated plants not only in sterilized soil but also in nonsterilized soil. Therefore, both isolates of *Glomus* sp. from this bioassay had enough viability and efficacy for starter preparation and inoculum production before application in the future.

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