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on

ROLE OF VA MYCORRHIZAE IN THE PHOSPHORUS NUTRITION OF ECONOMIC LEGUMINOUS CROPS IN THAILAND

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PROJECT PROFILE

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ABSTRACT

The VA mycorrhizal (VAM) spores were isolated from the soils of soybean and mungbean field in the North and Northeast of Thailand. Twelve selected isolates of VAM were used to inoculate soybean CV. S.J. 5 and mungbean CV. U-Thong 1 grown in a glasshouse. The objective was to compare their effectiveness on increasing grain yield and phosphorus absorption. Six of twelve isolates which showed highly effecttiveness were then further tested in paddy soils under field conditions at Chiang-Mai and Kalasin provinces in which the survival of VAM was also studied. Soybean/mungbean were inoculated with VAM and grown as the 1st crop during dry season and followed by rice CV. RD 23 during wet season as the 2nd crop. After rice had been harvested, soybean and mungbean were grown as the 3rd crop without reinoculation. The results showed that the number of VAM spores in soils increased by approximately 3-4 fold and 0.5 fold of indigenous number in soybean and mungbean field respectively. However, the spore number was decreased with condition which rice was grown as the second crop. Nevertheless, the number of spore was again automatically increased when the legumes were grown in the third crop. No root colonization was found in the rice roots, eventhough some spores were remained in the soils. This evidence suggested that VAM fungi could survive in soils under submerged conditions in form of sopre. *Glomus intraradices*, *Glomus mosseae*, and *Acaulospora scrobiculata # 22* seemed to be more superior to the other species on enhancing growth and yield through increasing phosphorus absorption, particularly on those legumes grown in the soil low in phosphorus content and low in indigenous VAM population.

The method of producing VAM inoculum was also studied and it was found that the number of spores in soil and infectivity were high when sand + clay, peat or coconut coir dust were used as the growth media. It was also observed that soybean, corn, or sorghum were the best host plants for producing inoculum followed by peanut and kudsu.

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1. INTRODUCTION

In Thailand only a few plants such as rice and corn are considered more important than the other grain legumes. Among the legumes, soybean and mungbean are the most valuable crops because they are used for producing a variety of consuming products such as vegetable, protein, oil and starch. At present, soybean and mungbean are already the major protein component in the staple diet of the rural population in Thailand. Improving yield of these grain legumes is the best quickest way to augment the production of food proteins. For increasing yield of these legumes a large amount of chemical fertilizer has to be used which created the big problem for farmers to afford the high expense for those fertilizers.

An advantage of legumes is that they perform symbiosis with rhizobia that give them being virtually self-sufficient for nitrogen Thai farmers could have rhizobium inoculum from Thai Department of Agriculture which produce the inoculum about 200 tons/year applying for 400,000 acres of legume cultivation field. A complementary symbiosis with VA mycorrhizae can increase greatly the ability of these crops to take up phosphorus in low phosphorus soils. Mycorrhizae fungi are species of fungi that intimately associate with plant roots forming a symbiotic relationship with the plant providing sugars for the fungi and the fungi providing nutrients, such as phosphorus, to the plant. Mycorrhizae fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue.

Under the undisturbed ecosystem, plant roots are normally mycorrhizal infected (Mosse et al.,1971) and this may be an important factor in the light nutrient cycle known to operate in this environment. Local rock phosphate which is relatively low solubility is low in cost. Thus improving the availability of rock phosphate through mycorrhizal activity is greatly benefit the farmers. Most Thai soils, the available P are usually low. To increase soybean and mungbean yields, heavy application of phosphorus is needed. The same effect can be achieved with VA mycorrhizal fungi.

This study was conducted to determine the extent to which the crops were mycorrhizae, the constraints to mycorrhizal performance and the opportunity for enhancing the benefits of mycorrhizae in field-grown soybean and mungbean. The studies were also included the mechanisms of infection and survival of mycorrhizal fungi in the flooded soil in Thailand which was still not known and was not brought to attention. From these studies, it was expected to show that mycorrhizal fungi might play an important role in tropical agriculture in solving phosphorus difficient problem or even the nitrogen.

2. OBJECTIVES

The overall objectives of this project were to improve the phosphorus nutrition of soybean *(Glycine max)*, and mungbean *(Vigna radiata)* in Thailand through assured symbiosis with phosphate absorbing mycorrhizal fungi. The study included:

A. Selection of the effective species of VA mycorrhizal fungi on enhancing the plant growth, yield and phosphorus absorption of soybean and mungbean.
B. Determine the survival of mycorrhizae during and following flooded condition of paddy soils.
C. Improving the method for producing the mycorrhizal inoculum for field trial.

3. REVIEW OF LITERATURE

3.1 Mycorrhizae

A mycorrhizae *("fungus-root")* is a type of endophytic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosystem. There are numerous reports in the literature on the incidence of this fungi on the root of plant including legumes (Suttons, 1973; Powell, 1977; Sparling and Tinker, 1975; Hayman et al., 1976; Rhodes and Gerdemann, 1978). There are three major groups of mycorrhizae, **ECTOMYCORRHIZAE, ECTENDOMYCORRHIZAE** and **ENDOMYCORRHIZAE.** Ectomycorrhizae and endomycorrhizae are important in agriculture and reforestation. (Harley, 1969; Smith, 1980). The ectomycorrhizae are generally associations of higher fungi (Basidiomycetes and Ascomycetes) typically involving the roots of woody perennials (Marks and Kozlowski, 1973). The fungi form intercellular ramifications of mycelium within the host cortex (the hartig net), and dense hyphal encapsulations of the fine roots (the sheath or fungal mantle). Ectomycorrhizae are the most important in forestry ecosystems.

Endomycorrhizae are characterized by fungal penetration of the host cells. There are two major groups (Harley, 1969; Smith, 1980) of septate and aseptate hyphae Endomycorrhizae. Septate hyphae occur in the Orchidaceae and the Ericaceae. The associated endophytes are higher fungi, aseptate hyphae Endomycorrhizae are the "phycomycetous" or vesicular-arbuscular (VA) mycorrhizae (Mosse, 1973; Gerdemann, 1975; Cooke, 1977). The fungi belonging to the family *Endogonaceae* of the order *Endogonales* and the class *Zygomycetes* (Trappe and Schenck, 1982). Four genera engage in known VA mycorrhizal interactions : *Acaulospora* Gerd. and Trappe, *Gigaspora* Gerd. and Trappe, *Glomus* Tul. and Tul., and *Sclerocystis.* Berk and Broome (Gerdemann and Trappe, 1944). One more genus, ***COMPLEXIPES*** had been reported by Smith (1982). Schenck and Smith (1982) divided VAM into two groups : *AZYGOSPORIC* genera including ***Gigaspora, Acaulospora, Entrophospora*** and *CHLAMYDOSPORIC* genera including ***Glomus, Sclerocystis,*** and ***Complexipes.*** Little knowledge of the life cycles of these organisms is known primarily according to non available of pure culturing method.

3.2 Distribution of VA Mycorrhizae.

VA mycorrhizae (VAM) were found indigenous in soils and remarkably widespread distributed geographically throughout the plant kingdom. VAM occur over a broad ecological range, from aquatic to desert environments (Mosse, 1981; Gianinazzi & Diem, 1982). Nopamornbodi et al. (1980) reported a mumber of plant species including field crop, vegetable crop and fruit crop associated with VAM. Bakshi (1974) reported VAM in plant family:– ***Araucariaceae, Aracuriaceae, Aceraceae, Acaceae, Altingiaceae, Bombaceae, Bignomaceae, Cupressaceae, Combretaceae, Leguminosae, Lauraceae, Magnoliaceae, Myrtaceae, Moraceae, Meliaceae, Papilinaceae, Proteaceae, Platanaceae, Taxodiaceae, Verbenaceae.*** Allen (1984) also stated that VA mycorrhiza may colonize stripmines and form the first stage of succession.

3.3 Extraction of VA mycorrhizal spores from soils.

There are several techniques to extract the VA mycorrhizal spores from soil. The pioneer was leaded by Gerdemann in 1955 on **"wet sieving and decanting technique"** to extract nematode cysts and larvae from soil. Later, many techniques were modified for

VA mycorrhizal spores extraction. Ohms (1957) using **"density gradient centrifugation"** method, seperated VAM spores by means of different concentration of sucrose. Gerdemann & Nicolson (1963) used **"applied wet sieving and decanting method"** to collect spores passing sieve number 420, 250, 100, 74 and 44 micrometer respectively. This method has been modified by Smith & Skipper (1979). Besides these techniques, **"differential sedimentation on gelatin column"** by Mosse & Jones, (1968); **"floatation-adhesion" technique** by Sutton & Barron, (1972); **"floatation-bubbling system"** with glycerol by Furlan & Fortin, (1975); **"discontinuous aqueous sucrose gradient"** by Mertz et al. (1979); and recently, Furlan, Bartschi & Fortin (1980) had **modified media for density gradient extracting technique.**

3.4 Counting of spore propagules in soils.

Dilution of soils are made from 10^{-1}, to 10^{-5} then drop the solution to the plant grown in vial. After 6 weeks, number of spores were calculated by Porter's table (Porter 1979; Powell, 1980). Smith & Skipper (1979) applied plate method by making the solution of soil and water by 1 : 9 ml and shaking thoroughly then drop 1 ml of soil solution on the filter paper in the petri dish. They streak the line from soil solution about 9 cm long and count spore propagules on the line.

3.5 Examine colonization of VA mycorrhizae in plant root.

To calculate the percent colonization of VAM in root, the examination of arbuscule or vesicle in the corticle cell has to be done, There are several methods to examine and calculate percent of root colonization. The general methods are as follow : the **Slide method** by Phillips & Hayman (1970) by cutting dyed root to 1 cm long about 50-100 pieces, count the number of **VAM** infected pieces then calculate the percentage of root colonization. **Visual method** by Hayman & Mosse (1971). **Yellow pigment forming** by Becker & Gerdemann (1977 b), by counted the yellow color root colonized by VAM but this method can be used only for some plant such as onion. **Colorimetric method** by Hepper (1977) by measure the light intensity of glucosamine from the fungi at 650 nm wavelength. **Gridline intersect method** by Giovanetti & Mosse (1980) by using Newman's formula (1966) to calculate the root length and percent root colonization.

3.6 Host plant for VA mycorrhizae.

The obligate symbiotic nature of VAM presently dictates that all VA mycorrhiza inoculum must be grown in roots of an appropriate host plant because VAM are inable to grow in pure culture. Mosse (1973) found that one VA mycorrhizal species can infect many host plant species, for example; *Endogone mosseae* from onion root can infect many other plants such as *Coprosma, Liquedambar, Fuchsia,* legumes, apple, tomato and many others.

VA mycorrhiza such as *Glomus mosseae*,*Glomus fasiculatum, Glomus etunicatum* isolated from citrus can infect many host plants e.g. sudan grass (*Sorghum valgare* Pers.) (Kleinschmidt & Gerdemann, 1972; Schenck & Tucker, 1974; Hattingh & Gerdemann, 1975; Menge et al., 1978 b; Nemec et al., 1981), *Zea mays.* L. (Maru et al., 1971), *Lycopersicon esculentum L., Glycine max, Phaseolus valgalis L., Coleus sp., Asparagus officinalis* L. (Menge & Johnson, 1978) and *Allium cepa* L. (Nemec et al., 1981).

Schenck & Kinloch (1980) found abundant spores of *Gigaspora magarita* in cotton, soybean, peanut, corn and sorghum but none of *G. magarita* was found in bahia grass

while the soil grown bahia grass will contain of *Glomus fasiculatum* and *Glomus clarum*. So VA mycorrhize will have a broad range of host plant.

3.7 Identification of VA mycorrhiza using immunofluorescence.

Most *Endogonaceae* have been described and are identified on the basis of the morphology of their sporocarps and/or spores (Gerdemann & Trappe, 1974; Hall, 1984). The exception is a few of the VA mycorrhiza species where characteristics of the morphology of the mycorrhizal infections can also be of value (Abbott, 1982). However, morphological features may be insufficient in distinguishing between closely related fungi species and a more specific identification method is required. Fluorescent antibody (FA) techniques have proved useful in identifying other soil fungi (Fitzell et al., 1980 a, b; Frankland et at., 1981). But because VA mycorrhiza are difficult to grow, they have not recieved the same attention. The FA technique should be useful in tracing the soil phase of VA mycorrhiza and spores.

3.8 Factors effecting the growth of VA mycorrhiza.

Daniels & Trappe (1980) studied the factors affecting spore germination of *Glomus epigaeus* then concluded that moisture, pH, temperature in soil and concentration of phosphate fertilizer will effect the germination of spores while species of host plant will not effect. Schenck *et al.* (1976) reported that temperature, light, pH and amount of nutrient will have an effect on spore germination in culture media.

Mellveen & Cole (1970) reported that in the soil applied with agricultural chemical such as captasol, carlondazin, flampropisapropol, dosanox and trifurafin will inhibit the spore germination of *Acaulospora laevis*, *Glomus caledonius* and *Glomus monosporus*. Daniels & Trappe (1980) applied N, P, K fertilizer in the form of NH_4NO_3, Ca $(H_2PO_4)_2$ and K_2SO_4 0, 25, 50, 100 and 200 ppm. respectively, after 1 week the spores of *Glomus epigaeus* have been applied, these factors do not have any effect on spore germination, but when P fertilizer was applied it increased some percents of spore germination while at 200 ppm the germination will be reduced.

Hayman (1970) found that in the agricultural land applied with N fertilizer will bare less formation of VA mycorrhizal spores than the bare land. He also found that fungicides will inhibit the growth of VA mycorrhiza in wheat and using methyl bromide 12,000 ppm about 400 lb/acre will kill VA mycorrhiza in 7 hrs.

Chlamydospore formation of *Glomus macrocarpum* will be decreased in the root when the plant root was infected by *Phytochuirium* and *Pythium* like fungus, and *Phytophthora* sp. also decrease root infection of VA mycorrhiza about 30-35% (Kruckelman, 1975). Sutton & Barron (1972) found the amount of VA mycorrhiza decrease with the depth of soil from the soil surface.

3.9 Effect of VA mycorrhiza on plant growth.

VA mycorrhiza plays a very important role onenhancing the plant growth and yield due to an increase supply of phosphorus to the plant. It has been shown that mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non-mycorrhizal (Mosse, 1981). VA mycorrhiza increase growth and yield of soybean about 30-40 percent when grown in sterile soil and mycorrhizal plant also accumulate phos-

phorus, potassium, calcium, copper, and manganese in the leaf at the higher amount than non-mycorrhizal plant (Ross & Harper, 1970). It was suggested that VA mycorrhiza may absorb phosphorus directly from fixed phosphate sources (Allen et al, 1981; Gianinazzi & Pearson, 1981). This phenomena depend very much on the host plant, VA mycorrhiza species and soil types (Krishna et al.,1985; Howeler et al.,1987).

Plant inoculated with VA mycorrhiza have been shown to be more resistant to some root diseases. *Glomus mosseae* or *Gigaspora margarita* can reduce the severity of onion root disease caused by *Pyrenochaeta terrestris* (Schenck & Kellam, 1978). *Glomus epunicatum* reduce the severity of soybean root disease cause by *Phytophthora megasperma* (Schenck, 1981). *Glomus mosseae* reduce the severity of *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* in soybean root. (Zambolim & Schenck, 1983; Zambolim *et al.*, 1983). *Glomus fasiculatum* reduce the severity of *Phytophthora parasitica* in citrus root (Davis & Menge, 1981).

3.10 Survival of VA mycorrhiza.

Daniels & Trappe (1980) studied the effect of water potential on spore germination by adding distilled water into the soil until moisture content was 8%, 17.4%, 26%, 34.8%, 43.5%, 52.5% and 69.6% the spore germinate well at field capacity or a little above field capacity. The spore germination will be reduced when water potential in soil lower than field capacity. There are some aquatic plants having quite a number of VA mycorrhiza (Sandergard & Laevard, 1977) In the rainy area or the area where the soils are always wet, including saline soil the amount of VA mycorrhizal spores will be low. (Mosse & Bowen, 1968; Khan, 1974 a). So no conclusion whether water potential having the effect on VAM spores germination

The amount of spores of VA mycorrhiza in soil will be varied by seasons. There are high number of VA mycorrhiza spores at the end of summer or autumn, then the amount of spores will be reduced in winter, spring and summer. Spore formation relates with the growth of host plant and climate (Sutton & Barron, 1972; Khan, 1974 b).

3.11 VA mycorrhiza inoculum production.

High concentration of N fertilizers can reduce mycorrhizal colonization in root (Hayman, 1982). The quality of VA mycorrhiza inoculum appears to be severely reduced under too wet or too dry condition. It is often better to under water mycorrhizal plants than over-water them (Mosse et al , 1981, Reed & Bowen, 1979).

Many VA Mycorrhiza will produce excellent inoculum over a wide pH range. *Glomus fasiculatum* prefer pH from 5.5 to 9.5 while *Glomus mosseae* and many other *Glomus* species prefer pH above 5.2 when *Acaulospora* species require pH below 5.0 (Lambert & Cole, 1980 ; Abbotts & Robson, 1977 ; Mosse, 1972)

High light intensity and long daylength improve mycorrhizal colonization or spore production in many plants due to increasing in photosynthesis which lead to increase carbohydrates concentration and exudation from root. (Graham et al., 1982; Ferguson, 1982). Temperature has a severe effect upon both host plant and VAM fungi growth. The soil temperature are more important in the VA mycorrhiza production than the air temperature. It is recommended that VA mycorrhiza inoculum should be produced at temperature slightly

above optimum for the host plant (Ferguson, 1981; Graham et al., 1982)

Pruning plant host reduce VA mycorrhizal infection and sporulation due to the consequent reduction in flow rate of photosynthate to root (Ferguson, 1981). It is desirable to produce VA mycorrhiza inoculum in large containers with deep not greater than 1 metre (Menge, 1984; Ferguson, 1987).

Many pesticides are detrimental to VA mycorrhizal infection and sporulation but some are quite compatible with VA mycorrhiza inoculum such as; DBCP 1-30, ethazole, captan, and metalaxyl (Menge, 1982). Most herbicides which damage host plant are also detrimental to VA mycorrhiza inoculum. However, some herbicides such as paraquat, simazine, atrazine and malic hydrazide have been found to increase VA infection due to increasing root exudation of amino acid or sugar (Menge 1982).

4. MATERIALS AND METHODS

The studies were concentrated on the following respectively.

I) Selection of the effective species of VA mycorrhizal fungi on enhancing the plant growth, yield and phosphorus absorption of soybean and mungbean.

II) Determine the survival of inoculated VAM fungi during and following flooded condition of paddy soils.

III) Producing mass inoculum of VA mycorrhizal fungi for field trial.

IV) Identification of VAM fungi by immunofluorescence

4.1 Investigation and inoculum preparation.

The VA mycorrhizae used in this study were collected from soybean and mungbean field. Surveys were made in several locations to observe the distribution of VA mycorrhizal fungi in legaminous fields and paddy fields in Thailand.

4.1.1 Sample collection.

Soil samples and plant root samples were sampling for sample collection from legume and rice field. Each field selected had been cultivated in cropping system of rice – legume – rice for many years. Thus VA mycorrhizal spores recovered from individual field soils are likely been produced by two kinds of cultivar, and have been colonized in soil for a number of years. These area were flooded once a year in rainy season

Soil and root plant samples were collected at the same time. Soils were sampling at the depth of 36 cm by digging the soil samples together with the root plant. Soils were collected from field in the Northern, Central, Northeastern and Eastern part of Thailand. Six composite samples were collected for represent soil sample of each site. The total samples were collected from 11 provinces with 54 sites and were stored individually in polyethylene bags at 2-5° C until the spores were extracted and identified.

4.1.2 Sample identification.

Soil and root samples were identified for VAM species and percent root colonization by the following methods :

4.1.2.1 Counting spore propagules in soils.

VA mycorrhizal spores were extracted from soil samples by wet sieving and decanting as described by Gerdemann (1963). Soil samples approximately 250 gm were suspended in 1 litre of water by gentle stirring. Heavier particles were allowed to settle for a few seconds and the liquid was decanted through a seive 250 micron to remove the large particles of organic matter and allow the desired spores to pass through. The suspension was passed again through sieve 100 micron and 63 micron. The spores and small amounts of debris remained on each sieve were transferred from the sieve to a shallow layer of water for examination under a stereoscopic microscope. The spores were picked out individually with a small tip capillary tube.

According to time consuming for yielding VA mycorrhizal spores by Gerdemann technique, the sucrose centrifugation method by Smith & Skipper (1979) was used. Soil suspension was screened through sieve 250 micron and 63 micron. The debris and spores remain on 63 micron sieve were poured into centrifuge tube containing water, centrifuged 4 min at 1,800 rpm. The upper solution was poured, the debris at the bottom was added with 4 % sucrose and centrifuged for 2 minute at 1,800 rpm. The upper solution was separated to examine under the stereoscopic microscope. This method could yield which more than thousand spores in one day.

The spores which were collected under the stereoscopic microscope were stored in Ringer's solution for further study. Spores from each sample were counted based upon 100 grams of soil. The propagule numbers for every samples were recorded.

4.1.2.2 Examination of vesicle and arbuscule in root tissue.

After the soil was washed from the plant roots, the feeder roots were removed from each plant. They were cleared and stained to determine the percent of root colonization by Phillips & Hayman method (1970). The roots were cleared in 10% KOH, boiled at 90° c about 15 minutes then stained in lactophenol trypan blue. Roots were cut in 1 cm long for 100 pieces randomly and placed on the slides for counting. Using a stereoscopic microscope (100 x) to estimate the percentage of root colonization by counted the pieces that obtained arbuscules or surface mycelia or clusters of echinulate vesicles.

4.1.2.3 Identification of VA mycorrhizal species.

Extracted spores were divided into groups of the same characteristics for identification. The species identified in this study followed the monograph of Gerdemann & Trappe (1974); Hall (1984); Trappe (1982) and Morton (1986). Five genera which were found in this study were: *Glomus* sp., *Acaulospora* sp., *Gigaspora* sp., *Scutellospora* sp. and *Entrophospora* sp. To determine a specie of a specimen of VA mycorrhizal fungi, a semipermanent slides were prepared. Semipermanent mountants, such as PVL (polyvinyl alcohol + lactophenol) or PVLG (polyvinyl alcohol + lactic acid + glycerol) were used. This mountants effected some certain spore characteristics, such as wall characteristics and spore color. The observations of spore color, spore diameter, attachment present, spore contents, auxilliary cell, sporocarp were recorded for species classification. The classified spores were multiplied in pot culture for further study.

4.1.3 Inoculum preparation.

Inoculum of each species were prepared by grown plant in steamed clay + sand in the ratio 1 : 1 in 12 inches clay pots. Soil was steamed three times on alternate days, each

time for 7 hr. at 82° c. *Sorghum vulgare* was used as host plant by growing first in paper cup for one week then transfered to grow in clay pot. Spores of VA mycorrhiza were applied at the bottom of the transplant hole. Each pots were fertilized with half strength of Hoagland's nutrient solution, and watering at alternate days. At about 80% flowering stage of host plant (about 3 months after germinated), root and soil samples were collected to examine the percent root colonization and number of spores produced. After harvesting, the infected root and soil were ready to use as inoculum for further studies.

4.2 Identification of VA mycorrhiza by using immunofluorescence.

The study of the soil phase of VA mycorrhiza was hampered by the difficulty of tracing and distinguishing their hyphae from those of the indigenouss fungi. Fluorescent antibody (FA) technique might be useful for examining the colonization of VA mycorrhiza in the root in the field experiment. The preparations for producing fugal antiserum were as follow :

4.2.1 Preparation of fungal antigen.

Mature spore of *Glomus mosseae* were obtained from the soil in pot cultures. The 80 mg of average weight of 7,000 fresh spores were washed in sterile distilled water, agitated, and centrifuged at 500 rpm for 2 minutes. This procedure was repeated 10 times with distilled water and with solution of gentamycin 200 ppm and streptomycin 200 ppm. Then the spores were extracted in 30 ml of NaCl 0.85% and ground in the tissue grinder. The spore solution was mixed with adjuvant at 1:1 ratio.

4.2.2 Immunization and determination of titre.

One milliliter of extracted wall suspension (30 mg/ml) was intravenously injected into the marginal vein of the rabbit's ear. The injections were repeated on the 1st, 2nd and 3rd day then the rabbits were rested for 3 days. On the 7th, 8th and 9th day the injection sequence was repeated as before. On the 18th and 26th day, samples of approximately 5 ml were withdrawn from the marginal vein of the ear to determine the titre. The injection sequence was repeated on the 18th day and the rabbit was bled on the 28th day. The blood was allowed to stand overnight to coagulate, after the red blood cells were spun down. The serum was collected and subsequently used for the antibody titre assay.

The antibody titre was determined by a modification of the agglutination technique. Mycelial wall preparations was described by Valajczuk et al. (1975). Chlamydospores suspended in PBS were ground with tissue grinder, then left for 2 hours to allow the larger fragments of cell walls to settle out. The remaining suspension was removed and used as an antigen in an agglutination test. The reaction with rabbit serum was carried out at 40° c in a coverd water bath for 12 h. The agglutination reaction was assessed by examination under a dissecting microscope. A positive reaction resulted in a diffuse cloud of agglutinated particles; a negative reaction was a clear suspension. Based on these results, all further serum collections were diluted with PBS (1:25) and stored in 10 ml aliquats at–10° c until needed.

4.2.3 Specificity tests with spores.

Chlamydospores of *Glomus mosseae* were collected as described above, but were not extracted. Spores of other VA mycorrhizal species were obtained by sieving and decanting from pot cultures. The samples preparation for examine were placed in a 25 mm

membrane filter apparatus (Millipore Inc.) equipped with a 0.45 μm nucleopore filter, then washed with 500 ml sterile PBS after which 0.5 ml rabbit serum (diluted 1:25 with PBS) was introduced. This combination was allowed to react in a covered water bath at 40° c for 1 h. Control fungal preparation with PBS but without rabbit serum or with non-immune rabbit serum were kept in the same chamber. The samples were then rinsed with 500 ml PBS, and 0.5 ml of 1:50 dilution of fluorescein-labelled goat anti rabbit serum (Antibodies Inc, Divis, CA) was added to all samples including the controls. They were then returned to the waterbath to react for 1 hour after which samples were rinsed with 500 ml of PBS, and stored in moist plastic seal bags in the dark at 4° c for examination. They could be kept up to 2 days for examination. All preparations were examined under incandescent and UV. illumination microscope.

4.3 Selection of effective species of VA mycorrhizal fungi.

To select the effective VA mycorrhiza species on enhancing plant growth, yield, phosphorus absorption, root colonization and VAM spores produced in the soil grown soybean and mungbean, the experiments were conducted in both glasshouse and field. Only soybean and mungbean were studied in this project. Field trials were established at three locations in Thailand. At Chiangmai, northern part of Thailand, soybean and peanut were grown. At Kalasin, northeastern part of Thailand, mungbean, were grown and at Prachinburi, middle part of Thailand, mungbean and soybean were grown. Chiangmai soil considered to be a fertile soil while Kalasin and Prachinburi soil are low in soil fertility. The Chiangmai soil is a clay loam texture with pH 5.5, organic matter 1.4%, Bray II extractable P 30 ppm. The soil at Kalasin is a loamy sand texture with pH 5.1, organic matter 1%, Bray II extractable P 8 ppm and the soil at Prachinburi is a clay texture with pH 4.8, organic matter 1.04%, Bray II extractable P 2 ppm. Cropping systems at Chiangmai were continuous soybean - rice rotation where as at Kalasin were mungbean-rice rotation and at Prachinburi were mungbean and soybean-rice rotation. Each crop was applied with a small amount of fertilizer.

4.3.1 Soil And plants preparation.

Plants used in this study were soybean *(Glycine max)* variety S.I.5 and mungbean *(Vigna radiata)* Variety U-Thong 1. Seeds were obtained from Field Crop Division, Department of Agriculture.

4.3.1.1 Glasshouse experiment .

Soil used in the glasshouse is loamy sand texture with pH 6.5, Bray II extractable P 7 ppm. Steam sterilized soil were filled in the 12 inch diameter clay pot. Twelve species of VA mycorrhiza selected from the pot culture after multiplied were used to study for their effectiveness. Forty spores of each VA mycorrhiza isolate were placed at the bottom of the plant hole about 3 cm below soil surface. Four seeds of each legume were placed above the spores and covered with soil. Five days after emergence, the plants were thinned down to only two plants per pot. All treatments were inoculatedwith peatbase rhizobium inoculum : *Bradyrhizobium japonicum* for soybean, and *Cowpea Rhizobia* for mungbean. One gram of rockphosphate (5% available P) was added to each pot.

4.3.1.2 Field experiment .

The field trial was carried out for soybean in Chiangmai, mungbean in Kalasin and soybean

and mungbean at Prachinburi. The field was kept free of vegetation by ploughing the soil 2 times with a disk harrow before planting. At planting time, 937.5 kg/ha of rock phosphate (5% P_2O_5) was applied only in fertilized plot while 18.75 kg N/ha of urea and 37.5 kg K_2O/ha of KCl were broadcasted and incorporated to the soil in every plot. Only 6 species of VA mycorrhiza which were selected in the glasshouse and showed a high efficiency in phosphorus nutrient absorbtion and yield increased for each cultivar were used for the field trial. Two hundreds spores of each VA mycorrhiza species from pot culture were applied in each plant hole at 1 inch below the legume seeds. Two plants were allowed in each hole. The plot size was 3 × 2 metres, plant spacing was 50 cm between rows and 25 cm between plants. The ditch was made around each split plot preparing for the further experiment on survival determination.

4.3.2 Experimental design and management.

4.3.2.1 Glasshouse experiment.

The plot experiments were conducted in Randomized Complete Block (RCB) design with 6 replications and 13 treatments for each cultivar, soybean S.J.5, mungbean U-Thong 1 as followed : -

Treatment 1, inoculated with *Glomus etunicatum* # 31 (ETU 31)
Treatment 2, inoculated with *Glomus deserticola* # 39 (DES 39)
Treatment 3, inoculated with *Gigaspora margarita* # 40 (MAR 40)
Treatment 4, inoculated with *Gigaspora gregaria* # 33 (GRE 33)
Treatmetn 5, inoculated with *Entrophospora* # 8 (ENTRO 8)
Treatment 6, inoculated with *Gigaspora gregaria* # 57 (GRE 57)
Treatment 7, inoculated with *Gigaspora* sp. # 56 (GI 56)
Treatment 8, inoculated with *Acaulospora scrobiculata # 22 (ASCR22)*
Treatment 9, inoculated with *Glomus mosseae* # 6 (MOS 6)
Treatment 10, inoculated with *Glomus intraradices* # 26 (INT 26)
Treatment 11, inoculated with *Glomus mosseae* (MOS)
Treatment 12, inoculated with *Glomus* sp. # 17 (GLO 17)
Treatment 13, non inoculated (control)

4.3.2.2 Field experiment.

Field experiment was conducted in split plot design with 6 replications and 7 treatments for every locations. The main plot was no fertilizer and fertilized with rock phosphate (5% P_2O_5) 937.5 kg/ha. Sub plot included non inoculation and inoculation with each species of the following VA mycorrhiza :

Treatment 1, inoculated with *Glomus mosseae* # 156 # 6 (MOS 156)
Treatment 2, inoculated with *(Glomus sp.* # 17 (GLO 17)
Treatment 3,inoculatedwith *Glomus intraradices* # (INT 26)
Treatment 4,inoculated with *Acaulospora scrobiculata* # 22 (ASCR22)
Treatment 5, inoculated with *Glomus etunicatum* # 31 (ETU 31)
Treatment 6, inoculated with *Glomus deserticola* # 39 (DES 39)
Treatment 7, non inoculated (control)

Pesticide, enthazole was applied at 30 and 60 days after inoculation. The weeds were controlled and eliminated by hand. In the glasshouse, water was showered everyday. In the field, water was applied into the ditch along the plant rows until harvested.

4.3.3 Data collection

Plant growth included height and dry matter of shoot, grain yield, percent root colonization and number of spores in soil were recorded every 30 days. Two replications were used for examining the plant growth, percent root colonization and spores produced in soil and the rest 4 replications were kept for the harvesting time. Nutrient content in shoot especially P content were analyzed at harvesting time.

The data were statistically analyzed by using ANOV and Duncan's multiple range test to compare the ability of VA mycorrhiza on phosphorus nutrient absorption ability and yield increased.

4.4 Survival of VA mycorrhiza in the 2nd crop in paddy field.

To study the survival of VA mycorrhiza under flooding condition, the experiment was done succeeding after the selection of effective VA mycorrhizal species for enhancing soybean and mungbean growth, which was conducted in the same plot by growing rice.

4.4.1 Soil and plants preparation.

Rice variety RD 7 was used for this study. Rice seeds were obtained from Rice Division, Department of Agriculture.

4.4.1.1 Glasshouse experiment.

To detect the exact number of spores survival in flooding condition with and without host plant, the host plant used was rice RD 7. Steam sterilized soil from paddy field was filled in the 18 inches diameter pot, and 400 spores of each VA mycorrhiza species were inoculated into the soil per pot then flooded the soil with water.

4.4.1.2 Field experiment.

Rice variety RD 7 was grown in spacing of 25 × 25 cm in the same plot of the first experiment. At planting time, 625 kg/ha of rock phosphate, 18.75 kg N/ha of urea and 37.5 kg N/ha of KCl were broadcasted and incorporated in the soil for every plot. Ditches were made around each split plot and flooded the plot with water, 15–20 cm high in level. Rice seedlings at 14 days were transplanted to grow in the prepared plot without VAM inoculation.

4.4.2 Experimental design and management.

4.4.2.1 Glasshouse experiment.

The experiment was employed in split plot design with 6 replications, main plot was growing rice and non growing rice, sub plot included 7 treatments of VA mycorrhizal species as follows :

Treatment 1, inoculated with *Glomus mosseae* # 156 # 6 (MOS 156)
Treatment 2, inoculated with *Glomus sp.* # 17 (GLO 17)
Treatment 3, inoculated with *Glomus intraradices* # (INT 26)
Treatment 4, inoculated with *Acaulospora scrobiculata* # 22 (ASCR22)
Treatment 5, inoculated with *Glomus etunicatum* # 31 (ETU 31)
Treatment 6, inoculated with *Glomus deserticola* # 39 (DES 39)
Treatment 7, non inoculated (control)

4.4.2.2 Field experiment.

After harvesting soybean and mungbean from first experiment, the root of those plants were left and were ploughed and incorporated into the soil. Each plot was seperated by the ditch made from the first trial. The same experimental design, split plot was employed in this experiment. The main plot were no fertilizer and fertilized with rock phosphate, subplots was flooded and non flooded plot. No VA mycorrhizal species were inoculated at this time.

4.4.3 Data collection.

Plant growth included height and dry matter of shoot, grain yield, percent root colonization and number of spores in soil were recorded after 60 days of transplanting rice. Phosphorus contents in the rice shoots were analyzed at harvesting time.

The data were statistically analyzed by using ANOV and Duncan's multiple range test to compare the effective on survival of each VA mycorrhizal species.

4.5 Survival of VA mycorrhiza in the 3rd crop of soybean and mungbean field following rice.

Soybean and mungbean, the same variety as in Materials & Methods 4.3, were used. These crops were planted following rice in the same plot.

4.5.1 Soil and plants preparation.

Soil preparation was the same as in Material and Method 4.3.1. In this field, instead of applied urea as N–fertilizer, rhizobium inoculant had been applied. The other treatments were the same as the 1st crop.

4.5.2 Experimental design and management.

Same design and management as in Material & Method 4.3.1 was employed.

4.5.3 Data collection.

Plant growth included height and dry matter of shoot, grain yield, percent root colonization and number of spores in soil were recorded every 30 days. Nutrient contents were analyzed at harvesting time. The data were statistically analyzed by using ANOV and Duncan's multiple range test to compare the ability of VAM on phosphorus absorption ability and yield increased.

4.6 VA mycorrhiza inoculum production.

Since VA mycorrhiza can not produce in the pure culture, pot culture technique by Menge had to be used for inoculum production. Spores density, appropriate media and suitable host plant were studied for mass production.

4.6.1 VA mycorrhizal spores density for plant growth.

Peanut Tainan 9 was used as a host plant grown in clay pot contained 10 kg sandy soil low in phosphorus content. The experiment was employed in Randomized Complete Block design with 4 replications and 7 treatments comprising various numbers of *Glomus mosseae* spores from 0, 10, 20, 30, 40, 50 and 100. Peanut seeds were surface steriled by 10% clorox before planting and using 3-4 seeds per one pot. Rock phosphate with 5% avialable P was applied 1 gm/pot and mixed thoroughly with the soil before planting. Spores were placed at the bottom of the hole under the peanut seeds then covered with soil. Seven

days after germination the plants were thinned to two plants per pot. Water was showered everyday and pesticide, Azodrin, was applied at 30 and 60 days after planting.

The plants were harvested at 100 days, dry matter for shoot, root and pod were recorded. Percent root colonization and number of spores produced in the soil were also recorded. All data were statistically analysed by using ANOV and Duncan's multiple range test.

4.6.2 Selection of appropriate media.

Appropriate media were selected from 7 plant growth media; sand, clay, peat, loamy sand, rice husks, coconut coir dust, vermiculite and two combination of sand + peat and sand + clay in ratio 1 : 1 by volume. The experiment was designed in Randomized Complete Block with 4 replications and 9 treatments of different media mentioned above. These media were steam sterilized 3 times successively and filled in the 10 inch clay pot about 3/4 of pot volume. Peanut Tainan 9 was used as the host plant and seed was surface sterilized by 10% clorox before using. Four to five peanut seeds were planted in each pot inoculated with 100 spores of *Glomus fasiculatum* by applied under the seed. At seven days, plants were thinned to two plants for each pot. Water was given everyday until harvested. Once a week, every pot was applied with the standard Hoagland's solution (1/2 strength) minus phosphorus.

Height, dry matter of shoot, percent root colonization and number of sporulation in soil were recorded at 30 and 60 days. All data were statistically analyzed by using ANOV and Duncan's multiple range test.

4.6.3 Selection of suitable host plant.

A suitable host plant was selected from 10 species of cultivar; onion, sorghum, corn, soybean, mungbean, peanut, sabi, bahia grass, kudzu and cabbage (which was a nonmycorrhizal plant) was included. The experiment was designed in Randomized Complete Block with 6 replications and 10 treatments of different host plant mentioned above. Each surface sterilized plant was grown in 12 inch clay pot contained 10 kgs of sterile sand + clay in the ratio 1 : 1 by volume. Plants in one pot were inoculated with 100 spores of *Glomus fasiculatum* by placing the spores beneath the seeds. Once a week, every pot was applied with the standard Hoagland's solution (1/2 strength) minus phosphorus.

At 60, 90 and 120 days, root dry weight, percent root colonization and number of spores in soil were recorded. The data were analyzed for statistical comparison of spore production by ANOV and Duncan's multiple range test.

5. RESULTS AND DISCUSSION

5.1 Selection of the effective species of VA mycorrhizal fungi on enhancing plant growth, yield and phosphorus absorption of soybean and mungbean.

To select the effect VA mycorrhizal species, spore samples were collected, multiplied and identified both via morphology of the fungi and immunofluorescent antibody (FA). The identified species were then chosen basing on their effectiveness on phosphorus absorption and enhancing growth of soybean and mungbean.

5.1.1 Description of collected VA mycorrhiza.

Soil and root samples were collected from several sites of soybean and mungbean field from May 1983 to April 1984. (Table 1). At each site, five soil samples were randomly collected for composite sample at 0–36 cm depths. Extracted spores and sporocarps were stored in Ringer's solution and were mounted in lactophenol on glass slides for identification. Number of VA mycorrhizal spores in soil at various location varied from 0 to over 27,200 spores per 100 gm of soil. Soil at Khonkaen (Utisols, pH 6.20–6.60) had least amount of VAMF compared to the other soils at another province. The highest VAMF spores were found at Saraburi (Vertisol, pH 6.5) where most soils contained low available P, less than 42 ppm available phosphorus. VAMF representing of 5 genera of *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and Sclerocystis were observed. Species of *Glomus*, *Gigaspora* and *Acaulospora* were the most frequent species observed. Identified species were included *Acaulospora scrobiculata* Trappe, *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus microcarpum* Tul. & Tul. *Glomus multicaulis* Gerd. & Bakshi, *Glomus melanosporum* Gerd. & Trappe, *Glomus intraradices*, *Glomus deserticola* Trappe, Bloss & Menge *Glomus fasiculatum* (Thaxter & Gerd.) Gerd. & Trappe *Glomus macrocarpum* Tul. & Tul., *Gigaspora magarita* Becker & Hall, *Sclerocystis coremioides* Berk. & Broome *Sclerocystis clavispora* Trappe, *Sclerocystis sinuosa*, *Scutellospora gregaria* (Schenck & Nicol.) Walker & Sanders, comb. nov., *Scutellospora heterogama* (Nicol. & Gerd.). Walker & Sanders.

Table 1 Soil characteristics of samples from paddy field survey with number of VA mycorrhizal spores from different locations.

Locations	Soil order	Soil texture	Soil pH	Available P by Bray II (ppm)	No.of spores per 100 g soil	VAM species Founded
Ayudthaya 1	Inceptisols	Clay	6.30	20	246	*Gigaspora sp., Scutellospora sp.*
Ayudthaya 2	Inceptisols	Clay	6.05	20	389	*Acaulospora sp., Glomus sp.*
Chiangmai 1	Alfisols	Sandy Clay loam	6.20	36	190	*Glomus sp., Acaulospora sp.*
Chiangmai 2	Alfisols	"	6.57	39	160	*Gigaspora sp.*
Kabinburi 1	Ustults	"	7.30	15	116	*Gigaspora sp., Acaulospora sp.*
Kabinburi 2	"	"	6.80	9	63	*Glomus sp. Sclerocystis sp.*
Kabinburi 3	"	"	6.72	14	79	
Kabinburi 4	"	"	7.50	13	132	
Kabinburi 5	"	"	6.90	14	263	
Kalasin 1	Ustults/	Loamy sand	4.60	13	340	*Gigaspora sp., Glomus sp.*
Kalasin 2	Aquults	"	4.99	13	104	*Acaulospora sp.*
Kalasin 3	"	"	4.88	17	54	
Kalasin 4	"	"	5.70	15	120	
Kalasin 5	"	"	6.00	12	174	
Kalasin 6	"	"	6.20	16	363	
Khonkaen 1	Utisols	Loamy sand	6.30	11	27 (dead)	*Glomus sp,*
Khonkaen 2	"	"	6.20	12	18 "	*Acaulospora sp.,*
Khonkaen 3	"	"	6.60	7	21 "	*Gigaspora sp.*
Mahasarakarm 1	Utisols	Loamy sand	5.69	5	230	*Gigaspora sp.,*
Mahasarakarm 2	"	"	5.50	3	170 (dead)	*Scutellospora sp.,*
Mahasarakarm 3	"	"	5.60	16	260	*Acaulospora sp.*
Mahasarakarm 4	"	"	4.80	8	175	
Nakonrajsima 1	Inceptisols	Clay	6.30	13	145	*Glomus sp,*
Nakonrajsima 2	"	"	6.50	20	180	*Acaulospora sp.*
Pisanuloke 1	Alfisols		4.49	36	20	*Gigaspora sp.*

Besides the soybean and mungbean roots which collected at the same time of soil samples, the root of weeds growing in the same area were also collected and examined for the colonization of the VA mycorrhiza fungi. Thirteen species of weeds were found to be infected by VA mycorrhiza in their root system (Table 2). The highest colonization were found in the root of *Heliotropium indicum* Linn. about 15%. Root of *Echinochoa colonum* (Linn.) Link and *Eriochloa procera* (Retz.) C.E. Hubbard were equally infected by VAM. All the weeds listed in Table 2 were commonly found in the soybean and mungbean fields, therefore these plants might be the alternated host plants which helping VA mycorrhizal fungi to survival from one cropping to another cropping without re-inoculation.

Table 2 Percent root colonization found in the root weed grown in paddy field.

plant species	percent root colonization
Heliotropium indicum Linn.	15%
Echinochoa colonum (Linn) Link	12%
Eriochloa procera (Retz) C.E. Hubbard	12%
Mimosa pudica Linn.	10%
Scoparia duleis Linn.	9%
Chrozaphora rottleri	9%
Hedyotics diffusa Roxb.	7%
Phyllanthis virgatus Forst.f.	5%
Sporoblus virgineus Linn.	4%
Physalis minima Linn.	4%
Euphobia hirta Linn.	3%
Euphobia hyerricifolia Linn.	2%
Dichanthium caricosum Linn.	1%

All spores of the species collected were multiplied in pot culture using *Sorghum vulgare* as the host plant. Twelve species listed in Table 3 were selected for use in the further experiment because they could sporulate well in pot culture not later than 4 months. *Acaulospora scrobiculata* could produce the highest number of 4,200 spores per 100 g of soil via direct count. *Gigaspora* species yielded spores in pot culture less than the other genera, *Acaulospora* sp. and *Glomus* sp.

5.1.2 Identification of VA mycorrhiza by immunofluorescence technique.

The serum collected was diluted 1 : 20 for use in the tests. Antiserum produced by spores of *Glomus mosseae* was hardly reacted with crushed spores of *Glomus mosseae*. The fluorescein-labelled was not uniformly distributed on the outer spore surface of *G. mosseae* and the spore of VAM species in other genera tested did not react although some had been reported on their success (Kough, 1983). According to the limitation of time to develop FA technique to identify VAM isolates, the identification of all species for this study was done after DR. N.C. Schenck and Dr.J.A. Menge the consultant of this project via using spores characteristics and morphology.

5.1.3 Comparison of the effectiveness of collected VAM species in glasshouse.

Twelve isolates of VAM identified by technique of Nicolson and Schenck (1979) were chosen to study for their effectiveness. These isolates were selected because they were able to sporulate and produced enough spores without contamination in the pot culture.

Table 3. Number of spore produced in soil by 12 effective VAM species in plot culture.

VAM species	no. of spores/100 g soil
1. *Acaulospora scrobiculata* # 22 (ASCR # 22)	4200
2. *Glomus intraradices* 183 # 26 (INT # 26)	250
3. *Glomus mosseae* (MOS # 6)	1400
4. *Glomus* sp. # 17 (GLO # 17)	1200
5. *Glomus mosseae* 156 # 6 (MOS # 6)	1450
6. *Glomus deserticola* 160 # 39 (DES # 39)	840
7. *Glomus etunicatum* 157 # 31 (ETU # 31)	2000
8. *Gigaspora margarita* 103 # 40 (MAR # 40)	134
9. *Gigaspora gregaria* 116 # 33 (GRE # 33)	123
10. *Gigaspora* sp. 011 # 57 (GRE # 57)	115
11. *Entrophospora* sp. # 8 (ENTRO # 8)	115
12. *Gigaspora* sp.002 # 56 (GI # 56)	175

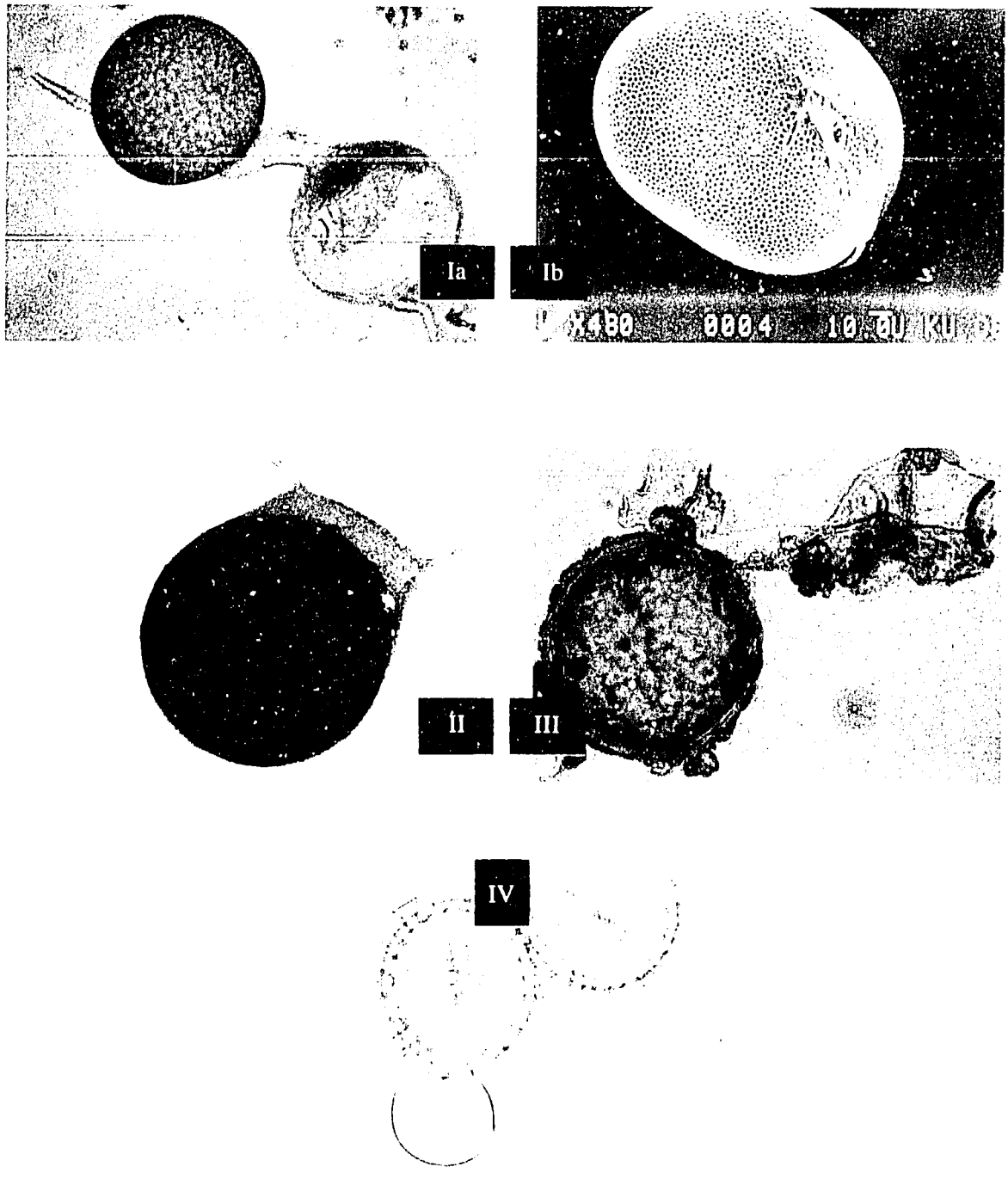

Figure 1. I.*Acaulospora scrobiculata* (a). mature spore with empty spore attached, x 40, (b). spore surface II. *Acaulospora spinosa*, x 20, III. *Acaulospora trappei*, x 40, IV. *Acaulospora* unknown specie, x 20

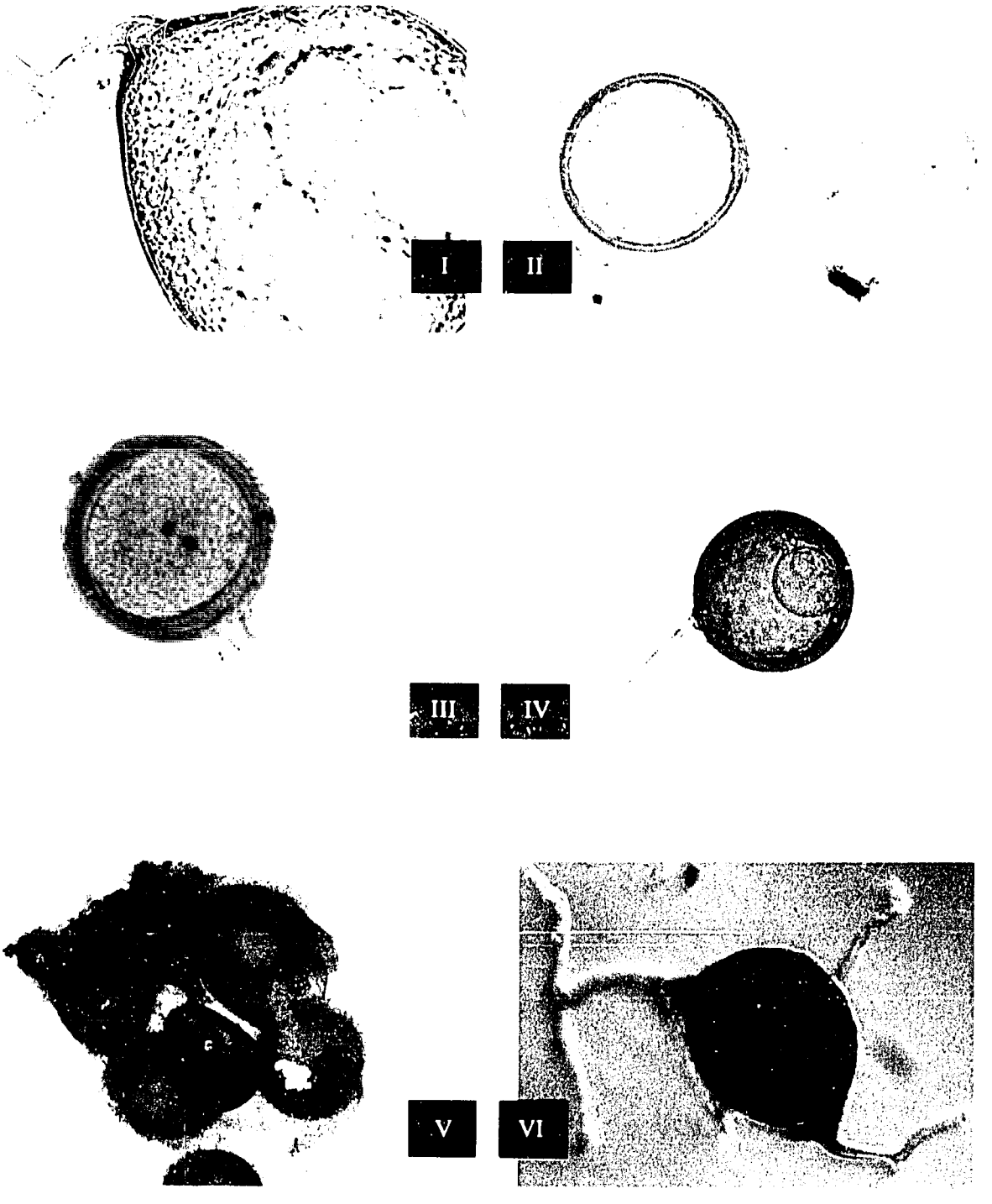

Figure 2. I. *Glomus mosseae*, x 40, II. *Glomus clarum*, x 20, III. *Glomus macrocarpum*, x 40, IV. *Glomus fasiculatum*, x 40, V. *Glomus melanosporum*, x 40, VI. *Glomus multicaulis*, x 20

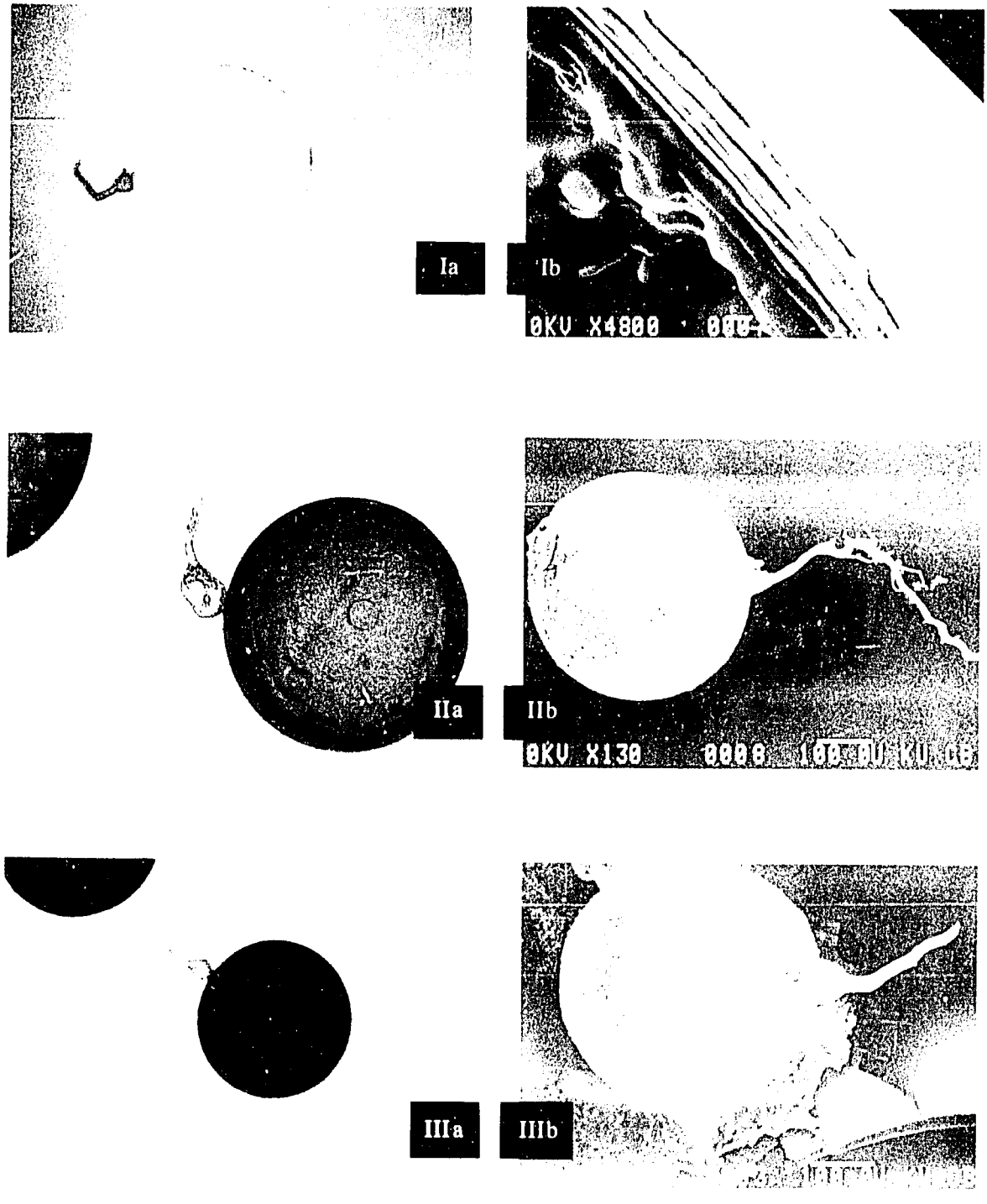

Figure 3. I. *Gigaspora margarita* (a). mature spore, 20 x, (b). spore layer. II. *Scutellospora heterogama*, (a). mature spore, 20 x, (b). mature spore by SEM. III. *Scutellosp gregaria*, (a). mature spore, 20 x, (b). mature spore from SEM.

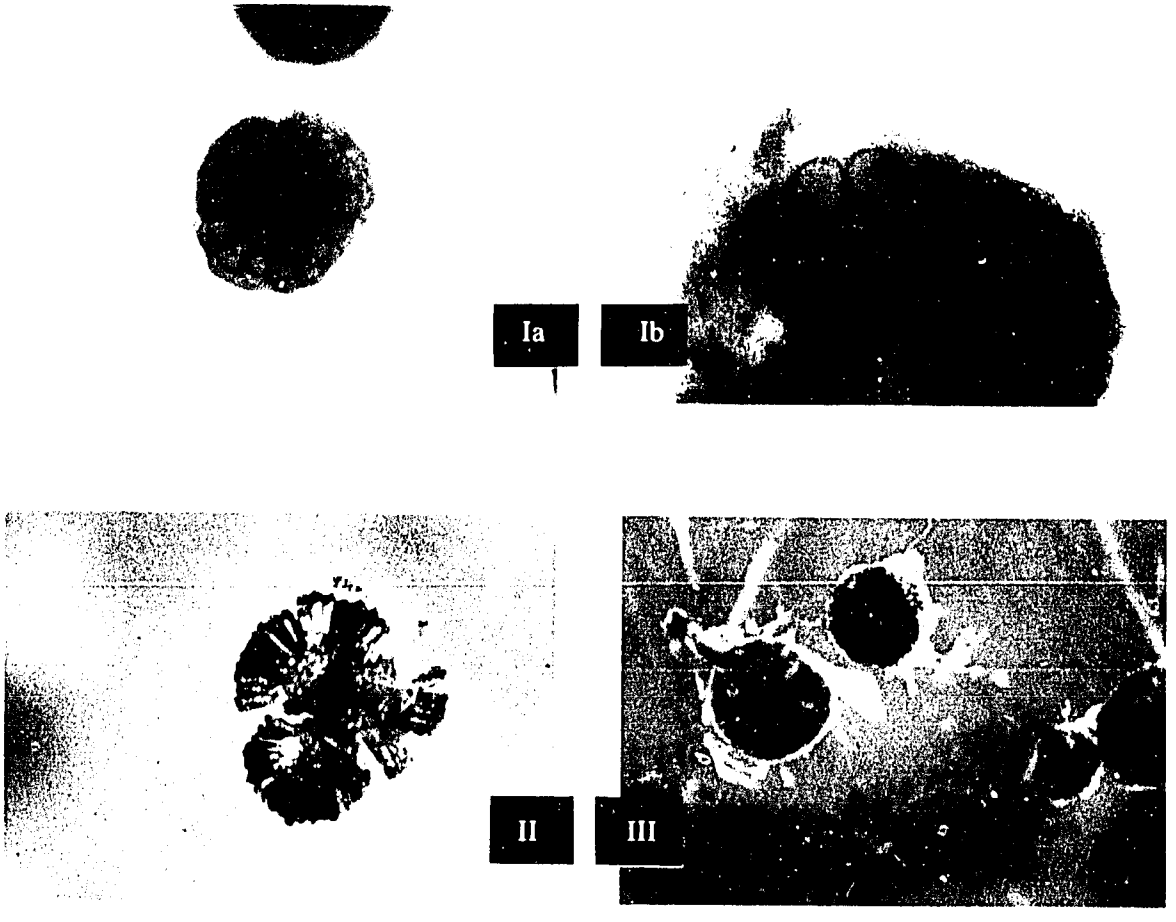

Figure 4. I. *Sclerocystis coremioides* (a). x 10, (b). x 20, II. *Sclerocystis clavispora*, x 20, III. *Sclerocystis sinnosa*, x 10.

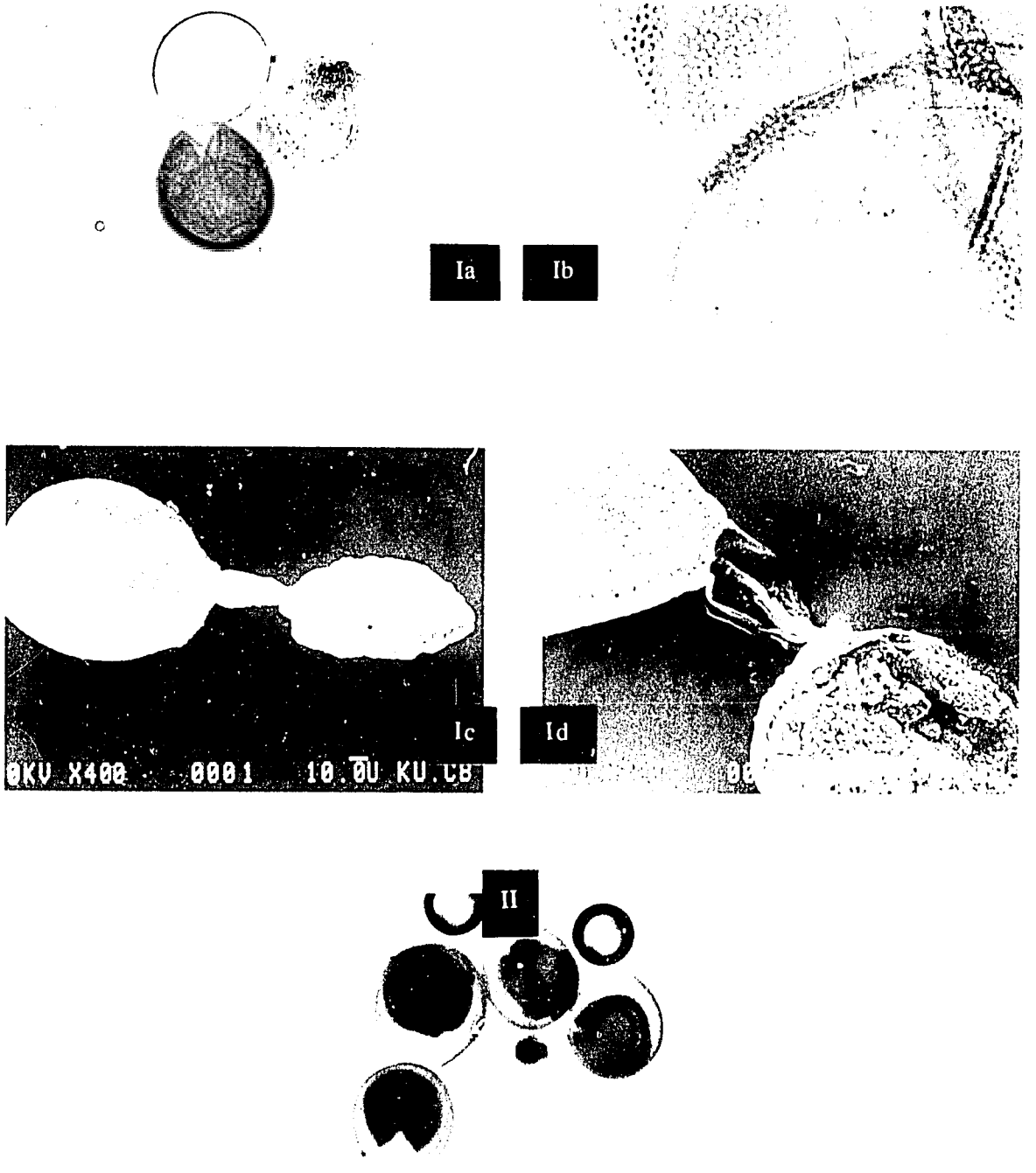

Figure 5. I. *Entrophospora infrequens* (a). mature spore, x 20, (b). surface spore (shell), x 40, (c). mature spore from SEM, x 400, (d). mature spore from SEM, x 720. II. *Entrophospora colombiana*, x 20

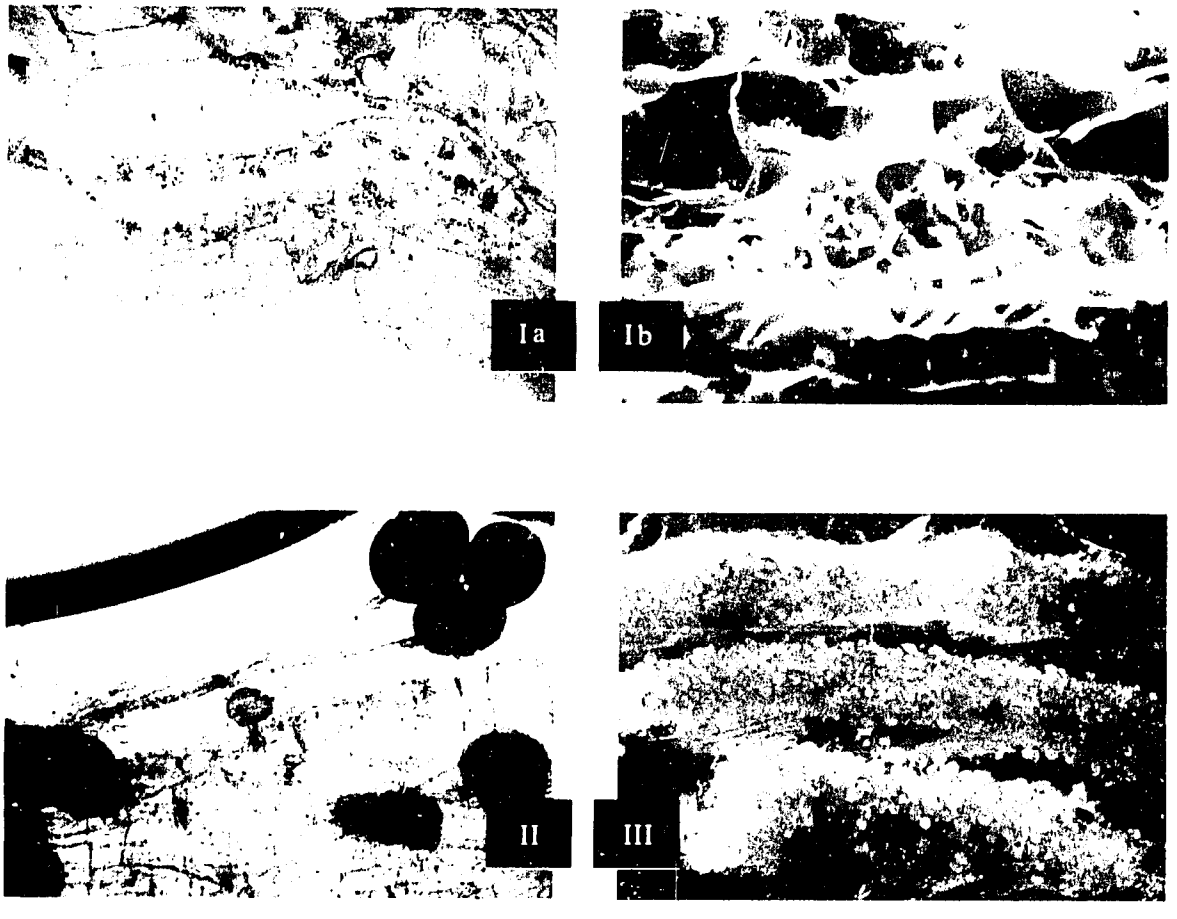

Figure 6. I. Arbuscule (a). in sorghum root, x 40, (b). arbuscule in sorghum root by SEM, x 720, II. vesicle in soybean root, x 40 III. vesicle in corn root infected by *Glomus fasiculatum*, x 10

5.1.3.1 Soybean.

Result in the glasshouse showed that many species of VAM fungi were effective. Data in table 4 showed that there were no significantly difference of soybean height at 30 and 60 days. *Entrophospora* # 8 and *Glomus mosseae* # 6 were not significantly different from the other VAM but were significantly different in height from the control at 15 and 12% respectively. Soybean inoculated with *Gigaspora gregaria* # 57 was the shortest and even shorter than control. There were no difference in average dry shoot weights among the inoculated and the noninoculated. The differences were found among the VAM isolates. Soybean inoculated with *Glomus mosseae* # 6 had the highest shoot dry weight of 16.5 grams at 100 days while *Glomus etunicatum* had the lowest shoot dry weight of 9.8 grams at the same age. It was shown that the soybean inoculated with *Glomus intraradices* # 26 had the highest pod dry weight which was higher than the noninoculated about 150%. This indicated that *Glomus intraradices* # 26 had the high efficiency in increasing soybean yield when grown under glasshouse condition with low phosphate and free from indigenous VAM contamination. Most selected VAM species used in this study had the capability to enhancing crop production except *Glomus* etunicatum # 31 and *Gigaspora gregaria* # 33 which did not show any effect on increasing soybean yield compared to noninoculated.

Examination of soybean root at 30 days found the occurrence of some VAM colonizations. *Glomus intraradices* # 26 seemed to perform infection in soybean root earlier than the other VAM isolates. At 60 days after planting the percent of root colonization was found up to 60%. At 90 days all the soybean roots were colonized by all VAM isolates. The highest colonization was found in soybean root inoculated with *Glomus intraradices* # 26 while the lowest was found with *Gigaspora gregaria* # 33. There were few contaminations about 1% in the noninoculated pot. There were correlation among the percent root colonization, dry shoot weight and yield. Soybean shoot dry weight and yield were increased with increasing in percent root colonization of *Glomus intraradices* # 20 and *Glomus mosseae* # 26, while the lowest percent root colonization of *Glomus etunicatum* # 31 resulted in the lowest plant dry weight.

There was no correlation between amount of spores in soil and percent root colonization. Isolate obtained from *Acaulospora scrobiculata* # 22 were found to produce highest number of spores up to 837 spores per 100 grams of soil with 60 percent root colonization while *Glomus intraradices* # 26 produced only 207 spores with 98% of root colonization. This showed that the VAM isolates which producing high amount of spores are not necessary to obtain the high infectivity and vice versa.

Considering the phosphorus content in soybean shoot and leaves founded that the VAM isolates which influenced on increasing soybean yield contained the greater phosphorus content than noninoculated soybean.

Table 4. Average height, shoot dry weight, yield, root colonization, number of spores and phosphorus content of soybean grown in glasshouse.

Treatments	Height (cm)			Shoot dry weight (g/pl)			Yield (g/pl)	
	30 days	60 days	100 days	30 days	60 days	100 days	fresh pod	dried pod
ETU # 31	21.8 a	28.9 a	28.8 ab	1.6 ab	9.1 a	9.8 b	2.9 c	1.6 b
DES # 39	21.1 a	29.1 a	28.9 ab	1.3 b	8.3 ab	13.5 ab	9.0 abc	3.8 ab
MAR # 40	22.3 a	29.9 a	28.3 abc	2.2 ab	9.8 a	11.9 ab	11.4 ab	4.9 a
GRE # 33	21.7 a	29.0 a	28.9 ab	1.6 ab	9.2 a	11.3 ab	3.5 c	1.7 b
ENTRO # 8	28.1 a	28.4 a	30.6 a	2.1 ab	7.4 ab	10.8 b	5.8 bc	3.3 ab
GRE # 57	18.4 a	25.2 e	24.6 c	1.2 b	6.4 b	10.8 b	5.5 bc	2.7 ab
GI # 56	20.9 a	28.4 a	27.5 abc	1.1 b	8.7 a	12.2 ab	6.3 bc	3.4 ab
ASCR # 22	18.4 a	26.2 a	27.0 abc	1.7 ab	6.4 b	16.0 a	10.0 ab	3.8 ab
MOS # 6	22.4 a	29.9 a	30.0 a	2.6 a	9.5 a	16.5 a	12.0 a	4.6 a
INT # 26	19.4 a	27.4 a	27.6 abc	1.4 ab	8.9 a	14.5 ab	12.9 a	5.0 a
MOS	19.2 a	27.6 a	27.9 abc	2.3 ab	9.2 a	12.0 ab	6.5 bc	3.8 ab
Glomus # 17	23.5 a	29.5 a	29.2 ab	1.8 ab	9.5 a	11.8 ab	9.5 ab	4.4 ab
Control	20.1 a	26.5 a	26.7 bc	2.1 ab	9.3 a	11.5 ab	3.8 c	2.0 b
% CV.	9.8	4.3	6.2	16.7	15.4	21.7	20.7	18.4

Table 4. cont.

Treatments	Root colonization (%)			No. of Spores/100 g soil		P content in plant tissue (%)
	30 days	60 days	100 days	60 days	100 days	100 days
ETU # 31	7.2 d	15 c	24 c	3 d	38 d	0.119
DES # 39	0 f	24 b	72 ab	292 a	443 b	0.148
MAR # 40	0 f	48 b	73 ab	0 c	128 c	0.161
GRE # 33	0.5 f	1 c	9 d	3 d	15 d	0.113
ENTRO # 8	4.0 e	3 c	23 c	16 c	518 b	0.139
GRE # 57	15.0 c	20 b	25 c	1 d	7 d	0.124
GI # 56	15.0 c	17 c	30 c	2 d	40 d	0.143
ASCR # 22	3.1 e	54 b	60 b	0 d	1837 a	0.154
MOS # 6	23 b	74 a	80 a	0 d	202 c	0.169
INT # 26	60 a	90 a	98 a	4 d	207 c	0.174
MOS	2.5 e	17 c	31 c	13 d	155 c	0.163
Glomus # 17	0.2 f	1 c	36 c	80 b	85 d	0.179
Control	0 f	0 c	1 d	0 d	0 d	0.120
% CV.	73.5	89.3	87.7	102.5	98.7	9.4

In a column, means followed by different letter are significantly differen at the 5% level by DMRT.

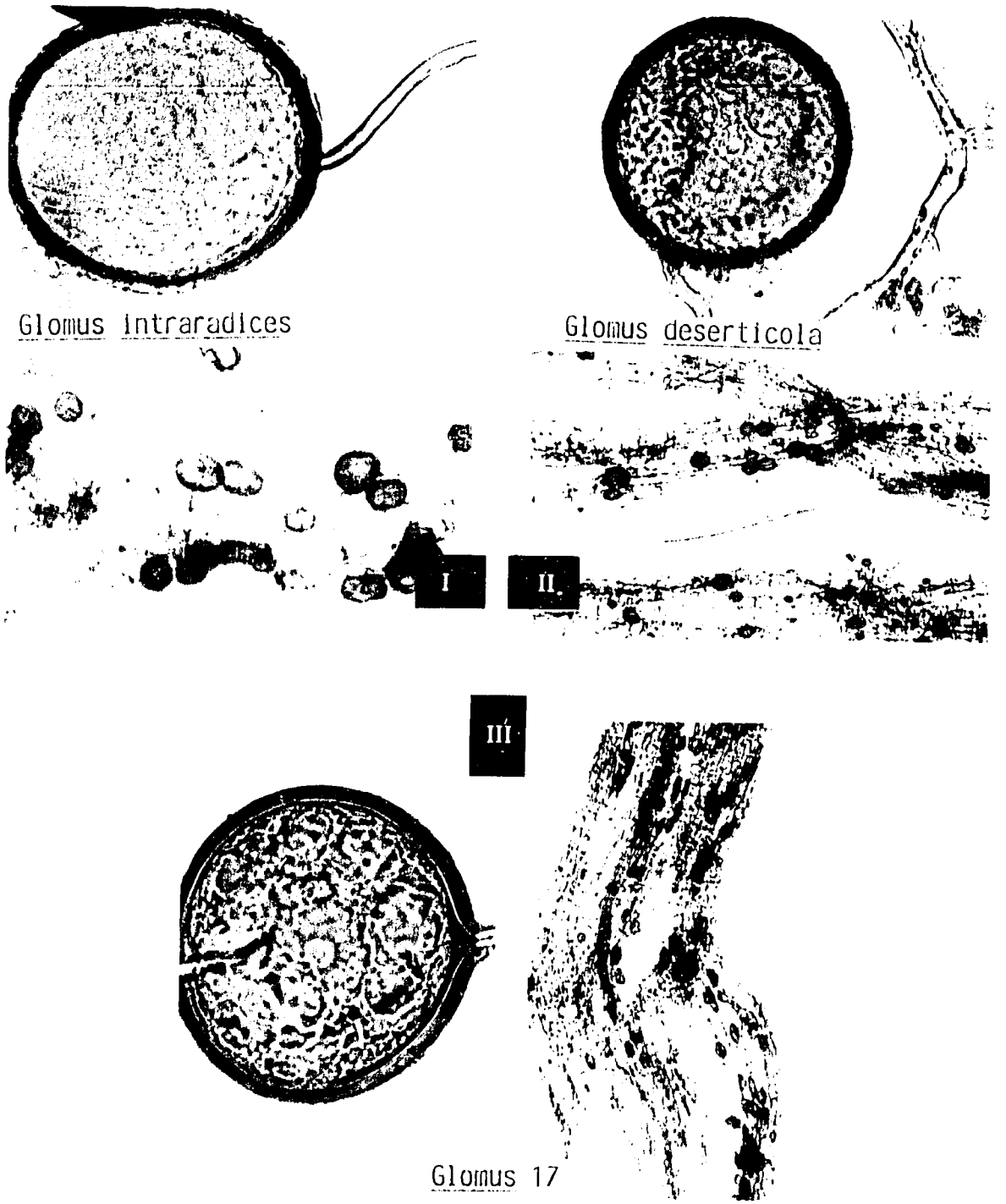

Figure 7. Vesicle and spore of inoculated VA mycorrhizal fungi in soybean root from field trial I. root infected by *Glomus intraradices*. II. root infected by *Glomus deserticola*. *III. root infected by Glomus* # 17.

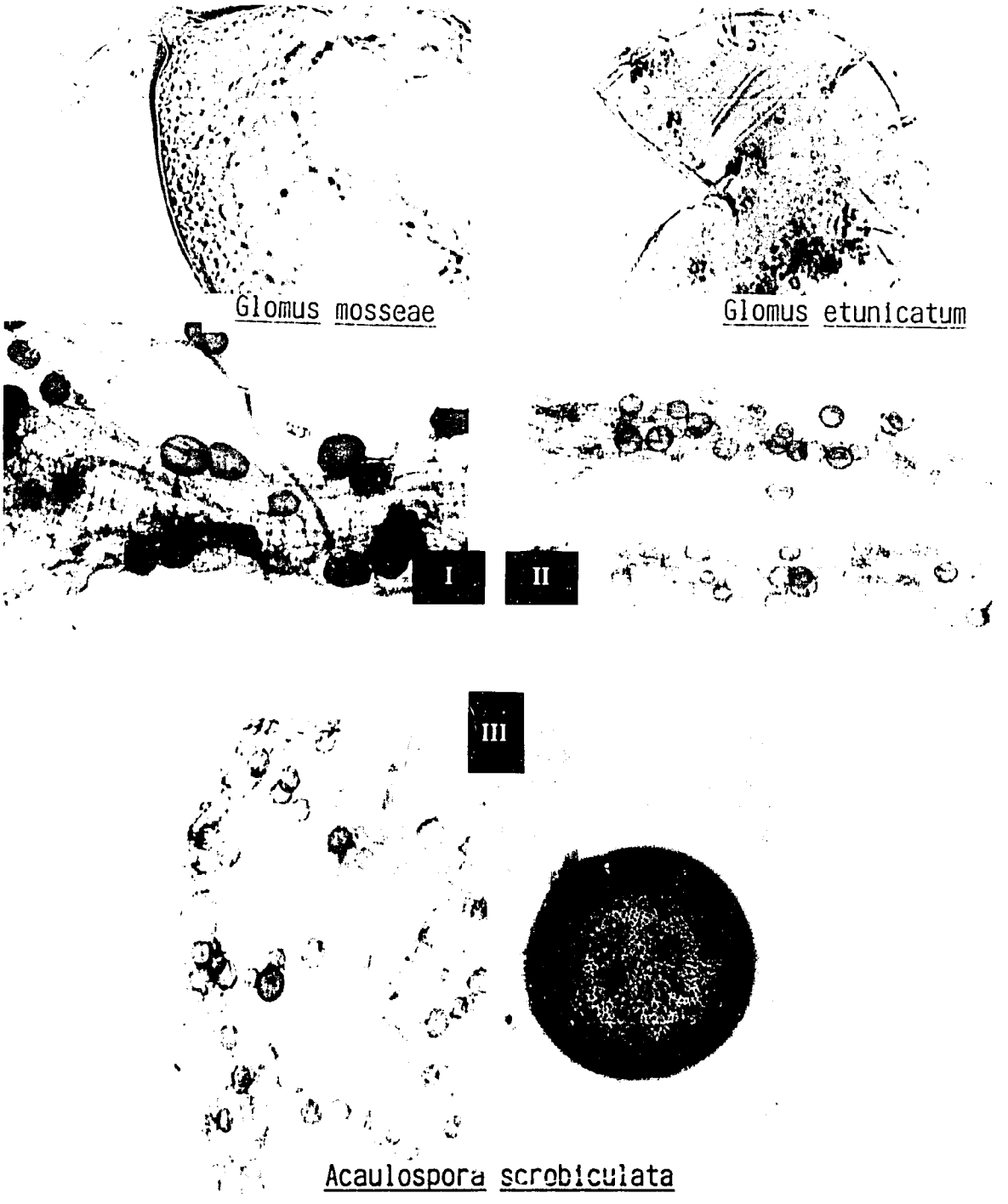

Figure 8. Vesicle and spore of inoculated VA mycorrhizal fungi in soybean root from field trial I. root infected by *Glomus mosseae*. II. root infected by *Glomus etunicatum*. III. root infected by *Acaulospora scrobiculata*

5.1.3.2 Mungbean.

In table 5 showed that there was no differences between inoculated and noninoculated on mungbean height at 30, 60 and 100 days. The differences were found in shoot dry weight at 60 days. Mungbean inoculated with *Acaulospora scrobiculata* # 22, had the highest weight which was higher than noninoculated about 119%. At 100 days, the highest weight gained from mungbean inoculated with *Glomus intraradices* # 26 and *Gigaspora margarita* # 40 were higher than noninoculated 44% and 42% respectively. *Glomus mosseae* could increase mungbean yield up to 103% over noninoculated, while *Glomus* # 17, *Gigaspora margarita* # 40, *Acaulospora scrobiculata* # 22 increased mungbean yield at 68%, 58% and 47% over control respectively. Some isolates such as *Glomus deserticola* # 39, *Gigaspora gregaria* # 33, *Entrophospora* # 8 and *Gigaspora gregaria* # 22 did not show any significantly difference from noninoculated.

The colonization of VAM in mangbean root was observed at 60 days. *Glomus intraradices* # 26 showed the earliest colonization in mangbean root. At 100 days after planting all the treatments were colonized by VAM. The percent root colonization by *Glomus intraradices* # 26 and *Gigaspora margarita* # 40, were found highly at 98 & 97% respectively. *Glomus mosseae* with 88% root colonization obtained the highest yield. *Acaulospora scrobiculata* # 22 produced highest number of spore in soil, 1005 spores per 100 grams of soil, while *Glomus intraradices* # 22, *Gigaspora margarita* # 40 and *Glomus mosseae* produced only 96, 37 and 182 spores respectively. No correlation between the number of spores in soil and percent root colonization was found in mungbean trial. The phosphourus content in mungbean shoots and leaves showed the greater P content in the effective VAM inoculated mungbean than the noninoculated.

Table 5. Average height, shoot dry weight, yield, root colonization, number of spores and phosphorus content of mungbean grown in glasshouse.

Treatments	Height (cm)			Shoot dry weight (g/pl)			Yield (g/pl)	
	30 days	60 days	100 days	30 days	60 days	100 days	fresh pod	dried pod
ETU # 31	17.3	20.5	22.4	1.6	3.8 ab	5.3 ab	7.8 ab	3.7 bc
DES # 39	15.7	18.2	20.1	1.6	3.0 ab	4.2 b	6.2 b	1.7 c
MAR # 40	18.1	21.5	23.7	2.0	3.3 ab	6.6 a	12.1 ab	6.0 ab
GRE # 33	16.8	18.2	20.2	1.6	1.7 b	4.9 ab	7.0 b	2.6 c
ENTRO # 8	16.0	19.5	21.6	1.6	4.1 ab	4.4 b	7.3 b	2.7 c
GRE # 57	16.9	19.5	21.7	2.4	3.9 ab	5.2 ab	6.8 b	2.2 c
GI # 56	16.4	19.0	21.1	1.9	3.1 ab	5.7 ab	7.0 b	2.1 c
ASCR # 22	17.1	21.3	23.7	1.8	4.6 a	5.5 ab	10.2 ab	5.6 b
MOS # 6	17.0	20.7	22.5	1.7	4.2 ab	5.9 ab	7.3 b	3.3 c
INT # 26	17.8	21.2	23.0	2.0	3.8 ab	6.2 ab	7.0 b	3.0 c
MOS	16.3	19.5	21.7	1.3	4.0 ab	4.9 ab	18.4 a	7.7 a
Glomus # 17	16.0	18.6	20.3	1.4	3.6 ab	4.2 ab	13.5 ab	6.4 a
Control	16.6	19.7	21.8	1.5	2.1 b	4.3 ab	8.0 ab	3.8 bc
% CV.	9.4	12.3	8.6	17.9	15.8	19.9	12.6	11.8

Table 5. cont.

Treatments	Root colonization (%)		No. of Spores/100 g soil		P content in plant tissue (%)
	60 days	100 days	60 days	100 days	100 days
ETU # 31	13 c	50 c	88 c	92 d	0.327
DES # 39	45 b	74 b	189 b	150 c	0.263
MAR # 40	35 bc	97 a	5 e	37 e	0.287
GRE # 33	1 d	16 d	2 e	15 f	0.290
ENTRO # 8	24 c	84 ab	5 e	4 f	0.280
GRE # 57	11 d	68 b	1 e	15 ef	0.295
GI # 56	3 d	14 d	4 e	50 e	0.430
ASCR # 22	20 c	70 b	193 b	1005 a	0.393
MOS # 6	54 b	68 bc	34 d	213 c	0.390
INT # 26	65 a	98 a	28 e	96 d	0.389
MOS	9 d	88 ab	55 d	182 c	0.436
Glomus # 17	21 c	49 c	296 a	360 b	0.399
Control	0 d	19 d	0 e	0 f	0.294
% CV.	69.3	72.5	93.4	91.4	23.0

In a column, means followed by different letter are significantly different at the 5% level by DMRT.

5.1.4 Comparison of the effectiveness of collected VAM species in field trial.

Six screening of VAM isolates from glasshouse experiment; MOS # 6, GLO # 17, INT # 26, ASCR # 22, ETU # 31 and DES # 39 were used for the field studies. *Gigaspora sp.* was discarded because of its poor sporulation.

5.1.4.1 Soybean

Data in table 6 showed that height of soybean grown in Chiangmai at 90 days after planting was statistically different when inoculated with different VAM. Soybean inoculated with Glomus # 17 was the shortest while inoculated with *Acaulospora scrobiculata* was the tallest. There was no difference between the plots applied with rock phosphate and non. At Prachinburi the different in height was found at 60 days of planting. Soybean inoculated with *Acaulospora scrobiculata* was the tallest and inoculated with *Glomus etunicatum* was the shortest. There was no statistically difference between the plot applied with rock phosphate and non.

There were statistically differences in shoot dry weight of soybean grown at Chiangmai at 30, 60 and 90 days (Table 7). The highest shoot dry weight was found in soybean inoculated with *Acaulospora scrobiculata* and higher than noninoculated 17% when grown at 60 days. *Glomus etunicatum* increased highest shoot dry weight higher than noninoculated 37% when grown at 90 days. At Prachinburi, soybean inoculated with *Acaulospora scrobiculata* had the highest dry weight at both 60 and 90 days. In contrast, *Glomus etunicatum* which showed the most effective at 90 days in increasing growth of soybean grown at Chiangmai showed the opposite result at Prachinburi, this isolate caused the lowest soybean weight.

Soybean inoculated with *Acaulospora scrobiculata* at Chiangmai also gave the highest yield, higher than noninoculated 50%. The other isolates showed no difference among isolates nor the noninoculated (Table 8). At Prachinburi, *Acaulospora scrobiculata* also increased the highest yield for soybean about 25%. There were no statistically difference between the plot applied with rock phosphate and non. The differences in yield obtained from Chiangmai and Prachinburi were mainly effected by the soil fertility. Besides soil fertility, the water stress was also involved. At Prachinburi during the growing season, the land was dried throughout the cultivation period. The effect of VAM application was more dominant on the treatment of applied phosphate. The yield of the treatments recieved rock phosphate and inoculated with *Acaulospora scrobiculata*, *Glomus etunicatum*, *Glomus* # 17, *Glomus deserticola* # 39 and *Glomus mosseae* # 6 were 280, 67, 61, 46 and 23 kg/ha respectively, which were higher than the treatments with non phosphate application. This result indicated that under dry condition where the water was not adequated to dissolve phosphorus available to plant, VAM might play the significant role on absorbing phosphorus for plant.

Percent root colonizations of soybean grown at Chiangmai were the highest when inoculated with *Acaulospora scrobiculata* and *Glomus deserticola* and they were higher than the noninoculated about 40% and 37% (Table 9). The same results were found at Prachinburi where soybean roots were infected with *Acaulospora scrobiculata* and *Glomus deserticola* were 15% and 14% higher than noninoculated. At *Chiangmai*, *Glomus deserticola* and *Acaulospora scrobiculata* were found to produce higher number of spores in soil. The similar results were also found at Prachinburi. At both places, the amount of spores in soil were higher when soybeans were inoculated with VAM (Table 10)

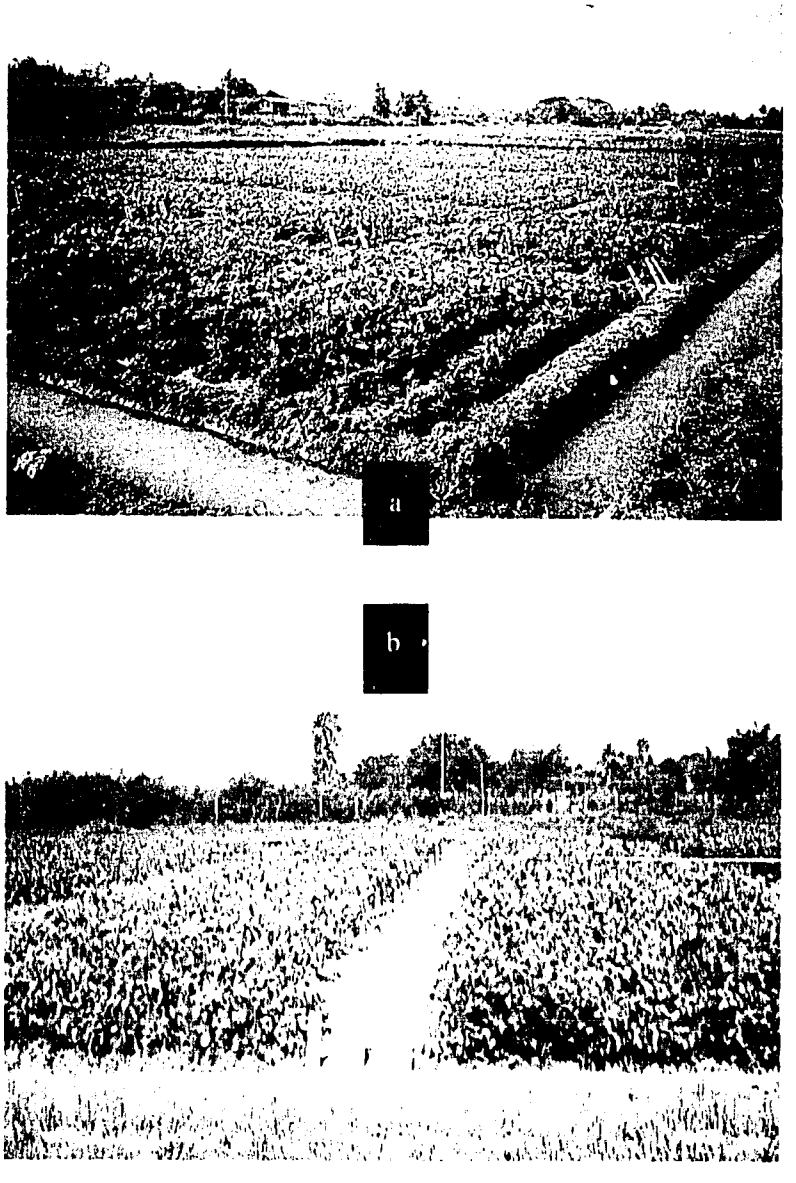

Figure 9. Soybean field (lst crop) (a). Soybean grown at Chiangmai inoculated with different V. mycorrhizal fungi. (b). Soybean grown at Prachinburi inoculated with *Glomus mosse* applied rock phosphate (left), *Glomus etunicatum* with no rock phosphate (right).

Table 6. Average soybean height (cm) inoculated with VA mycorrhizal species at Chiangmai and Prachinburi (1st crop).

Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	25.7	28.6	27.2	35.2	37.8	36.5	45.7	42.3	44.0
GLOMUS # 17	26.3	28.3	27.3	33.7	31.0	32.3	41.1	40.1	40.6
INT 183 # 26	24.7	27.3	26.9	34.0	34.4	34.2	45.7	42.1	43.9
ASCR # 22	25.7	30.0	27.8	34.0	37.3	35.7	46.8	44.9	45.8
ETU 157 # 31	23.7	29.3	26.5	35.2	35.7	35.4	44.1	42.0	43.1
DES 160 # 39	28.7	24.6	26.6	38.3	35.7	37.0	46.6	41.4	44.0
Control	30.0	28.3	29.2	39.1	36.7	37.9	46.0	43.1	44.6
Mean	28.1	26.4	27.2	35.6	35.5	35.6	45.1	42.3	43.7
CV.P (%)		25.5			14.3			11.1	
CV. Species (%)		19.8			13.3			5.1	

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	17.9	15.7	16.8	28.5	33.5	31.0	29.5	29.5	29.5
GLOMUS # 17	16.5	14.2	15.3	27.7	31.8	29.7	32.2	30.7	31.5
INT 183 # 26	14.9	16.9	15.9	29.0	28.5	29.8	29.3	25.7	27.5
ASCR # 22	16.8	15.3	16.1	36.6	30.3	33.5	30.1	27.6	28.9
ETU 157 # 31	18.0	14.2	16.1	28.0	26.5	27.2	28.9	27.4	28.1
DES 160 # 39	16.7	15.0	15.9	27.1	28.0	27.6	26.6	29.9	28.2
Control	14.9	15.5	15.2	28.3	30.2	29.2	29.2	25.4	27.3
Mean	16.5	15.3	15.9	29.3	29.8	29.6	29.4	28.0	28.7
CV.P (%)		16.7			18.1			7.8	
CV. Species (%)		14.5			15.0			15.1	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

39'

Table 7. Average shoot dry weight of soybean (gm) inoculated with VA mycorrhizal species at Chiangmai and Prachinburi (1st crop).

Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	1.36 ab	2.07 a	1.71 ab	14.20 b	24.26 ab	19.23 b	10.88	10.01 ab	10.44 ab
GLOMUS # 17	1.41 ab	2.04 a	1.72 ab	20.02 ab	18.79 ab	19.41 b	7.72	9.47 ab	8.59 ab
INT 183 # 26	1.06 ab	1.16 b	1.11 b	24.16 ab	16.08 ab	20.12 b	10.23	6.18 b	8.21 ab
ASCR # 22	1.13 ab	2.18 a	1.66 ab	29.57 ab	26.69 a	29.13 a	6.08	3.90 b	4.99 b
ETU 157 # 31	0.95 b	2.14 a	1.55 ab	26.81 ab	13.85 ab	20.33 b	9.94	17.76 a	13.85 a
DES 160 # 39	1.49 ab	1.77 b	1.63 ab	32.80 a	10.20 b	21.50 ab	8.75	6.84 b	7.80 ab
Control	2.05 a	1.91 a	1.98 a	30.00 a	20.74 ab	25.37 ab	8.06	12.18 ab	10.12 ab
Mean	1.35	1.90	1.62	25.37	18.66	22.01	8.8	9.5 a	9.1
CV.P (%)		69.1			61.9			58.8	
CV. Species (%)		34.6			37.8		61.8		

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	2.27	1.97	2.17	7.02	9.77	8.39 ab	4.28	4.13 a	4.21 ab
GLOMUS # 17	2.16	1.52	1.05	7.02	7.68	7.35 ab	4.32	3.86 a	4.09 ab
INT 183 # 26	1.83	2.29	2.11	4.85	5.78	5.32 a	4.23	4.48 a	4.36 a
ASCR # 22	1.99	1.74	1.87	11.33	7.64	9.48 a	6.39	3.53 a	4.96 a
ETU 157 # 31	2.57	1.63	2.10	5.25	4.83	5.04 b	2.79	3.50 a	3.15 b
DES 160 # 39	2.17	1.87	2.02	5.10	5.96	5.53 b	3.52	3.56 a	3.54 b
Control	1.08	1.97	1.92	6.36	6.05	6.21 ab	4.22	3.77 a	3.99 ab
Mean	2.14	1.86	2.00	6.70	6.81	6.76	4.25	3.83	4.04
CV.P (%)		32.6			45.3			60.9	
CV.Species (%)		29.8			45.2			29.1	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 8. Average pod dry weight and yield of soybean inoculated with mycorrhizal species at Chiangmai and Prachinburi (1st crop).

Chiangmai

Treatments	Dry pod (g/plant)			Yield (Kg/ha)		
	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	45.12	43.37 ab	44.24 ab	1250 b	1489 ab	1369.40 b
GLOMUS # 17	34.74	23.36 bc	29.05 b	1191 b	1214 b	1202.40 b
INT 183 # 26	40.38	16.34 c	28.36 b	1342 b	1236 b	1288.68 b
ASCR # 22	50.08	46.25 ab	48.17 a	1834 a	1653 a	1743.76 a
ETU 157 # 31	29.41	49.25 a	39.33 ab	1411 b	1230 b	1320.69 b
DES 160 # 39	29.69	29.93 abc	29.81 b	1603 ab	1275 ab	1438.98 b
Control	37.68	37.59 ab	37.63 ab	1575 ab	1244 ab	1409.76 b
Means	38.16	35.16	36.66	1458	1334	1396
CV.P (%)		23.2			9.6	
CV.Species (%)		33.6			16.0	

Prachinburi

Treatments	Dry pod (g/plant)			Yield (Kg/ha)		
	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	6.47 a	5.12 a	5.80 a	355 b	331	343
GLOMUS # 17	7.06 a	4.68 a	5.87 a	368 b	306	337
INT 183 # 26	6.86 a	4.68 a	5.87 a	260 b	270	265
ASCR # 22	11.28 a	6.27 a	8.78 a	529 b	249	389
ETU 157 # 31	7.18 ab	4.71 a	5.95 a	283 b	217	250
DES 160 # 39	5.33 b	6.09 a	5.71 a	275 b	230	252
Control	6.48 ab	3.59 a	5.03 a	265 ab	259	262
Means	7.09	5.02	6.06	334	266	300
CV.P (%)		45.7			30.3	
CV.Species (%)		58.1			27.4	

Table 9. Percent root colonization of soybean inoculated with mycorrhizal species at Chiangmai and Prachinburi (1st Corp).

Chiangmai

Treatments	30 days				60 days				90 days			
	P_1	P_0	Mean		P_1	P_0	Mean		P_1	P_0	Mean	
MOS 156 # 6	53	50	51		76	81	78	a	80	78	79	a
GLOMUS # 17	58	51	55		45	48	46	b	43	44	44	b
INT 183 # 26	44	58	51		50	63	56	b	42	55	49	b
ASCR # 22	61	60	60		82	76	80	a	82	86	84	a
ETU 157 # 31	67	38	53		71	70	70	a	50	56	53	b
DES 160 # 39	57	61	59		69	79	73	a	80	84	82	a
Control	53	69	61		74	85	79	a	42	78	60	b
Mean	56.0	55.3			66.4	71.7			59.9	68.7		
CV.P (%)		31.5				15.7				20.5		
CV.Species (%)		19.2				11.0				12.0		

Prachinburi

Treatments	30 days				60 days				90 days		
	P_1	P_0	Mean		P_1		P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	32	26	28	c	68	ab	57	62	79	87	83
GLOMUS # 17	57	45	51	ab	62	abc	51	57	78	84	81
INT 183 # 26	70	45	58	a	77	a	58	68	82	86	84
ASCR # 22	23	46	34	bc	48	c	62	55	86	84	85
ETU 157 # 31	50	26	38	abc	73	a	57	65	76	89	82
DES 160 # 39	34	28	31	c	53	bc	60	57	80	88	84
Control	31	32	32	c	63	abc	48	55	76	72	74
Mean	42	35			63		56		79	84.1	
CV.P (%)		53.4					18.2			6.9	
CV.Species (%)		39.7					13.0			11.9	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 10. Number of spores in 100 gram soil grown VAM inculated at Chiangmai and Prachinburi (1st corp).

Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	204	92	148	165	117	141 ab	118	104	111 cd
GLOMUS # 17	275	90	183	67	65	66 d	218	144	181 bc
INT 183 # 26	185	141	163	81	87	84 cd	177	164	171 bc
ASCR # 22	209	119	164	177	124	151 a	323	168	246 ab
ETU 157 # 31	125	199	162	126	94	110 abc	124	89	107 d
DES 160 # 39	128	141	135	95	97	96 bc	235	279	257 a
Control	109	80	95	134	100	117 abc	144	178	161 bcd
Mean	176.4	111.7		120.7	97.7		191.3	160.9	
CV.P (%)		29.4			16.5			7.7	
CV.Species (%)		15.2			8.7			8.3	

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	144 ab	78 bc	96	119	95	107 a	221	190	206
GLOMUS # 17	135 ab	83 bc	109	50	77	64 b	81	90	86
INT 183 # 26	86 bc	101 b	94	109	115	112 a	97	127	112
ASCR # 22	92 b	105 b	99	108	155	132 a	247	227	237
ETU 157 # 31	58 c	197 a	128	149	103	126 a	188	153	171
DES 160 # 39	186 a	58 c	122	41	108	75 b	123	120	122
Control	113 ab	132 ab	123	102	121	112 a	188	101	145
Mean	112	107.7		96.9	110.6		163.6	144	
CV.P (%)		5.2			8.4			9.4	
CV.Species (%)		7.3			9.6			7.1	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Data from Table 11 and 12 are showing the nutrients in soybean shoots and leaves and N, P, K, content in soil. There wre no differences on nitrogen, phosphorus and potassium content between inoculated and noninoculated soybean at Chiangmai. There was also no difference on phosphorus content in plant tissue among the plot applied with rock phosphate and non. At Prachinburi where the soil was low in P content, the difference P content in inoculated soybean was greater than the noninoculated by the mean value but not great enough to show the difference in statistics.

5.1.4.2 Mungbean.

Height of inoculated mungbean grown at Kalasin was different from noninoculated at 30 days after planting (Table 13). Height of mungbean inoculated with *Acaulospora scrobiculata* was the greatest. After 30 days, there were no significantly differences between inoculated and noninoculated mungbean. The same results were found at Prachinburi.

Shoot dry weights of inoculated mungbean were not statistically different from noninoculated but some isolates such as *Glomus deserticola*, *Glomus mosseae* were greater than non inoculated in the average mean of shoot dry weight at Kalasin (Table 14). At Prachinburi, the dry weights, of mungbean inoculated with *Acaulospora scrobiculata* and *Glomus intraradices* were greater than the noninoculated.

Table 11. Nitrogen, phosphorus and potassium content in VAM inoculated soybean shoot grown at Chiangmai and Prachinburi.

Chiangmai

Treatments	% N			% P			% K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.896	1.020	0.958	0.093	0.107	0.100	0.523	0.597	0.560
GLO # 17	0.900	0.971	0.935	0.088	0.091	0.090	0.547	0.528	0.537
INT 183 # 26	0.982	0.857	0.920	0.100	0.089	0.094	0.484	0.648	0.566
ASCR # 22	0.833	0.891	0.862	0.078	0.086	0.082	0.447	0.587	0.517
ETU 157 # 31	0.917	0.822	0.870	0.099	0.076	0.087	0.523	0.557	0.540
DES 160 # 39	0.959	1.059	1.009	0.089	0.117	0.103	0.553	0.643	0.598
CK	0.912	0.854	0.883	0.094	0.095	0.095	0.567	0.581	0.574
Mean	0.914	0.925		0.091	0.094		0.521	0.591	
CV.P (%)	4.9			10.8			47.6		
CV. Species (%)	16.1			13.4			13.9		

Prachinburi

Treatments	% N			% P			% K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.806	0.918	0.862	0.084	0.096	0.090	0.471	0.537	0.504
GLO # 17	0.815	0.873	0.844	0.079	0.080	0.080	0.490	0.475	0.483
INT 183 # 26	0.895	0.871	0.883	0.097	0.096	0.097	0.465	0.583	0.524
ASCR # 22	0.750	0.820	0.785	0.080	0.087	0.084	0.459	0.528	0.494
ETU 157 # 31	0.870	0.729	0.800	0.089	0.068	0.079	0.471	0.501	0.486
DES 160 # 39	0.860	0.943	0.902	0.080	0.086	0.083	0.496	0.579	0.538
CK	0.810	0.768	0.789	0.052	0.051	0.052	0.510	0.520	0.515
Mean	0.829	0.846		0.085	0.081		0.480	0.532	
CV.P (%)	5.7			11.9			26.2		
CV. Species (%)	15.3			14.6			15.8		

Table 12. Nitrogen, phosphorus and potassium content in soil after grown VAM inoculated soybean at Chiangmai and Prachinburi.

Chiangmai

Treatments	% N			P(ppm)			K(ppm)		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	2.35	2.15	2.25	188	29	108.0	52	66	59
GLO # 17	2.15	2.15	2.15	203	37	120	60	64	62
INT # 26	2.22	2.15	2.19	173	25	99	68	76	72
ASCR # 22	2.22	2.22	2.22	198	26	112	71	60	66
ETU 157 # 31	2.42	2.22	2.32	220	32	126	70	64	67
DES 160 # 39	2.22	2.22	2.22	250	25	138	68	60	64
CK	2.22	2.15	2.19	270	37	154	84	65	75
Mean	2.26	2.18		215	30		68	65	
CV.P (%)	5.8			26.4			15.3		
CV. Species (%)	15.6			17.9			13.2		

Prachinburi

Treatments	% N			P(ppm)			K(ppm)		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	2.01	2.00	2.01	63	29	46	67	70	69
GLO # 17	1.90	2.90	2.40	67	28	48	60	61	61
INT 183 # 26	2.15	2.10	2.13	67	23	45	60	61	61
ASCR # 22	2.15	2.11	2.13	58	26	42	60	59	60
ETU 157 # 31	2.24	2.20	2.22	67	29	48	59	61	60
DES 160 # 39	2.24	2.20	2.22	59	24	42	61	61	61
CK	2.24	2.20	2.22	71	26	49	63	61	62
Mean	2.13	2.24		62	26		61	62	
CV.P (%)	4.2			7.4			7.6		
CV. Species (%)	17.1			15.4			19.5		

Table 13. Average height of mungbean inoculated with VA Mycorrhizal species at Kalasin and Prachinburi (1st crop).

Kalasian

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	14.3 ab	14.5 ab	14.4 b	24.9	25.9	25.4 b	25.1 b	26.0 bcd	25.6 a
GLOMUS # 17	12.9 b	12.8 ab	12.8 bc	24.9	25.8	25.3 b	28.4 ab	28.7 bc	28.5 a
INT 183 # 26	16.9 a	16.6 a	16.8 a	27.3	29.5	28.4 ab	31.8 a	30.9 ab	31.4 a
ASCR # 22	15.1 ab	11.3 b	13.2 bc	27.2	26.6	26.9 ab	28.0 ab	26.0 bcd	27.0 a
ETU 157 # 31	13.5 b	10.8 b	12.1 c	28.6	30.9	29.8 a	33.2 a	24.8 d	29.0 a
DES 160 # 39	14.4 ab	14.8 ab	14.6 b	27.8	29.0	28.4 ab	28.0 ab	34.6 a	31.3 a
Control	14.9 ab	12.3 ab	13.6 bc	26.1	28.9	27.5 ab	27.9 ab	30.9 ab	29.4 a
Mean	14.6	13.3	13.9	26.6	28.8	28.1	28.9	28.4	28.7
CV.P (%)		12.4			24.9			12.9	
CV. Species (%)		12.8			16.2			12.9	

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	13.7	10.2	12.0	28.9	19.0	23.9	32.4	24.1 ab	28.2
GLOMUS # 17	13.9	12.2	13.1	29.2	21.7	25.4	33.4	21.8 ab	27.6
INT 183 # 26	15.6	12.6	14.1	31.7	24.1	27.9	33.7	26.5 ab	30.1
ASCR # 22	12.3	11.2	11.7	27.0	20.5	23.7	28.7	24.3 ab	26.5
ETU 157 # 31	12.3	10.7	11.5	29.2	20.0	24.6	32.5	21.5 b	27.0
DES 160 # 39	12.3	12.5	12.4	29.1	24.4	26.7	29.2	27.4 ab	28.3
Control	13.3	12.7	12.9	25.7	26.5	26.1	32.8	30.2 a	31.5
Mean	13.3	11.7	12.5	28.7	22.3	25.5	31.8	25.1 a	28.5
CV.P (%)		18.1			28.0			17.4	
CV. Species (%)		19.4			22.9			18.5	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 14. Average shoot dry weight of mungbean inoculated with VA mycorrhizal species at Kalasin and Prachinburi (1st crop).

Kalasin

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.75 b	0.60	0.67 ab	6.37	7.61	6.99	6.45	4.57 a	5.51 a
GLOMUS # 17	0.63 b	0.68	0.65 ab	5.65	6.53	6.09	4.71	5.13 a	4.92 a
INT 183 # 26	1.18 a	0.67	0.92 a	4.77	8.06	6.41	6.12	4.40 a	5.26 a
ASCR # 22	0.83 ab	0.52	0.67 ab	5.95	7.77	6.86	4.06	3.42 a	3.74 a
ETU 157 # 31	0.62 b	0.45	0.54 b	5.39	7.12	6.25	6.63	2.98 a	4.80 a
DES 160 # 39	0.66 b	9.53	0.59 b	7.66	6.45	7.05	6.51	6.11 a	6.31 a
Control	0.74 a	0.50	0.62 a	6.10	6.21	6.15	3.74	6.71 a	5.23 a
Mean	0.77	0.56	0.67	5.98	7.10	6.54	5.46	4.76	5.11
CV.P (%)		13.5			27.5			16.9	
CV. Species (%)		38.9			44.9			48.8	

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	2.06	1.27	1.66	3.51	1.67 ab	2.59 ab	2.43	1.52	1.97
GLOMUS # 17	1.64	1.36	1.50	3.19	2.62 ab	2.90 ab	3.26	1.51	2.39
INT 183 # 26	2.09	1.56	1.83	3.22	2.79 ab	3.00 ab	3.75	2.02	2.88
ASCR # 22	1.43	1.35	1.39	2.65	1.22 b	1.94 b	3.42	2.39	2.90
ETU 157 # 31	1.74	1.30	1.52	2.71	1.41 b	2.06 ab	2.55	1.58	2.06
DES 160 # 39	1.35	1.32	1.33	1.95	·2.03 ab	1.99 ab	2.67	1.44	2.06
Control	2.06	1.87	1.97	3.28	3.63 a	3.46 a	2.96	2.39	2.68
Mean	1.77	1.43	1.6	2.93	2.19	2.56	3.00	1.84	2.42
CV.P (%)		35.8			34.0			32.2	
CV. Species (%)		43.6			50.2			63.7	

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

At Kalasin, yields of mungbean inoculated with *Glomus deserticola*, *Acaulospora scrobiculata*, *Glomus intraradices* and *Glomus mosseae* were greater than the noninoculated 11%, 10%, 10% and 5% respectively. At Prachinburi, only mungbean inoculated with *Glomus intraradices* was greater than noninoculated 91% and the effect of rock phosphate on mungbean was higher than the nonfertilized 38% (Table 15).

The similar effect of dry condition also observed in the mungbean field as of the soybean field mentioned above. Regarding in sufficiency of water to irrigate the cultivar field resulted in the very low yield of mungbean. Under this condition the inoculated plots showed more cleary effect by VAM, and rock phosphate which is slow releasing in available phosphorus should be used when the effective VAM fungi inoculum was applied.

Mungbean roots were found to be colonized by VAM at 30 days after planting. At Kalasin site (Table 16), mungbean root infected with *Glomus intraradices*, *Glomus mosseae* and *Acaulospora scrobiculata*, roots were colonized at 85%, 81% and 78% respectively greater than noninoculated. At Prachinburi, mungbean root infected with *Glomus intraradices* was colonized greater than noninoculated 35%. The noninoculated plants were found to be colonized by the indigenous species. Number of spores produced in Kalasin soil of mungbean inoculated with *Glomus intraradices* and *Acaulospora scrobiculata* were greater than noninoculated while in Prachinburi soil, only *Glomus deserticola* was found greater sporulated than noninoculated. From the number of spores formed by inoculated VAM showed the superimposement of effective VAM isolates over the indigenous VAM population which resulted in a stimulation of increasing growth and crop yield. (Table 17).

Data in Table 18 which showing the plant nutrient analysis of nitrogen, phosphorus and potassium indicated a similar result as found in soybean. There were no differences among treatments at Kalasin. At Prachinburi, however, there were some differences between the mean data of some isolates inoculated and the non-inoculated but was not different in statistic. Differences were found among the N, P, and K content in soil. The plots applied with effective VAM species had nutrient left over in soil (Table 19) less than the control plot.

Table 15. Average pod dry weight and yield of mungbean inoculated with VAM species at Kalasin and Prachinburi (1st crop).

Kalasin

Treatments	Dry pod (g/pl)			Yield (kg/ha)		
	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	7.74	4.86 ab	6.09	617.9 bcd	643 ab	630 ab
GLOMUS # 17	8.96	7.42 ab	8.19	588.7d	618 ab	603 b
INT 183 # 26	9.24	6.11 ab	7.68	701.4 a	622 ab	662 a
ASCR # 22	6.18	4.14 b	5.16	663.8 ab	664 a	664 a
ETU 157 # 31	9.47	3.90 b	6.68	605.4 bcd	589 b	579 b
DES 160 # 39	6.56	9.18 a	7.87	668.0 ab	668 a	668 a
Control	6.79	8.28 ab	7.54	601.2 cd	605 ab	603 b
Mean	7.8522 a	7.0464 a	7.0464	635.18 a	629.82 a	632.51
CV.P (%)		59.2			4.1	
CV. Species (%)		43.2			6.5	

Prachinburi

Treatments	Dry pod (g/pl)			Yield (kg/ha)		
	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	5.79 a	2.33	4.06 ab	381	144	263
GLOMUS # 17	4.66 ab	2.84	3.75 ab	356	158	257
INT 183 # 26	5.41 ab	3.51	4.46 a	495	221	358
ASCR # 22	3.08 b	2.38	2.73 b	269	138	203
ETU 157 # 31	4.69 ab	2.92	3.80 ab	341	162	251
DES 160 # 39	4.69 ab	3.33	4.01 ab	277	160	219
Control	3.39 b	2.56	2.98 b	259	260	260
Means	4.53	2.84 b	3.68	340	178	259
CV.P (%)	40.6			51.7		
CV. Species (%)	70.2			60.5		

In a column, means followed by a different letter are significantly different at the 5% level by DMRT

Table 16. Percent root colonization of mungbean inoculated with mycorrhizal species at Kalasin and Prachinburi (1st crop)

Kalasin

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	12	9	11	29	23	26 abc	44	29.0	36 a
GLOMUS # 17	13	10	11	22	19	21 bc	31	25.8	29 ab
INT 183 # 26	12	10	11	27	33	30 ab	37	39.0	38 a
ASCR # 22	13	16	15	31	36	24 a	37	33.8	35 a
ETU 157 # 31	10	9	9	25	27	26 abc	22	30.3	26 b
DES 160 # 39	9	14	11	20	34	27 abc	38	34.0	36 a
Control	6	12	9	17	21	19 c	17	23.3	20 b
Means	11	11		25	28		32	31	
CV.P (%)	89.2			12.1			21.0		
CV. Species (%)	28.0			18.9			14.9		

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	15	14	15	20	17	18	16	38	27
GLOMUS # 17	21	25	23	32	16	24	34	24	29
INT 183 # 26	28	16	22	46	17	31	40	40	40
ASCR # 22	20	25	22	17	26	19	31	25	28
ETU 157 # 31	19	15	17	24	18	21	26	36	31
DES 160 # 39	15	15	15	13	21	17	31	33	32
Control	12	21	17	15	26	20	30	30	30
Means	19	19		24	19		30	32	
CV.P (%)	119.8			37.7			33.9		
CV. Species (%)	35.6			35.0			29.5		

In a column, means followed by a different letter are significantly different at the 5% level by DMRT

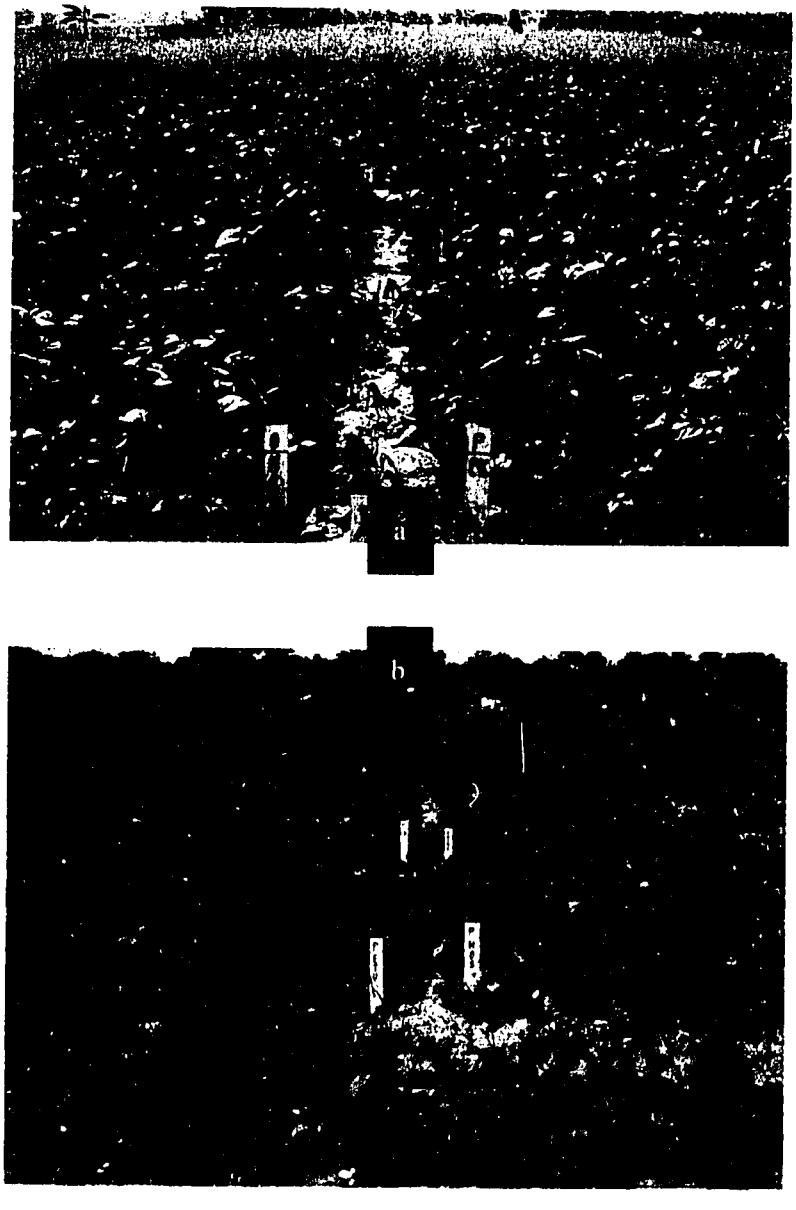

Figure 10. Mungbean field (1 st crop) (a). Mungbean grown at Kalasin inoculated with *Glomus mosseae* applied no rock phosphate (left), uninoculated with rock phosphate (right). (b). Mungbean grown at Kalasin inoculated with, *Glomus etunicatum* applied rock phosphate (left),*Glomus mosseae* with rock phosphate (right).

Table 17. Number of spores in 100 gram soil grown VAM inoculated mungbean at Kalasin and Prachinburi (1st crop)

Kalasin

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	44 b	99 ab	72	83	131	107	157	170	163 c
GLOMUS # 17	42 b	83 ab	62	80	127	103	189	100	194 abc
INT 183 # 26	42 b	92 ab	67	138	122	130	197	189	193 abc
ASCR # 22	44 b	119 ab	82	101	149	125	212	255	233 ab
ETU 157 # 31	30 b	69 b	49	107	114	110	217	165	191 abc
DES 160 # 39	80 a	147 a	113	111	141	126	288	206	247 a
Control	71 a	42 c	57	137	108	122	219	126	172 bc
Mean	50	93		108	127		211	187	
CV.P (%)		10.3			8.3			7.2	
CV.Species (%)		8.6			5.6			5.2	

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	102	86	43	87	60	74	33	110	72
GLOMUS # 17	81	69	35	50	82	25	138	134	136
INT 183 # 26	72	62	31	100	100	100	123	141	132
ASCR # 22	53	59	56	51	113	57	108	106	107
ETU 157 # 31	79	64	72	68	83	76	129	103	116
DES 160 # 39	74	79	77	110	70	90	161	151	156
Control	13	68	41	74	83	78	170	99	135
Mean	74	70		77	84		123	121	
CV.P (%)		1.8			10.4			3.8	
CV. Species (%)		11.1			10.8			7.4	

In a column, means followed by a different letter are significant different at the 5% level by DMRT

Table 18. Nitrogen, phosphorus, and potassium content in VAM inoculated mungbean shoot grown at Kalasin and Prachinburi.

Kalasin

Treatments	% N			% P			% K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	2.675	2.428	2.551	0.214	0.183	0.199	2.675	2.428	2.551 bc
GLO # 17	2.662	2.665	2.664	0.204	0.198	0.201	2.662	2.665	2.664 abc
INT 183 # 26	2.618	2.245	2.431	0.211	0.158	0.185	2.618	2.245	2.431 c
ASCR # 22	2.957	2.770	2.864	0.235	0.225	0.230	2.957	2.770	2.864 a
ETU 157 # 31	2.692	2.488	2.596	0.248	0.200	0.228	2.692	2.488	2.590 bc
DES 160 # 39	2.540	2.913	2.726	0.216	0.200	0.208	2.540	2.913	2.726 ab
Control	2.480	2.457	2.469	0.257	0.190	0.223	2.480	2.457	2.469 bc
Mean	2.661	2.566		0.228	0.193		2.661	2.566	
CV.P (%)	13.5			19.9			19.4		
CV. Species (%)	16.3			21.2			19.1		

Prachinburi

Treatments	% N			% P			% K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	1.343	1.214	1.279	0.107	0.092	0.100	1.330	1.210	1.270
GLO # 17	1.331	1.333	1.332	0.100	0.099	0.100	1.311	1.302	1.307
INT 183 # 26	1.309	1.123	1.216	0.107	0.082	0.095	1.309	1.122	1.216
ASCR # 22	1.479	1.385	1.432	0.119	0.110	0.115	1.470	1.380	1.425
ETU 157 # 31	1.346	1.244	1.295	0.110	0.101	0.106	1.337	1.244	1.291
DES 160 # 39	1.270	1.457	1.364	0.106	0.097	0.102	1.289	1.457	1.373
Control	1.240	1.229	1.235	0.100	0.090	0.095	1.240	1.221	1.231
Mean	1.331	1.284		0.107	0.096		1.327	1.277	
CV.P (%)	13.0			20.6			21.3		
CV. Species (%)	15.4			24.9			16.4		

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 19. Nitrogen, phosphorus, and potassium contents in soil grown VAM inoculated mungbean at Kalasin and Prachinburi.

Kalasin

Treatments	% N			P(ppm)			K(ppm)		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	1.58	1.34	1.46	69 b	10	40 b	80	41	61
GLO # 17	1.51	1.48	1.50	175 a	10	93 a	51	70	61
INT 183 # 26	2.68	1.68	2.18	65 b	11	38 b	65	80	73
ASCR # 22	1.28	1.41	1.35	43 b	17	30 b	41	82	62
ETU 157 # 31	1.54	1.41	1.48	54 b	11	33 b	67	60	64
DES 160 # 39	1.54	1.34	1.44	108 ab	10	59 ab	55	55	55
Control	1.54	1.68	1.61	158 a	11	85 a	67	87	77
Mean	1.67	1.48		96	11		61	68	
CV.P (%)	14.6			21.7			23.6		
CV. Species (%)	9.5			16.9			19.3		

Prachinburi

Treatments	% N			P(ppm)			K(ppm)		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	1.30	1.40	1.35	132	60	96	86	80	83
GLO # 17	1.42	1.48	1.45	98	64	81	74	75	75
INT 183 # 26	1.56	1.42	1.49	96	60	78	60	65	63
ASCR # 22	1.60	1.39	1.50	87	67	77	43	60	52
ETU 157 # 31	1.60	1.56	1.58	84	60	72	60	58	59
DES 160 # 39	1.60	1.31	1.46	160	53	107	59	58	39
Control	1.58	2.01	1.80	107	61	84	65	65	65
Mean	1.52	1.51		109	61		64	66	
CV.P (%)	8.7			24.9			16.9		
CV. Species (%)	19.6			18.7			21.4		

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

5.2 SURVIVAL OF VA MYCORRHIZA IN PADDY FIELD UNDER FLOODING CONDITION.

To determine the survival of VA mycorrhiza in paddy field under flooding condition, the same plots trial from 4.1 were maintained. The 1st crop of inoculated soybean and mungbean fields were harvested, the same fields were grown rice (without VAM inoculation) as a 2nd crop under flooding condition for 4 months. After rice was harvested, the 3rd crop of soybean and mungbean were grown without reinoculation. The survival of inoculated VAM was determined by the examining number of spores in soil and percent root colonization of VAM in the 3rd crop. To confirm the number of VAM survival from flooded condition, the exact number of VAM spores survival were detected via pot experiments in the glasshouse.

5.2.1 Survival of VAM species under flooding soil condition in pot in the glasshouse.

Data showing in Table 20 revealed that VAMF did not infect the rice root. After rice was grown in the pot under flooded condition for four months, the spores of 6 VAMF inoculated species did not increase in the number in soil and found that some of the spores still survived. There were no differences in height, weight and seed between inoculated and noninoculated rice. The results showed that when VAMF were applied to the rice VAMF did not infect the rice root, and VAMF spores applied, could survive in soil under flooded condition even no host plant. Under the field condition there were some other plants such as grasses or weeds which could be the alternate host for the survival VAM spores to infect and multiply the spores in soil after flooded condition. In this case, VAMF that could survive and can play some role on enhancing the nutrient absorption in the third crop that grown after rice even no reinoculation of VAM fungi.

Table 20. Average height, dry weight, seed yield, % root colonization, number of spores in soil and N P K content in rice grown in glasshouse.

Treatments	Height (cm)	Dry weight (gm/pl)	Yield (gm/pot)	% root colonization	No. of spores/pot
MOS 156 # 6	132 ab	282.0	50.4	0	160
GLO # 17	126 ab	174.8	47.1	0	180
INT # 26	136 ab	251.8	46.5	0	60
ASCR # 22.	124 b	189.3	91.6	0	200
ETU 157 # 31	136 ab	226.0	42.3	0	220
DES 160 # 39	139 a	231.5	25.2	0	220
Control	128 ab	245.2	57.7	0	10
CV.	3.75	27.47	20.08	–	9.43

Talbe 20 cont.

	% N	% P	% K
MOS 156 # 6	0.753	0.139	1.700
GLOMUS # 17	0.890	0.160	2.163
INT # 26	1.003	0.156	3.003
ASCR # 22	0.893	0.150	1.913
ETU 157 # 31	0.857	0.144	2.080
DES 160 # 39	0.780	0.150	2.440
Control	0.873	0.145	1.823
CV.	3.7	5.4	39.6

In a column, means followed by a different letter are significantly different at the 5% levelby DMRT.

5.2.2 Survival of VAM species under flooding condition in the 2nd crop of rice following the first crop of VAM inoculated soybean/mungbean.

5.2.2.1 Soybean

Height of rice RD 23 at both Chiangmai and Prachinburi (Table 21) was not significantly different among inoculated plots nor between inoculated and non inoculated plots at all stages of rice growth. The same results were found on shoot dry weight of rice at both places (Table 22). Eventhough yields of inoculated soybean (Table 19) in 1st crop were greater than the noninoculated but no significantly differences were found in this crop. Only mean yield of rice grown in the plot inoculated with *Glomus intraradices* and *Glomus deserticola* were greater than the noninoculated about 4.2% and 4.1% respectively at Chiangmai. At Prachinburi yield of rice grown in the plot inoculated with *Glomus intraradices* was greater than the noninoculated about 3.3 %. (Table 23).

The colonization of VAM was not found in any rice root eventhough quite a number of spores were survival in the soil but no infection occurred (Table 24, 25).

Data in Table 26 are showing the nutrient content in plant tissue of rice grown at Chiangmai and Prachinburi. No differences were found on the nitrogen, phosphorus and potassium content among the inoculated plots and between the inoculated and noninoculated plots. For Phosphorus content the mean data in the plot applied with rock phoaphate were a little bit greater than the non phosphate applied plots.

Table 21. Average height (cm) of rice grown after mycorrhizae inoculated soybean at Chiangmai and Prachinburi (2nd crop)

Chiangmai

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	70.9	68.3	69.6	79.0	80.4	79.7	111.2	108.5	109.9
GLOMUS # 17	69.8	68.3	69.1	79.7	79.8	79.8	110.2	110.2	110.2
INT 183 # 26	71.7	70.2	71.0	79.8	80.2	80.0	112.0	108.7	110.4
ASCR # 22	68.3	72.8	70.6	79.3	79.2	79.3	105.3	108.0	106.7
ETU 157 # 31	68.7	66.0	67.4	79.5	79.7	79.6	108.8	107.0	107.9
DES 160 # 39	68.4	70.2	69.3	79.7	78.8	79.3	110.2	108.9	109.6
Control	69.1	66.4	67.8	82.0	79.6	80.9	110.1	107.8	108.9
Mean	69.6	66.9	69.2	79.9	79.7	79.8	109.7	108.4	109.1
CV.P (%)		4.1			5.8			2.7	
CV.Species (%)		6.9			2.8			3	

Prachinburi

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	60.2	59.7	60.0	95.8	90.3	93.1	108.7	100.5	104.6
GLOMUS # 17	62.9	61.9	62.4	94.2	89.0	91.6	106.5	98.7	102.3
INT 183 # 26	60.5	60.0	60.3	95.3	90.0	92.7	105.4	99.0	102.2
ASCR # 22	61.7	59.8	60.6	94.3	88.5	91.4	104.3	96.4	100.4
ETU 157 # 31	62.5	60.3	61.4	94.5	87.8	91.2	103.7	96.0	100.6
DES 160 # 39	62.4	61.0	61.7	93.4	89.6	91.5	103.0	102.3	102.7
Control	61.0	60.0	60.5	92.1	87.9	90.0	103.2	100.2	101.7
Mean	61.6	60.4	61.0	94.2	89.0	91.6	105.3	99.0	102.1
CV.P (%)		4.6			10.7			7.5	
CV.Species (%)		12.0			13.7			9.2	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT

Table 22. Average shoot dry weight (gm/pl) of rice grown after mycorrhiza inoculated soybean at Chiangmai.

Chiangmai

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	14.4	8.6	11.5	35.7	25.3	30.5	30.1	24.1	27.1
GLOMUS # 17	18.3	10.8	14.55	29.1	29.6	29.35	29.2	29.7	29.45
INT 183 # 26	14.6	12.5	13.55	26.1	33.9	30.00	25.2	28.6	26.9
ASCR # 22	14.5	11.3	12.9	26.1	28.3	27.2	30.7	24.8	27.75
ETU 157 # 31	16.9	13.7	15.2	28.8	28.7	28.75	31.3	21.9	26.6
DES 160 # 39	15.0	15.0	17.1	27.6	24.1	25.85	27.2	30.2	28.7
Control	19.2	15.0	17.1	27.6	24.1	25.85	27.2	30.2	28.7
Mean	16.12	12.51		28.75	29.3		29.15	25.9	
CV.P (%)		17.7			25.0			14.2	
CV.Species (%)		51.1			30.1			23.7	

Table 23. Average yield of rice grown after mycorrhizae inoculated soybean at Chiangmai and Prachinburi (2nd crop).

Treatments	Yield (kg/ha)					
	Chiangmai			Prachinburi		
	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	4909	4471	4690	3927	3580	3754
GLOMUS # 17	4884	4508	4696	3910	3607	3759
INT 183 # 26	4816	4802	4809	3867	3854	3860
ASCR # 22	4386	4491	4441	3840	3725	3783
ETU 157 # 31	4817	4575	4696	3853	3660	3757
DES 160 # 39	4939	4671	4805	3952	3738	3845
Control	4726	4507	4616	3760	3710	3735
Mean	4782	4576		3873	3696	
CV.P (%)	31.5	15.7		20.5	23.5	
CV.Species (%)	19.2	11.0		12.0	17.3	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT

Table 24. Percent root colonization of rice grown after VAM inoculated soybean at Chiangmai and Prachinburi (2nd crop).

Chiangmai

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0	0	0	0	0	0	0	0	0
GLOMUS # 17	0	0	0	0	0	0	0	0	0
INT 183 # 26	0	0	0	0	0	0	0	0	0
ASCR # 22	0	0	0	0	0	0	0	0	0
ETU 157 # 31	0	0	0	0	0	0	0	0	0
DES 160 # 39	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0	0	0	0	0	0	0	0	0
GLOMUS # 17	0	0	0	0	0	0	0	0	0
INT 183 # 26	0	0	0	0	0	0	0	0	0
ASCR # 22	0	0	0	0	0	0	0	0	0
ETU 157 # 31	0	0	0	0	0	0	0	0	0
DES 160 # 39	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0
CV. (%)	–	–		–	–		–	–	
CV.Species (%)	–	–		–	–		–	–	

Table 25. Number of spore in 100 gram soil grown rice after VAM inoculated soybean at Chiangmai and Prachinburi (2nd crop).

Chiangmai

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	103	90	97 cd	61	53	57 c	58	36	47
GLOMUS # 17	158	109	134 bc	50	59	55 c	42	71	57
INT 183 # 26	273	118	196 a	79	67	73 ab	88	80	84
ASCR # 22	152	121	137 ab	59	73	66 abc	72	53	63
ETU 157 # 31	90	69	80 d	66	54	60 bc	81	64	43
DES 160 # 39	185	169	177 a	73	83	78 a	116	73	95
Control	106	125	116 bc	65	74	70 abc	106	59	83
Means	152	114		65	66		80	62	

Prachinburi

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	102	81	92	64	57	61	52	56	54
GLOMUS # 17	39	65	52	30	49	40	37	40	39
INT 183 # 26	91	101	96	60	83	72	51	70	61
ASCR # 22	90	134	112	59	92	76	43	84	64
ETU 157 # 31	117	89	103	67	80	75	56	73	65
DES 160 # 39	35	91	63	23	74	49	37	65	51
Control	84	95	90	45	69	57	40	53	47
Mean	80	94	87	50	72	61	45	63	54
CV.P (%)		10.9			15.4			21.2	
CV. Species (%)		14.7			8.3			20.9	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 26. Average nitrogen, phosphorus and potassium content in rice (2nd crop) grown after VAM inoculated soybean at Chiangmai and Prachinburi

Chiangmai

Treatments	N			P			K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.540	0.537	0.539	0.103	0.096	0.100	2.468	2.591	2.530
GLO # 17	0.550	0.532	0.541	0.101	0.093	0.097	2.493	2.541	2.517
INT 183 # 26	0.471	0.558	0.514	0.103	0.113	0.108	2.224	2.370	2.297
ASCR # 22	0.528	0.528	0.528	0.093	0.078	0.085	2.273	2.493	2.383
ETU 157 # 31	0.518	0.528	0.523	0.099	0.096	0.098	2.444	2.503	2.473
DES 160 # 39	0.564	0.507	0.536	0.101	0.089	0.095	2.517	2.419	2.468
Control	0.562	0.551	0.557	0.099	0.103	0.101	2.555	2.322	2.438
Mean	0.533	0.534		0.100	0.095		2.425	2.463	
CV.P (%)		2.6			6.8			5.8	
CV.Species (%)		8.4			9.3			9.4	

Prachinburi

Treatments	N			P			K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.521	0.581	0.551	0.100	0.109	0.104	2.370	2.175	2.273
GLO # 17	0.518	0.507	0.513	0.095	0.112	0.104	2.175	2.150	2.163
INT 183 # 26	0.561	0.537	0.549	0.096	0.110	0.103	2.346	2.224	2.285
ASCR # 22	0.569	0.613	0.591	0.106	0.115	0.110	2.150	2.224	2.187
ETU 157 # 31	0.556	0.573	0.565	0.073	0.105	0.089	2.224	2.224	2.224
DES 160 # 39	0.576	0.546	0.561	0.102	0.103	0.103	2.273	2.321	2.297
Control	0.564	0.542	0.553	0.111	0.099	0.105	2.248	2.273	2.260
Mean	0.552	0.557		0.097	0.108		2.255	2.227	
CV.P (%)		3.2			30.7			4.7	
CV.Species (%)		11.4			6.9			6.5	

5.2.2.2 Mungbean field.

The same results were found in mungbean field as in soybean field. No differences were found on shoot height and shoot dry weight of rice at Kalasin and Prachinburi (Table 27, 28). At Kalasin, rice yield in the mungbean plot inoculated wjth *Glomus intraradices* was 3.4% greater than noninoculated plot (Table 29). At Prachinburi rice yield in the mungbean plot inoculated with *Glomus intraradices* was only 1% greater than noninoculated plot.

There was no explanation on the effect of some increasing in rice yield compared to the result in Table 27 which showed no infection in the rice root (Table 30). So VAM might act in some way in the soil to increasing rice yield as shown in *Glomus intraradices* treatment. The spores of VAM were found in consider amount in both inoculated and noninoculated but lesser than in the 1st crop (Table 31).

No difference on nitrogen, phosphorus and potassium content in rice, grown after mungbean, were found between incoulated and noninoculated plot at both Kalasin and Prachinburi (Table 32).

Table 27. Average height (cm) of rice grown after VAM inoculated mungbean at Kalasin and Prachinburi (2nd crop).

Kalasin

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	65.7	62.7	64.2	99.1	86.9	93.0	107.5	102.1	104.8 ab
GLOMUS # 17	69.0	61.6	65.3	96.8	94.5	95.7	105.7	101.8	103.8 b
INT 183 # 26	66.3	68.7	67.5	97.7	97.5	97.6	104.7	103.0	103.9 ab
ASCR # 22	68.6	62.6	65.6	98.1	93.1	95.6	105.4	102.9	104.2 ab
ETU 157 # 31	68.7	62.4	65.5	97.4	90.1	93.8	104.7	101.8	103.3 ab
DES 160 # 39	67.7	65.0	66.4	95.2	92.6	93.9	105.2	104.7	105.0 ab
Control	67.6	65.4	66.5	96.4	97.2	96.8	105.7	105.7	105.7 a
Mean	67.7	64.1		97.2	93.1		105.1	103.1	
CV.P (%)		4.6			9.8			2.5	
CV. Species (%)		6.9			5.4			2.4	

Prachinburi

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	60.4	60.0	60.2	86.5	81.7	84.1	99.7	87.3	93.5
GLOMUS # 17	65.0	63.7	64.4	85.3	80.5	82.9	96.5	90.2	93.4
INT 183 # 26	63.7	61.5	62.6	86.4	80.5	83.5	98.2	95.6	96.9
ASCR # 22	64.4	61.3	62.9	85.7	81.3	83.5	97.3	90.9	94.1
ETU 157 # 31	61.3	60.2	60.8	84.5	80.4	82.5	96.2	87.9	92.1
DES 160 # 39	65.0	61.3	63.2	83.2	79.3	82.3	94.9	90.0	92.5
Control	64.3	61.0	62.7	85.0	80.7	48.3	95.3	91.7	93.5
Mean	63.4	61.3	62.4	85.2	80.6	78.2	96.9	90.5	93.7
CV.P (%)		5.9			17.2			8.6	
CV. Species (%)		10.6			9.0			5.9	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 28. Average shoot dry weight (g/pl) of rice grown after VAM inoculated mungbean at Kalasin and Prachinburi (2nd crop).

Kalasin

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	30.3 a	22.1 abc	26.2	23.9	14.8	19.4	24.6	17.6	21.1
GLOMUS # 17	21.0 b	17.1 c	19.1	20.4	20.2	20.3	17.5	16.0	16.8
INT 183 # 26	21.1 b	25.8 a	23.5	19.0	19.4	19.2	19.4	19.9	19.7
ASCR # 22	27.4 a	19.3 bc	23.4	22.9	18.9	20.9	21.9	18.3	20.1
ETU 157 # 31	21.2 b	24.5 ab	22.9	16.7	16.1	16.4	17.2	17.9	17.6
DES 160 # 39	26.1 ab	20.4 abc	23.3	17.2	17.5	17.4	21.0	18.9	20.0
Control	21.0 b	23.4 ab	22.2	17.3	19.2	18.3	17.9	22.6	20.3
Mean	24.0	21.8		19.6	18.0		19.9	18.7	
CV.P (%)		10.4			25.0			10.5	
CV.Species (%)		16.8			26.7			34.3	

Prachinburi

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	23.5	21.8	22.7	29.8	27.3	28.6	31.2	29.3	30.3
GLOMUS # 17	22.7	20.5	21.6	30.0	27.7	28.9	29.2	28.5	28.9
INT 183 # 26	23.2	22.7	23.0	27.9	25.8	26.9	30.5	26.5	28.5
ASCR # 22	21.8	20.3	21.1	29.3	27.3	28.3	30.9	28.7	29.8
ETU 157 # 31	22.6	20.7	21.7	28.4	24.7	26.6	28.3	25.9	27.1
DES 160 # 39	22.5	20.5	21.5	27.5	25.9	26.7	29.3	27.3	28.3
Control	20.7	20.0	20.4	27.0	24.8	25.9	29.9	27.0	28.5
Mean	22.4	20.9	21.7	28.6	26.2	27.4	29.9	27.6	28.8
CV.P (%)		7.8			12.7			17.8	
CV.Species (%)		13.2			15.9			19.6	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 29. Average yield of rice grown after VAM inoculated mungbean at Kalasin and Prachinburi (2nd crop).

Treatments	Yield (Kg/ha)					
	Kalasin			Prachinburi		
	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	4054	3524	3789	4506	4104	4305
GLOMUS # 17	3676	3336	3506	4303	4062	4184
INT 183 # 26	3900	4242	4071	4499	4179	4339
ASCR # 22	4334	3482	3908	4183	4007	4095
ETU 157 # 31	3566	3190	3378	4321	4175	4248
DES 160 # 39	3766	4083	3925	4476	4100	4288
Control	3991	3883	3937	4213	4018	4115
Mean	3898	3677		4357	4092	
CV.P (%)		15.9			22.7	
CV.Species (%)		19.0			19.3	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 30. Percent root colonization of rice grown after VAM inoculated mungbean at Kalasin and Prachinburi (2nd crop).

Kalasin

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0	0	0	0	0	0	0	0	0
GLOMUS # 17	0	0	0	0	0	0	0	0	0
INT 183 # 26	0	0	0	0	0	0	0	0	0
ASCR # 22	0	0	0	0	0	0	0	0	0
ETU 157 # 31	0	0	0	0	0	0	0	0	0
DES 160 # 39	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0

Prachinburi

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0	0	0	0	0	0	0	0	0
GLOMUS # 17	0	0	0	0	0	0	0	0	0
INT 183 # 26	0	0	0	0	0	0	0	0	0
ASCR # 22	0	0	0	0	0	0	0	0	0
ETU 157 # 31	0	0	0	0	0	0	0	0	0
DES 160 # 39	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0
CV.P (%)	–	–		–	–		–	–	
CV.Species (%)	–	–		–	–		–	–	

Table 31. Number of spores in 100 gram soil grown rice after VAM inoculated mungbean at Kalasin and Prachinburi (2nd crop).

Kalasin

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	74	34	54	48	70	59 a	18	20	19
GLOMUS # 17	57	41	49	29	41	35 ab	37	28	33
INT 183 # 26	113	72	93	24	58	41 ab	48	22	35
ASCR # 22	42	50	46	42	36	39 b	21	56	39
ETU 157 # 31	50	50	50	42	158	110 a	12	50	31
DES 160 # 39	54	58	56	50	39	45 ab	20	35	28
Control	56	64	60	34	44	39 ab	25	23	24
Mean	64	53		38	64		26	33	
CV.P (%)		36.3			51.5			12.3	
CV.Species (%)		26.5			22.9			30.3	

Prachinburi

Treatments	60 days			90 days			120 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	84	99	92	65	72	69	53	52	53
GLOMUS # 17	120	125	123	69	65	67	46	50	48
INT 183 # 26	107	109	108	92	90	91	59	47	53
ASCR # 22	95	97	96	84	76	80	67	51	59
ETU 157 # 31	103	117	110	66	70	68	50	48	49
DES 160 # 39	136	121	129	76	67	72	61	56	59
Control	125	85	105	68	69	69	60	59	60
Mean	110	108	109	74	73	74	57	52	54
CV.P (%)		12.3			11.7			3.0	
CV.Species (%)		6.4			10.2			6.4	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 32. Average nitrogen, phosphorus, potassium content in rice (2nd crop) grown after VAM inoculated mungbean at Kalasin and Prachinburi

Kalasin

Treatments	% N			% P			% K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.682	0.554	0.618	0.175	0.102	0.138	1.122	0.697	0.910
GLOMUS # 17	0.608	0.543	0.576	0.173	0.144	0.159	1.306	1.013	1.159
INT 183 # 26	0.538	0.597	0.568	0.157	0.146	0.152	1.100	1.100	1.100
ASCR # 22	0.493	0.520	0.506	0.147	0.135	0.141	1.051	1.022	1.036
ETU 157 # 31	0.603	0.531	0.567	0.167	0.113	0.140	1.156	0.819	0.988
DES 160 # 39	0.583	0.532	0.558	0.167	0.124	0.145	0.981	1.147	1.064
Control	0.610	0.660	0.635	0.154	0.188	0.171	1.148	1.359	1.254
Mean	0.588	0.562		0.163	0.136		1.124	1.023	
CV.P (%)	16.5			67.1			35.2		
CV.Species (%)	20.6			21.2			29.7		

Prachinburi

Treatments	% N			% P			% K		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	0.530	0.526	0.528	0.130	0.095	0.113	1.671	1.944	1.808
GLOMUS # 17	0.492	0.490	0.491	0.129	0.093	0.111	1.129	1.120	1.125
INT 183 # 26	0.562	0.506	0.534	0.139	0.079	0.109	1.571	1.970	1.771
ASCR # 22	0.541	0.540	0.541	0.137	0.079	0.108	1.744	2.006	1.875
ETU 157 # 31	0.491	0.552	0.522	0.102	0.088	0.095	1.629	1.845	1.737
DES 160 # 39	0.489	0.480	0.485	0.118	0.102	0.110	1.378	1.420	1.399
Control	0.520	0.530	0.525	0.116	0.090	0.103	1.495	1.734	1.615
Mean	0.518	0.518		0.124	0.089		1.517	1.720	
CV.P (%)	6.8			17.5			37.3		
CV.Species (%)	9.3			15.2			43.5		

Figure 11. Rice field (2nd crop) (a). Rice grown after mungbean at Kalasin. (b). Rice grown after soybean at Chiangmai, in *Acaulospora scrobiculata with rock phoshate plot (left), in Glomus mosseae with no rock phosphate plot (right).*

5.2.3 Survival of VAM species in the third crop of soybean/mungbean following the second crop of rice.

The 3rd crop of soybean and mungbean fields were established only at Chiangmai and Kalasin because at Prachinburi had not enough water to irrigate the plants.

5.2.3.1 Soybean field.

Data in Table 33 and 34 showed that there were no differences on height and plant dry weights of soybean between inoculated and noninoculated plots at Chiangmai. The differences were found on soybean yield inoculated with *Glomus intraradices*, *Acaulospora scrobiculata* and *Glomus etunicatum* which were greater than the noninoculated about 10%, 8% and 3% respectively (Table 35).

The infection of VAM isolates were found in soybean root at 30 days of soybean growth (Table 36). At 90 days root infection were found in all plots including the noninoculated plots. The number of spores produced in soil were found in all plots and the number in uninoculated plot was the greatest (Table 37). Even there were spores sporulated and colonized in soybean root of uninoculated plot, the higher yield of soybean grown in inoculated plot was still obtained, this evident indicated that the VAM isolates of *Glomus intraradices*, *Glomus etunicatum* and *Acaulospora scrobiculata* were superior to the indigenous VAM.

At Chiangmai the nitrogen content in soybean was high compared to the other crops. This might be due to the left over of rice straw in the second crop. No difference was found in the phosphorus and potassium content in soybean plant (Table 35).

Table 33. Average height(cm) of soybean (3rd crop) grown after rice at Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	36.1	34.5	35.3	56.4	51.2	53.8	60.3	55.3	57.8
GLOMUS # 17	34.6	33.0	33.7	57.0	49.5	53.3	60.5	54.3	57.4
INT 183 # 26	35.2	32.2	33.7	54.6	50.8	52.7	59.3	54.6	56.9
ASCR # 22	37.3	36.0	36.6	56.4	51.5	54.0	61.3	55.3	58.3
ETU 157 # 31	37.2	35.6	36.4	58.3	49.8	54.0	61.7	52.9	57.3
DES 160 # 39	34.2	34.7	34.4	50.3	48.2	49.2	54.4	52.6	53.5
Control	35.3	30.2	32.7	55.4	53.2	54.3	58.8	56.6	57.7
Mean	35.7	33.7	34.7	55.5	50.6	57.0	59.5	54.5	53.0
CV.P (%)		7.1			7.4			6.8	
CV.Species (%)		10.9			9.5			9.2	

Table 34. Average dry weight of soybean (3rd crop) grown after rice at Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	7.8	4.3	6.1	40.8	38.7	39.7	68.0	81.8	74.9 ab
GLOMUS # 17	4.7	4.9	4.8	44.9	41.5	43.2	67.7	83.3	75.5 ab
INT 183 # 26	7.3	5.5	6.4	43.6	32.6	38.1	68.6	66.1	67.3 b
ASCR # 22	7.2	4.9	6.0	47.7	37.5	42.6	89.2	68.5	78.8 ab
ETU 157 # 31	7.1	6.3	6.7	48.2	26.0	37.1	87.1	88.9	88.0 a
DES 160 # 39	5.9	5.8	5.8	32.1	40.8	36.5	61.8	83.6	72.7 ab
Control	6.1	5.1	5.6	44.2	38.9	41.6	72.3	59.8	60.0 b
Mean	6.6	5.2	5.9	43.1	36.6	39.8	73.5	76.0	74.8
CV.P (%)		48.0			50.0			67.5	
CV.Species (%)		27.5			24.2			32.0	

In a column, means followed by a different letter are signigicantly different at the 5% level by DMRT.

Table 35. Average yield and nitrogen, phosphorus and potassium content in soybean (3rd crop) grown after rice at Chiangmai.

Treatments	Yield (kg/ha)			N content (%)			P content (%)			K content (%)		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	2400	2119	2260	3.035	3.210	3.123	0.255	0.260	0.258	2.830	2.850	0.284
GLOMUS # 17	2404	2179	2292	3.030	3.013	3.022	0.241	0.257	0.249	2.827	2.824	2.826
INT 183 # 26	2499	2431	2465	3.003	3.030	3.017	0.230	0.262	0.246	2.695	2.821	2.758
ASCR # 22	2501	2331	2416	3.100	3.113	3.107	0.245	0.251	0.248	2.680	2.690	2.685
ETU 157 # 31	2333	2292	2308	3.068	3.003	3.036	0.249	0.256	0.253	2.670	2.740	2.705
DES 160 # 39	2183	2260	2222	3.060	3.042	3.051	0.238	0.251	0.245	2.663	2.782	2.723
Control	2310	2180	2245	3.008	3.010	3.009	0.240	0.250	0.245	2.665	2.771	2.718
Mean	2375	2256	2315	3.043	3.060		0.243	0.255		2.719	2.783	
CV.P (%)		7.4			8.6			9.3			8.9	
CV.Species (%)		12.0			11.5			10.9			7.3	

Table 36. Average percent root colonization of soybean (3rd crop) grown after rice at Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	9	10	10	24	22	23	55	59	37
GLOMUS # 17	8	7	8	28	29	28	64	57	60
INT 183 # 26	10	10	10	21	23	22	56	41	49
ASCR # 22	9	9	9	30	28	29	68	50	59
ETU 157 # 31	8	8	8	20	23	25	45	47	46
DES 160 # 39	8	8	8	27	28	27	60	33	47
Control	8	8	8	24	27	26	74	58	66
Mean	9	9	9	25	26	25	60	49	55
CV.P (%)		9.8			37.9			54.3	
CV. Species (%)		17.0			20.3			26.9	

Table 37. Average number of spores in soil grown soybean (3rd crop) after rice at Chiangmai

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 16	63	58	61	203	170	186	260	181	221
GLOMUS # 17	50	49	50	158	148	153	181	225	203
INT 183 # 26	56	60	58	163	242	202	211	136	174
ASCR # 22	69	61	65	207	126	166	184	242	213
ETU 157 # 31	43	60	52	146	184	165	196	194	195
DES 160 # 39	49	54	52	175	220	198	173	328	251
Control	65	60	63	136	154	145	282	205	244
Mean	56	57	57	170	178	174	212	216	214
CV.P (%)	15.7			10.9			32.1		
CV. Species (%)	21.9			10.3			12.9		

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5.2.3.2Mungbean field.

No difference was found on the mungbean height at 30 and 60 days after planting (Table 38). At 60 days, mungbean inoculated with*Glomus* # 17 was taller than the noninoculated one. No differences were found on the mungbean shoot dry weight at any stage (Table 39). The differences were found on mungbean yield in the plot inoculated with *Glomus mosseae*, *Acaulospora scrobiculata* and *Glomus deserticola* which were greater than the mungbean yield grown in noninoculated plot about 4%, 2%, and 2% respectively (Table 40). The incidence of percent root colonization in mungbean root and the number of spore in soil were the same as the soybean experimental plot (Table 41,42)

At Kalasin, no differences were found in the nutrient content of nitrogen, phosphorus and potassiun (Table 40). For phosphorus content, the plots applied with rock phosphate were greater in P content than the nonphosphate application. This was probably due to rock phosphate could be released more available phosphorus to plant and the Kalasin soil also contained low phosphorus.

Survival incidence of inoculated VAM isolates and indigenous species were similar in both soybean and mungbean plots. Yield of inoculated soybean and mungbean were significantly greater than noninoculated in the 1st crop, but no significantly differences were found in rice yield of the 2nd crop except for some isolates that greater in mean yield when compared to the mean value of noninoculated. For the 3rd crop, soybean and mungbean yield in some inoculated plot had higher yield than the noninoculated plot. Although the differences were not shown statistically but the mean data showed some evident of superimposement of the inoculated VAM isolates over the indigenous VAM.

Root colonization was highest in the 1st crop. The inoculum placed below the seed provided a greater opportunity for introduced fungi to infect, the root prior the indigenous fungi which were abundant throughout the soil. Therefore, both fields where soil contained the indigenous fungi at the same level of inoculum, plant growth and yield responded significantly to inoculation. The colonization in the root were found in the 1st and 3rd crop of soybean and mungbean, but not in the root of rice grown as the 2nd crop.

Quantity of spores in the soil from the 1st crop were higher and decreased with time during flooding condition. Number of spores were lowest when the land was flooded about 20-30 inches of water for four months. Then they were increased again in the 3rd crop when soybean and mungbean were grown. This showed that some VAM spore could survive under flooded condition. Harley and Smith (1983) also stated the most VAMF could survive as spores or other resting structures and may persist as mycelium in perviously active root. In this experiment the survival VAMF showed their effectiveness of some isolates on increasing soybean and mungbean yield in some extent over the indigenous VAM which naturally always presented in the soil.

Table 38. Average height (cm) of mungbean (3rd crop) grown after rice at Kalasin

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	42.8	41.2	42.5 bc	53.2	52.0	52.6	66.4	66.9	66.6 ab
GLOMUS # 17	43.3	43.4	43.4 a	51.4	53.4	52.4	67.3	67.6	67.4 a
INT 183 # 26	42.1	42.1	42.1 cd	52.3	50.0	51.1	66.2	66.3	66.2 b
ASCR # 22	43.0	42.8	42.9 ab	53.2	51.0	52.1	67.0	65.8	66.4 b
ETU 157 # 31	42.9	42.8	42.8 ab	53.0	53.8	53.4	66.1	65.6	66.9 ab
DES 160 # 39	42.9	42.0	42.5 bc	53.9	53.1	53.5	66.2	66.7	66.4 b
Control	41.6	41.7	41.6 d	52.1	52.3	52.2	66.0	65.8	65.9 b
Mean	42.6	42.4	42.5	52.7	52.2	52.2	66.5	66.4	66.4
CV.P (%)	3.0			2.7			1.6		
CV. Species	1.4			2.4			1.3		

In a column, means followed by a different letter are signigicantly different at the 5% level by DMRT.

Table 39. Average shoot dry weight (g/pl) mungbean (3rd crop) grown after rice at Kalasin

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	4.7	4.7	4.7	13.2	15.1	14.2	17.6	16.9	17.2
GLOMUS # 17	5.1	4.8	4.9	11.6	11.7	11.6	16.2	16.2	16.2
INT 183 # 26	4.8	4.7	4.7	12.3	12.6	12.4	16.9	17.0	17.0
ASCR # 22	4.7	4.6	4.7	16.0	12.1	14.1	17.3	16.5	16.9
ETU 157 # 31	5.0	4.9	5.0	18.7	11.7	14.7	16.7	15.9	16.3
DES 160 # 39	5.0	4.8	4.9	12.7	14.0	13.4	16.7	16.6	17.0
Control	4.7	4.7	4.7	16.9	14.6	15.7	17.3	15.8	16.2
Mean	4.9	4.7	4.8	14.5	13.0	13.8	16.7	16.4	16.7
CV.P (%)		36.2			76.7			26.7	
CV. Species (%)		14.25			26.5			15.3	

Table 40. Average yield, nitrogen, phosphorus and potassium content of mungbean (3rd crop) grown after rice at Kalasin

Treatments	Yield (kg/ha)			N content (%)			P content (%)			K content (%)		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	750	750	750 a	1.191	1.224	1.208	0.109	0.092	0.101	1.695	1.690	1.693
GLOMUS # 17	704	692	698 b	1.189	1.016	1.103	0.105	0.084	0.095	1.680	1.664	1.672
INT 183 # 26	713	725	719 ab	1.053	1.021	1.037	0.115	0.083	0.099	1.843	1.825	1.834
ASCR # 22	746	721	733 ab	1.116	1.184	1.150	0.117	0.106	0.112	1.726	1.830	1.778
ETU 157 # 31	721	692	706 b	1.044	1.209	1.127	0.108	0.091	0.100	1.710	1.813	1.762
DES 160 # 39	738	729	733 ab	1.113	1.200	1.157	0.107	0.090	0.099	1.820	1.764	1.792
Control	692	709	700 b	1.028	1.140	1.084	0.110	0.096	0.103	1.250	1.510	1.380
Mean	723	717	720	1.105	1.142		0.110	0.092		1.675	1.728	
CV.P (%)		2.4										
CV.Species (%)		4.5										

Table 41. Average percent root colonization of mungbean (3rd crop) grown after rice at Kalasin.

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	12	49	30	14	18	16	20	21	21
GLOMUS # 17	20	22	21	25	20	23	30	32	31
INT 183 # 26	14	23	18	20	27	24	27	43	35
ASCR # 22	18	18	18	24	29	27	30	31	31
ETU 157 # 31	14	20	17	16	19	17	19	26	22
DES 160 # 39	21	34	27	19	27	23	20	29	25
Control	18	34	26	21	35	28	34	40	37
Mean	17	29	23	20	25	22	26	32	29
CV.P (%)		89.4			52.7			91.0	
CV. Species (%)	35.6			45.2			43.3		

Table 42. Average munber of spores in soil grown mungbean (3rd crop) after rice at Kalasin

Treatments	30 days			60 days			90 days		
	P_1	P_0	Mean	P_1	P_0	Mean	P_1	P_0	Mean
MOS 156 # 6	23	31	27	59	66	62.5 ab	85	99	92 ab
GLOMUS # 17	20	27	24	42	54	48 b	59	84	72 ab
INT 183 # 26	19	24	22	41	47	44 b	54	68	61 b
ASCR # 22	20	32	26	73	80	77 a	96	118	107 ab
ETU 157 # 31	16	29	23	68	79	74 a	58	108	83 ab
DES 160 # 39	20	38	29	43	45	44 b	50	63	57 b
Control	20	27	24	86	110	98 a	116	135	125 a
Mean	30	30	25	59	69	64	74	96	85
CV.P (%)		21			37			20	
CV. Species (%)		16			23			14	

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Figure 12. Soybean field (lst corp) after harvested.

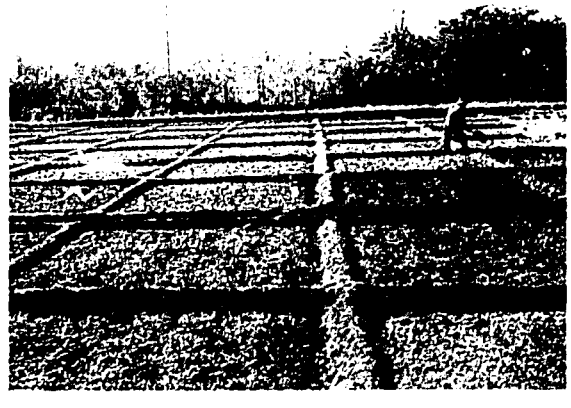

Figure 13. Land prepared for the 3rd crop.

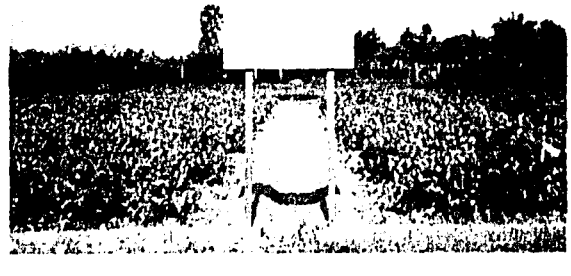

Figure 14. Soybean field (3rd crop) at Chiangmai

5.3 IMPROVING THE METHODS FOR PRODUCING VA MYCORRHIZA INOCULUM FOR FIELD TRIAL.

Pot culture is the practical method for VAM inoculum production at present. The pure collected spores were multiplied in pot culture. The spores produced in soil and root fragments were used as the inoculum. The various culture methods were described by many researchers. Manipulation of VAMF rooting media, hosts, fertilizeres and enveronment to produce VAM inoculum were resemble an art form rather than true science. Many scientists beleived that the difficulties involve in producing clean, high quality VAM inoculum on a commercial scale may prevent any large scale agricultural use of these fungi (Menge, 1984; Johnson and Hummel, 1985; Azcon, 1986; Adelman and Morton, 1985; Daneils & Trappe, 1978; Daneils & Menge, 1980; Graham & Timmer, 1984; Manjunath 1984; Morton, 1985; Ferguson, 1981). These experiments were achieved to find: first, the suitable number of spores to be used in the pot experiment. The result from this experiment was used to estimate the quantity of inoculum to be used in the field experiment. The second experiment was designed to produce high number of VAM inoculum by using the appropriate media instead of only soil or sand for grow plant host. The third experiment was to select the suitable host plant to produce enough inoculum to be used in the further field experiment. The VAM isolates used in this study were the isolates from Dr. Menge's laboratory at California.

5.3.1 Study the appropriate VAM spores density for inoculum size.

Results from Table 43 showed that the percent root colonization of peanut root were increased with the increasing of VAM inoculum. The plant inoculated with the highest rate of 100 spores pot gave the maximum root infection, shoot, root dry weight and yield, but increasing amount of VAM inoculum seemed to inhibit the height of plant. In general, it appeared that increasing inoculum dosage resulted in increasing the percentage of root colonization. However when optimun limit of root colonization was reached, greater inoculum size was not effect on increasing root colonization (Ferguson 1981). In annual crops with short growing season, high inoculum dosage may be required if maximum plant growth stimulation is to be achieved. Menge and Timmer (1982) suggested that 10 to 20 spores are usually sufficient to inoculate roots of young seedling grown aseptically in the greenhouse when the spores are placed in contract with the root. From this result the experiment in the field trial was used the double VAM inoculum size, 200 spores per plant to compete with the indigenous population in the soil

Table 43. Effect of VAM spores density on plant growth, yield and root colonization of peanut Tainan-9 at 100 days.

Treatments	plant growth			Shoot/root ratio	dry pod yield (g/pl)	root colonization (%)
	height (cm)	shoot dry wt. (g/pl)	root dry wt. (g/pl)			
1. 0 Spore	39.5 c	5.0 c	1.4 ab	1:0.27	0.43 b	3.4 c
2. 10 Spores	43.8 ab	7.9 abc	1.6 ab	1:0.20	0.29 bc	8.9 bc
3. 20 Spores	46.5 a	7.8 abc	1.9 a	1:0.25	0.14 c	4.0 c
4. 30 Spores	42.0 bc	10.3 a	1.8 ab	1:0.17	0.18 bc	6.5 bc
5. 40 Spores	40.3 c	7.5 abc	1.7 ab	1:0.22	0.15 c	32.3 ab
6. 50 Spores	39.9 c	7.0 bc	1.1 b	1:0.16	0.11 c	33.6 ab
7. 100 Spores	39.8 c	9.2 ab	2.0 a	1:0.22	0.91 a	44.6 a
% CV.	4.9	23.6	29.3		25.2	60.5

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

5.3.2 Selection of appropriate media for pot culture.

The results in table 44 showed that a growth medium of sand and clay mixing was the best in all parameters: plant growth, root infection and sporulation, follwed by peat. Cocounut coir dust was also good on root infection and sporulation but moderately on plant growth. The porus media such as peat, rice husk, coconut coir dust, sand + peat except vermiculite were good for fungi infection the same as clay or clay + sand. Furthermore the sporulation showed the same pattern as root infection except in rice husk medium which showed very low in sporulation eventhough the percent root infection was rather high. Vermiculite medium was even provided low in percent root colonization but high number of spores could be produced in soil and plant growth responded the vermiculite medium.

5.3.3 Selection of suitable host plant for pot culture.

The results from Table 45 showed that soybean, corn, sorghum and onion could be used as the suitable host plants for VAM production in pot culture because of their root system were suitable for the infection, colonization and sporulation of VAMF. Corn was the best host plant, better than sorghum, soybean and onion respectively. Kudzu was the second alternative, bahia grass and sabi grass were not suitable for using as the host plants to produce mass inoculum. If the purpose was to maintain the VAM inoculum in pot culture, these grasses could be used as the host plants as well as kudzu. For pineapple, they were harvested after the other plants because no infection and growth response were found by inoculation. After 8 months there were very few infections found in the roots. So it was concluded that pineapple can not be used as the host plant for VAM production.

Table 44. Average height, dry weight, number of spores and % root colonization of VAM inoculated peanuts grown in different media.

Treatments	Height (cm)		Dry weight/(g/pl)		No. of spores/100 g.growth media		% Root colonization	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
1. Sand	29.7 de	34.5 e	1.82 cd	1.38 c	105 b	160 a	6 b	27 b
2. Clay	48.3 abc	58.0 abc	4.72 ab	3.49 abc	284 a	315 a	8 b	35 b
3. Peat	54.9 a	66.5 a	2.98 bcd	4.73 a	345 a	351 a	17 a	58 a
4. Loamy sand	28.5 e	34.0 e	1.08 d	1.50 c	21 b	164 a	6 b	25 b
5. Rice husks	38.8 cde	39.8 de	2.24 bcd	1.67 bc	2 c	12 b	11 ab	40 ab
6. Coconut coir dust	42.7 bc	49.0 bcde	3.06 abc	2.34 bc	139 a	140 a	14 a	48 a
7. Sand + Peat	40.7 bc	43.5 cde	3.57 abc	2.59 bc	245 a	294 a	9 b	39 b
8. Sand + clay	51.9 ab	63.3 ab	5.20 a	5.03 a	313 a	370 a	12 a	60 a
9. Vermiculite	49.6 abc	52.7 abcd	3.86 abc	3.72 ab	144 a	156 a	4 b	16 b
% CV.	11.59	7.83	17.61	11.45	53.74	59.31	67.93	65.24

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Table 45. Effect of different kinds of host plant on root dry weight, % root colonization and spores in soil when infected by *Glomus fasiculatum*

Plant host	Root dry weight (g pl)				Root colonization (%)			Spores/100 g. soil		
	30 days	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days
1. Onion	6.85 a	6.94 a	4.92 b	3.80 c	46 a	80 a	80 a	253 c	342 cd	312 c
2. Sorghum	6.60 a	8.38 a	8.29 a	8.11 b	12 bc	60 a	80 a	293 b	413 a	410 a
3. Corn	4.87 a	1.30 c	3.91 b	9.01 a	20 b	76 a	80 a	269 bc	365 bc	415 a
4. Soybean	1.67 c	3.59 b	3.60 b	3.68 c	32 a	64 a	84 a	333 a	421 a	431 a
5. Mungbean	1.54 c	0.51 c	0.34 c	1.28 d	6 c	10 c	14 cd	205 de	313 d	329 c
6. Peanut	2.56 b	3.80 b	1.72 c	1.20 d	10 bc	46 b	20 c	253 c	372 bc	379 b
7. Sabi	0.42 d	0.72 c	0.71 c	1.20 d	6 c	6 cd	8 d	137 cf	284 d	310 c
8. Bahia	1.17 c	0.46 c	0.25 c	1.18 d	8 c	10 c	10 d	170 e	319 d	325 c
9. Kudzu	1.37 c	2.42 b	2.89 b	4.59 c	10 bc	27 b	56 b	250 c	368 bc	390 ab
10. Cowpea	1.52 c	3.50 b	3.75 b	3.87 c	20 b	31 b	54 b	210 d	300 d	313 c
11. Sudangrass	3.06 b	3.87 b	7.34 a	8.00 b	19 b	43 b	85 a	287 b	400 ab	410 a
12. Chinese cabbage	0.61 d	0.78 c	1.23 c	1.36 d	0 d	0 d	0 d	130 f	139 e	120 d
CV. (%)	11.0	8.7	15.9	17.6	39.7	27.0	35.2	17.9	20.3	10.5

In a column, means followed by a different letter are significantly different at the 5% level by DMRT.

Figure 15. Pot culture in the clay pot using (a). corn *(Zea mays)* as the host plant, (b). grasses *(Dactyloctenium aegyptium* L. Richer), (c). pot culture in Leonard jar using Arachis hypogaea as the host plant

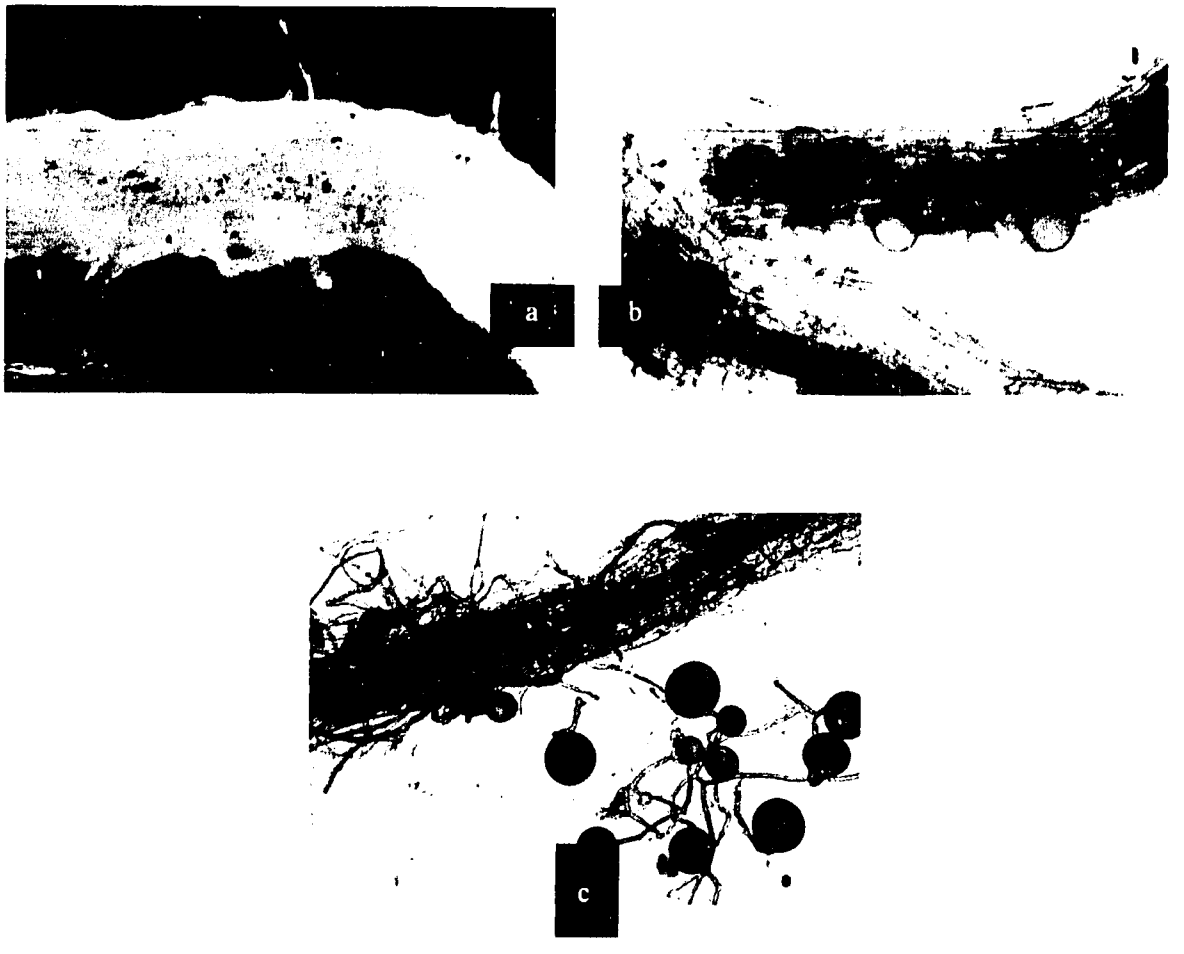

Figure 16. Chlamydospore from the infected root (a). spores in the root, (b). chlamydospore in the grasses root (c). spores released from the root.

Figure 17 Sporocarp (a). sporocarp of Glomus mosseae (b). sporocarp of *Glomus fasiculatum*

6. SUMMARY AND CONCLUSIONS

6.1 Selection of effective spceies of VA myorrhizal fungi on enhancing the plant growth, yield and phosphorus absorption of soybean and mungbean.

For soybean the result in glasshouse shown that the effective species for height were GRE # 33 and ASCR # 22, for shoot dry weight was MOS and for yield were INT # 26, MAR # 40 and ASCR # 22 (Table 4). In the field at Chiangmai the effective species for dry weight was DES # 39, for yield was ASCR # 22. VAM ASCR # 22 also produced the highest quantity of spores in soil and obtained the highest percent root colonization. (Table 8 and 9).

For mungbean the result in glasshouse showed that no effective species for height, effective species for shoot dry weight was MAR # 40, for yield were MOS, Glomus # 17 and MAR # 40 (Table 5). In the field at Kalasin the effective species were DES # 39 and ASCR # 22, INT # 26 (Table 15). INT # 26 obtained the highest root colonization while ASCR # 22 produced the highest amount of spores (Table 16 and 17).

No significantly difference was found between the main plot of applied and non applied phosphate in both locations. At Chiangmai where phosphorus content was higher than 15 ppm, no effect of VAM was shown on phosphorus content in shoot while it was shown at Kalasin where the phosphorus content in soil was about 7 ppm. VAM isolates INT # 26, MOS # 6 and ASCR # 22 were the effective species on increasing yield when grown in the low phosphorus soil and applied only small amount of available phosphorus (Table 8 and 15).

The result indicated that the phosphorus absorption ability in VAM inoculated plant grown in soil high of phosphorus content (> 15 ppm) was shown no different when phosphate fertilizer was applied where in the soil low in phosphorus absorption in plant was increased.

6.2 Determination of the survival of mycorrhizal fungi during and following flooding condition of paddy field.

The survival incidence of inoculated VAM and indigenous species in this experiments were similar in both soybean and mungbean field. Spores quantity in the soil from the 1st crop were decreased with time. Number of spores were lowest in the second crop when rice were grown and the fields were covered about 20-30 inches of water for four months. Then it increased again in the 3rd crop when beans were grown (Figure 18).

Root colonization was also found highest in the 1st crop. The VAM inoculum which places below the seed provided a high inoculum potential for the introduced fungi, giving them and advantage over the indigenous fungi which werespreadthroughout the soil. So at both field where soil became colonized by the indigenous fungi at the same level of inoculated, plant growth and yield responded significantly to inoculation with some of the introduced fungi in the first crop. Most sample found no colonization of VAM in the rice root. (Figure 18)

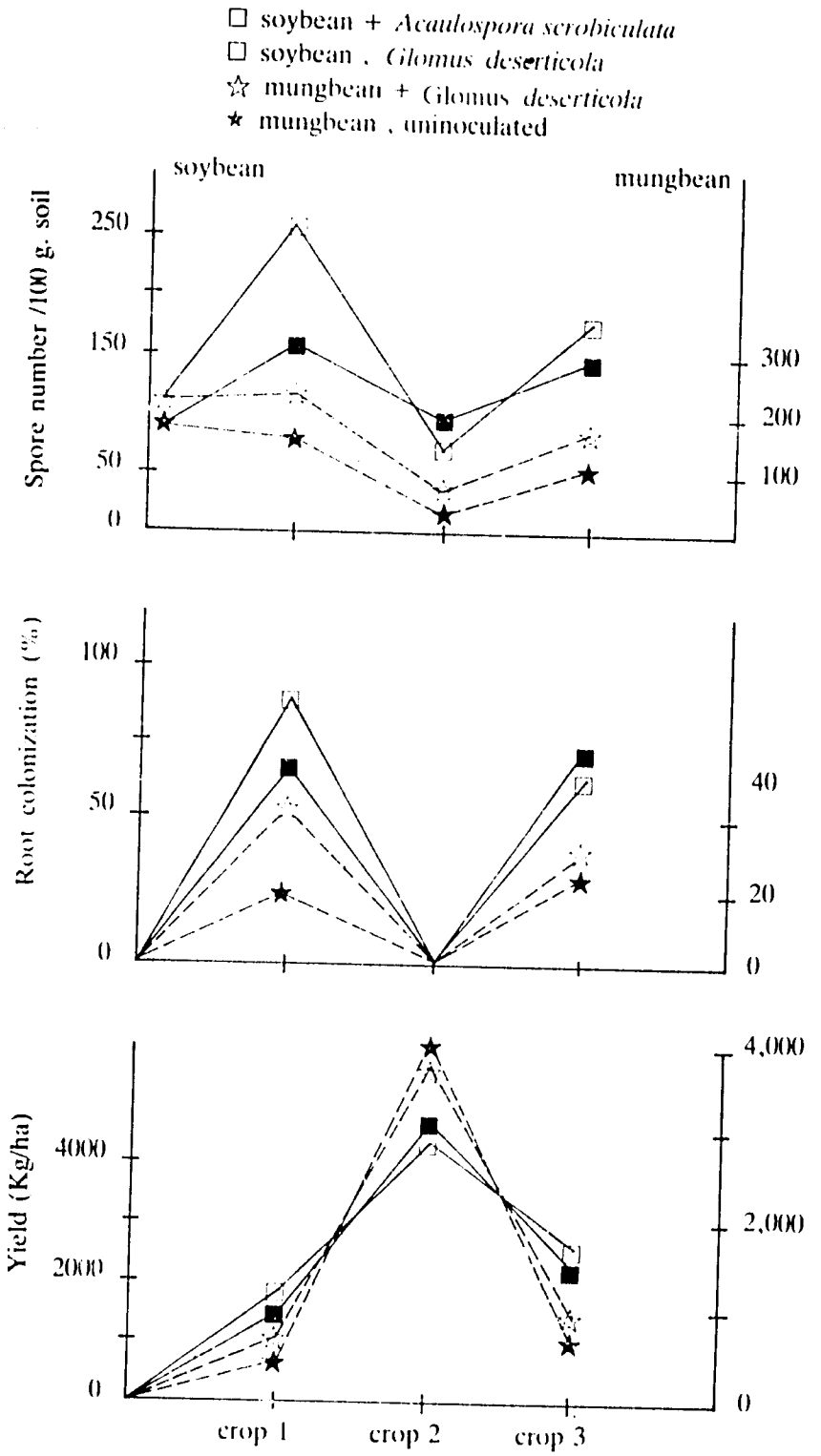

Figure 18 Comparison of VAM spores, root colonization and yield in the field of soybean/ mungbean (crop I), rice (crop II) and soybean/mungbean (crop III).

Yield of inoculated soybean and mungbean are significantly higher than uninoculated in the 1st crop but no significance were shown on the 2nd crop & the 3rd crop. The total infection and spore number after the 3rd crop showed the VAM fungi could survived from flooding. The number of VAM spore decreased by flooded condition and also by some microfauna in the soil. To obtain the highest benefit from VAM according to this phenomena, the crop after paddy rice should be remoculate with VAM. (Figure 18)

6.3 Improving the method for producing VAM inoculum for field trail.

The result on determination the suitable growth medium for host plant shown that sand + clay and sterilized peat were the best media for produce VAM spore in pot culture. For the appropriate host plant determination, the result shown that soybean, corn and sorghum were the most satisfactory host. Peanut and Kuzsu were the secondary (Table 44 and 45).

All the results are summarized as follow.

1. In glasshouse condition, some selected species of VAMF showed the ability on enhancing height, dry weight and yield of soybean. For mungbean the effectiveness species showed the effect only on dry weight and yield.

2. In field condition, the effectiveness on increasing yield of VAMF decreased because of the effect of indigenous species in the soils and some other uncontrol conditions.

3. Evidence from glasshouse and field experiment showed that individual species differ significantly in their effectiveness to promote growth with changing plant species and soil fertility.

4. No infection was found in the paddy rice root so the effectiveness of VAMF did not occure on the rice (2 nd crop) grown in the flooding condition

5. Most VAMF survived after flooding condition from the rice field.

6. Survival VAMF infected the bean root in the 3 rd crop but less effectiveness was found.

7. It is evident from the glasshouse and field experiment that the addition of VAMF to soils significantly affects soybean and mungbean growth in the 1 st crop but VAMF should be reappplied to the 3 rd crop grown after the paddy rice.

8. In low phosphorus location (Prachinburi, Kalasin) the effect of VAMF was more evident on enhancing growth and yield of soybean and mungbean than the high phosphorus location (Chiangmai).

9. The effective species for soybean were *Glomus intraradices*, *Glomus deserticola* and *Acaulospora scrobiculata*. For mungbean the effective species were *Glomus mosseae*, *Glomus intraradices*, *Glomus deserticola* and *Acaulospora scrobiculata*. These species produced high quantity of spores for inoculum used, were superimposement over the indigenous VAMF and could survived over flooding condition in rice field.

10. Sand + clay was the best growth medium for host plant and the appropriate host plant for VAMF were corn and sorghum.

7. APPENDIX

7.1 List of equipments obtained from this project

- One microbus
- One microscope
- One microscope with automatic camera set
- One cool light for microscope
- One incubator
- One centrifuge
- One electric controller
- One stirring hot plate
- One laminar flow
- One water bath
- One water purification
- One calculator
- One camera
- One microcomputor and accessary
- Two air-conditioners

8. REFERENCES.

Abbott, L.K. 1982.Comparative anatomy of vesicular-arbuscular mycorrhizas formed on subterranean clover. ***Aust. J. Bot.*** 30 : 485-499.

Abbott, L.K. and A.D Robson. 1977. Growth stimulation of subterranean clover with vesicular arbuscular mycorrhizas. ***Aust. J. Agric. Res.*** 28 : 639-649.

Allen, M.F. 1984. Physiology of mycorrhizae of arid zone plants : a key to successful reclamation. In : VA Mycorrhizae and Reclamation of Arid and Semi Arid Lands. Edited by S.E. Williams and M.F. Allen. University of Wyoming, Agr. Expt. Sta. Laramie. Wyoming (in press).

Allen, M.F.; W.K. Smith; T.S. Moore, Jr. and M. Christensen. 1981. Comparative water relations and photosynthesis of mycorrhizal and nonmycorrhizal Bouteloua gracilis. ***New Phytol.*** 88 : 683.

Bakshi, B.K. 1974. Mycorrhiza and its role in foresty. Forest Research Institute College, Dehradun. 86 p.

Becker, W.N. and J.W. Gerdemann. 1977. Colorimetric quantification of vesicla-arbuscular mycorrhizal infection in onion. ***New phytol.*** 78 : 289-295.

Davis, R.M. and J.A. Menge. 1981. Phytophthora parasitica inoculation and intensity of vesicular-arbuscular mycorrhizae in citrus. ***New phytol.*** 87 : 705-715.

Daniels, B.A. and J.M. Trappe. 1980. Factor affecting spore germination of the vesicular arbuscular mycorrhizal fungi, ***Glomus epigaeus. Mycologia*** 72 : 457-471.

Ferguson, J.J. 1981. Inoculum production and field application of vesicular-arbuscular mycorrhizal fungi. Ph. D. Dissertation. University of California, Riverside. 117 p.

Ferguson, J.J. 1982. The use of mycorrhizal fungi in citrus nurseries. ***The citrus industry*** 63 (12) : 8-13.

Fitzell, R.; P.C. Fahy and G. Evans. 1980a. Serological studies on some Australian isolates of ***Verticillium spp. Aust. J. Bot. Sci.*** 33 : 115-124.

Fitzell, R.; G. Evans and P.C. Fahy. 1980b. Studies on the colonization of plant roots by ***Verticillum dahliae*** Klebahn with use of immunofluorescent staining. ***Aust.J. of Bot.Sci.*** 28 : 357-368.

Frankland, J.C.; A.D. Bailey; T.R.G. Gray and A.A. Holland 1981. Development of an immunological technique for estimating mycelial biomass of *Mycena galopus* in leaf litter. ***Soil Biol. Biochem.*** 13 : 87-92.

Furlan, V.; H. Bartschi and J.A. Fortin. 1980. Media for density gradient extraction of Endomycorrhizal spores. *Trans. Br. Mycol. Soc. 75 : 336-338.*

Furlan, V. and J.A. fortin. 1975. A flotation-bubbling system for collecting Endogonaceae spores from sieved soil. ***Naturaliste Can.*** 102 : 663-667.

Gerdemann, J.W. 1955. Relation of a large soil-borne spore to phytomycetous mycorrhizal infections. ***Mycologia.*** 47 : 619-632.

——————. 1975. Vesicular-arbuscular mycorrhizae. Pages 575-591. *In* : The development and function of roots. Edited by J.G. Torrey and D.T. Clarkson. Academic Press. London.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of Mycorrhizal *Endogone extracted* from soil by wet sieving and decanting. ***Trans. Br. Mycol. Soc.*** 46 : 235-244.

Gerdemann, J.W. and J.M. Trappe. 1974. The Endogonaceae in the Pacific Northwest. ***Mycol. Mem.*** No. 5. 76 p.

Giannazzi-Pearson, V. and and S. Giannazzi. 1981. Role of endomycorrhizal fungi in phosphorus cycling in the ecosystem. Pages 637-652. *In* : The fungal community. *Edited by*. D.T. Wicklow and G.C. Caroll. Marcel Dekker Inc., New York and Basel.

Giannazzi-Pearson, V. and H.G. Diem. 1982. Endomycorrhizae in the tropics : Pages 209-251. *In* : Microbiology of Tropical Soils and Plant Productivity. Y.R. Dommergues and H.G. Diem. Eds., Martinus Nijhoff Dr. W. Junk Publishers, The Hague, Boston, London.

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

Graham, J.J.; R.G. Linderman and J.A. Menge. 1982. Developments of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of Troyer citrange. ***New Phytol.*** 90 : (in press).

Hall, I.R. 1984. Taxonomy of vesicular-arbuscular mycorrhizal fungi. Pages. 57-94. *In* : Taxonomy of Vesicular-Arbuscular Mycorrhizal Fungi. Ed., C.L. Powell & D.J. Bagyaraj. CRC Press. Boca Raton, Fl.

Harley, J.L. 1969. The biology of mycorrhiza: Leonard Hill, London. 334 p.

Hattingh, M.J. and J.W. Gerdemann. 1975. Inoculation of Brazilian sour orange seed with and endomycorrhizal fungus. *Phytopathology* 65 : 1013-1016.

Hayman, D.S. 1970. *Endogone* spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Trans. Br. Mycol. Soc.* 54 : 55-63.

Hayman, D.S. 1982. Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Amer. Phytopatho. Soc.* 72 (8) : 119-1125.

Hayman, D.S. and B. Mosse. 1971. Plant growth responses to vesicular-arbuscular mycorrhiza. I. Growth of *Endogone*-inoculated plants in phosphate-deficient soils. *New Phytol.* 70 : 19.

Hepper, C.M. 1977. A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots. *Soi. Biol. Biochem.* 9 : 15-18.

Howeler, R.H; E. Sieverding and S. Saif. 1987. Practical aspects of mycorrhizal technoloty in some tropical crops and pastures. *Plant and Soil* 100 : 249-283.

Khan, A.G. 1974. The occurrence of Mycorrhizas in halophytes, hydrophytes and xerophytes and of *Endogone* spores in adjacent soil. *J. Gen. Microbiol.* 18 : 14.

————. 1975. The effect of vesicular-arbuscular mycorrhizal association on growth of cereals. *Ann. Appl. Biol.* 80 : 27-36.

Kleinschmidht, G.D. and J.W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. *Phytopathology* 62 : 1447-1453.

Krishna, K.R.; K.G. Shetty; P.J. Dart and D.J. Andrews. 1985. Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet. ***Plant and Soil*** 86 : 113-125.

Knickelman, H.W. 1975. Effect of fertilizers, soil, soiltillage and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soil. Pages. 511-525. *In* : Endomycorrhizas. Ed., F.E. Sanders. University of Leeds, England.

Lambert, D.H.; H. Cole and D.E. Baker. 1980. Adaptation of vesicular-arbuscular mycorrhizae to edaphic factors. ***New Phytol.*** 85 : 513-520.

Malajczuk, N.; A.J. McComb and C.A. Parker. 1975. An immunofluorescence technique for detecting *Phytophthora cinnamomi* Rands. *Aust. J. Bot.* 23 : 289-309.

Marks, G.C. and T.T. Kozlowski. 1973. Ectomycorrhizae : Their Ecology and Physiology. Ed., G.C. Marks and T.T. Kozlowski. Academic Press, London.

Mellveen, W.D. and H. Cole. 1979. Influence of zinc on development of the endomycorrhizal fungus ***Glomus mosseae*** and it mediation of phosphorus uptake by ***Glycine max*** "Amsoy 71". *Agric. Environ.* 4(4) : 245-256.

Menge, J.A. 1982. Effect of soil fumigants and fungicides on vesicular-arbuscular fungi. *Phytopathology* 72 : 1125-1132.

Menge. J.A. 1984. Inoculum Production in VA mycorrhiza conway. Pages. 188-204. *In* : Taxonomy of VA Mycorrhizal fungi. Ed., C.L. Powell & D.J. Bagyyaraj. CRC Press. Boca Raton, Fl.

Menge, J.A.; E.L.V. Johnson and R.G. Platt. 1978. Mycorrhizal dependency of serveral citrus cultivars under three nutrient regimes. *New Phytol* 81 :'553-559.

Mertz, S.M.Jr.; Heithaus III, J.J. and R.L. Bush. 1979. Mass Production of axenic spores of the endomycorrhizal fungus *Gigaspora margarita*. ***Trans. Br. Mycol. Soc.*** 72 : 167-169.

Morton, J.B. 1986. Three new species of ***Acaulospora*** (Endogonaceae) from high aluminum, low pH soils in West Verginia. *Mycologia* 78 : 641-648.

Mosse, B. 1972. The influence of soil type and *Endogone* strain on the growth of mycorrhizal plants in phosphate deficient soils. *Rev. Ecol. Biol. Soil.* 9 : 529-537.

Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Ann. Phytopathol.* 11 : 171-196.

Mosse, B. 1981. Vesicular-Arbuscular Mycorrhiza Research for Tropical Agriculture. Res. Bull. 194. Hawaii Inst. Trop. Agric and Human Res., University of Hawaii.

Mosse, B. and G.D. Bowen. 1968. A key to the recognition of some *Endogone* spore type. *Trans. Br. Mycol. Soc.* 51 : 469-483.

Mosse, B. and G.W. Jones. 1968. Separation of *Endogone* spores from organic soil debris by differential sedimentation in gelatin columns. *Trans Br. Mycol. Soc.* 51 : 604-608.

Nopamornbodi, O., Y. Vasuvat, and S. Yanvaree. 1980. Investigation on the endomycorrhiza infection of economic crops in Thailand. Pl. Path. Div. Dept. of Agri. Annual Report for 1980, Bangkok, Thailand (In Thai). Pages 1-5.

Nemec, S.; J.A. Menge; R.G. Platt and E.L.V. Johnson. 1981. Vesicular-arbuscular mycorrhizal fungi associated with citrus in Florida and California and notes on their distribution and ecology. *Mycologia* 7 : 112-127.

Newman, E.T. 1966. A method of estimating the total length of root in a sample. *J. of Applied Ecology* 3 : 139.

Ohms, R.E. 1957. A flotation method for collecting spores of a phycomycetous mycorrhizal parastic from soil. *Phytopathology* 47 : 751-752.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. br. Mycol. Soc.* 55 : 158-161.

Porter, W.M. 1979. The most probable number method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Aust. J. Soil. Res.* 17 : 515-519.

Powell, C.L. 1977. Mycorrhizas in hill country soils. III. Effect of inoculation on clover growth in unsterile soils. ***NZ. J. Agric. Res.*** 20 : 343-348.

_____. 1980. Mycorrhizal infectivity of eroded soils. *Soil Biol. Biochem.* 12 : 247-250.

Reid, C.P.R. and G.D. Bowen. 1979. Effect of soil moisture on VA mycorrhizae formation and root development in *Medicago*. Pages 211-219. *in* : The Soil-root Interface. Ed., J.L. Harley and R.S. Russel. Academic Press, London.

Rhodes, L.H. and J.W. Gerdemann. 1978. Influence of phosphorus nutrition on sulfur uptake by vesicular-arbuscular mycorrhizae of onion. *Soil. Biochem.* 10 : 361-364.

Ross, J.P. and J.A. Harper. 1970. Effect of Endogone mycorrhiza on soybean yields. ***Phytopathology*** 60 : 1552-1556.

Sandergard, M. and S. Laeyaard. 1977. Vesicular arbuscular mycorrhizal on some aquatic vascular plants. ***Nature*** 268 : 232-233.

Schenck, N.C. 1981. Can mycorrhizae control root disease? *Plant disease* 65 (3) : 230-234.

Schenck, N.C.; S.O. Graham and N.F. Green. 1975. Temperature and light effect on contamination and spore germination of vesicular-arbuscular mycorrhizal fungi. ***Mycologia*** 67 : 1189-1192.

Schenck, N.C. and M.K. Kellam. 1978. The influence of vesicular-arbuscular mycorrhizae on disease development. *Fla. Agric. Exp. Stn. Tech. Bull.* 798 p.

Schenck, N.C. and R.A. Kinloch. 1980. Incidence mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. ***Mycologia*** 72 : 445-456.

Schenck, N.C. and G.S. Smith. 1982. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. ***Mycologia*** 74 : 77-92.

Schenck, N.C. and D.P.H. Tucker. 1974. Endomycorrhizal fungi and the development of citrus seedlings in Florida fumigated soils. *J. Amer. Soc. Hort. Sci.* 99(3) : 284-287.

Smith, T.F. 1980. The effect of season and crop rotation on the abundance of spores of vesicular-arbuscular mycorrhizas endophytes. ***Plant & Soil*** 57 : 457-479.

Smith, R.A. 1982. Nutritional study of ***Pisolithus tinctorius***. ***Mycologia*** 74 : 54-58.

Smith, G.W. and H.D. Skipper. 1979. Comparison of method to extract spores of vesicular-arbuscular mycorrhizal fungi. *Soil Sci. Soc. Amer. J.* 43 : 722-725.

Sparling, G.P. and P.B. Tinker. 1975. Mycorrhizas in Pinnine grassland. Pages. 545-560. *In* : Endomycorrhizas. Ed., F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London.

Sutton, J.C. 1973. Development of vesicular-arbuscular mycorrhizae in crop plants. ***Can. J. Bot.*** 51 : 2487-2493.

Sutton, J.C. and G.L. Barron. 1972. Population dynamics of *Endogone* spores in soil. ***Can. J. Bot.*** 50 : 1909-1914.

Trappe, J.M. 1982. Synoptic keys to the genera and species of Zygomycetes (vesicular-arbuscular) mycorrhizal fungi *Phytopathology* 72 : 1-24.

Trappe, J.M. and N.C. Schenck. 1982. Taxonomy of the fungi forming endomycorrhizae. Pages 1-9. *In* : Methods and Principles of Mycorrhizal Research. Ed., N. Schenck. American Phytopathological Society, St. Paul, Minn.

Zambolim, L; N.C. Schenck and D.J. Mitchell. 1983. Inoculum density, pathogenicity and interactions of soybean root-infecting fungi. *Phytopathology* 73 : 1398-1402.