STUDIES ON ENHANCING THE EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON MAIZE UNDER FIELD CONDITIONS

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are generally found in many soils and they form a symbiosis association with plant roots. AM fungi are obligate symbionts. They grow and reproduce only in the roots. The identification of over 160 AM fungal species had been made basing on different spore morphotypes and process of sporulation in pot culture (Morton and Benny, 1990; Brundrett *et al*., 1996). To identify AM fungi correctly, fungal taxonomists required over four months to increase new spores of this fungi in host plants. Feature variation of AM fungi within plant root depended on developmental stages and their changes depending on the genome of the host plants (Hetrick *et al*., 1985; Gianinazzi-Pearson *et al*., 1991; Giovannetti and Gianinazzi-Pearson, 1994). Merryweather and Fitter (1998) reported that colonized roots in the absence of spores could be identified only at a family level. To decrease time in identification of AM fungi, molecular techniques have been developed. The small subunit ribosomal DNA or 18S rDNA is highly conserved and not easily changed by environmental factors. Moreover, the variation in 18S rDNA sequences in each organism could indicate the genetic differences among individuals (Goosen and Debets, 1996). The polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques have been used to find the 18S rDNA patterns from spores and colonized maize roots for detection and identification of the AM fungal species at different stages of their life cycles.

Benefits to plants from mycorrhizal associations occur from increased nutrient supply, especially phosphorus (P), improved drought and pathogen tolerances and reduced uptake of heavy metals. However, the benefits of AM fungi to plants depend on the specific plant and fungal genotypes and their abiotic and biotic environments (Jones and Smith, 2004). Application of nitrogen (N) and P fertilizers have been generally found to reduce root colonization and spore production of AM fungi (Jensen and Jakobsen, 1980; Antunes and Cardoso, 1991; Suwanarit *et al*., 2000). Na

Bhadalung *et al*. (2005) found that the long-term N and P fertilization in a maize (*Zea mays* L.) cropping system caused decreases in total spore numbers of AM fungi and sensitivity to fertilization in each AM fungal species were different. However different researchers have reported different relative N and P supplies that were most efficient in promoting root colonization of AM fungal species and mycorrhizal plant response. Hays *et al.* (1982) reported that root colonization of *G. fasciculatum* was highest at high rate of N fertilizer and low rate of P fertilizer. Sylvia and Neal (1990) reported that P fertilization did not affect root colonization of *G. etunicatum* when the plant was deficient in N whereas P fertilization inhibited root colonization of the fungus when the plant was supplied with adequate N. According to Baath and Spokes (1989), mycorrhizal plant showed the highest growth response to colonization at low rate of P and medium rate of N fertilizers. Daft (1992) found that increasing concentration of urea applied to AM fungal inoculated maize plants increased spore production. Whereas, Olsson *et al*. (2005) found that high N availability reduced the mycelial development of *G. intraradices*. Reynolds *et al*. (2005) found that AM fungi can act as a parasite when P availability is relatively high. The disagreement of the above findings suggested that different AM fungi may give different responses to different combinations of N and P supplies. It is therefore worth-while to study effects of N and P levels on root colonization and sporulation of AM fungal species and their effects on maize growth.

Differences in response to AM fungi of plants can be found not only among plant species but also among cultivars of the host plant (Tawaraya, 2003). In Thailand, resistance to downy mildew, a fungal disease, has been developed in maize (Sriwatanapongse *et al*., 1993). In addition a maize cultivar low in fertilizer requirement and more drought tolerant has been introduced (Suwanarit *et al*., 1985; Thiraporn, 1990). It is therefore worth-while to examine effects of difference in downy mildew resistance (DMR) and fertilizer requirement of maize cultivars on the efficiency of AM fungal species on the plants.

Application of AM fungi in maize production are not extensive because the benefit of each AM fungal species to maize was very inconsistent. The effects might

not be found when AM fungi were inoculated in the maize field in the presence of indigenous AM fungi (Suwanarit *et al.*, 1997). In addition, production of AM fungal inoculum is very expensive because there is no effective method for fast multiplication and production (Kuwada *et al*., 2006). And, the transport expenses, and cost of application of the inocula to the crop will be very high if the benefit of the mycorrhizal association lasted for a short period of cropping (Safir, 1994). Therefore, it may be worth-while to examine change with time of the residual effects of AM fungal inoculation. Furthermore, it is worth-while to study the factors or methods of enhancing the effects of AM fungi, such as repetitive inoculation, in order that AM fungal inocula may be used more effectively.

With the above perspectives, the following six experiments were carried out:

Experiment 1: Identification of AM fungi by PCR technique.

Experiment 2: Effects of AM fungal species, nitrogen and phosphorus fertilizers on maize growth, root colonization and spore production.

Experiment 3: Comparative responses to AM fungi of maize cultivars different in downy mildew resistance and fertilizer requirement.

Experiment 4: A study on time courses of the effects on maize of AM fungi applied to maize in the field.

Experiment 5: Verification of AM fungi showing effects on maize grown on Rhodic Kandiustox in the field and pot experiments.

Experiment 6: A study on effects of AM fungal repetitive inoculation on maize grown in the field.

OBJECTIVES

Ultimate Objectives

1) To identify factors affecting efficiency of AM fungi applied to maize under field conditions.

2) To examine effects of some cultivation practices on the efficiency of AM fungi.

The objectives of the individual experiments were as follows.

Experiment 1: Identification of AM fungi by PCR technique

1) To obtain biochemical characteristics of spores of some selected AM fungal species by using Polymerase Chain Reaction (PCR) technique for identification of AM fungal spores in the other projects and for future reference.

2) To obtain biochemical characteristics of maize roots infected by some selected AM fungal species by using PCR technique for identification of AM fungi infecting maize roots in the other projects and for future reference.

Experiment 2: Effects of AM fungal species, nitrogen and phosphorus fertilizers on maize growth, root colonization and spore production

1) To examine effects of N and P fertilizers on the efficacy of AM fungi in promoting maize growth.

2) To examine effects of N and P fertilizers on the on the colonization and spore production of two AM fungi.

Experiment 3: Comparative responses to AM fungi of maize cultivars different in downy mildew resistance and fertilizer requirement

1) To compare responses to AM fungi of maize cultivars different in downy mildew resistance.

2) To compare responses to AM fungi of maize cultivars different in fertilizer requirement.

Experiment 4: A study on time courses of the effects on maize of AM fungi applied to maize in the field

To examine change with time of the effects of inoculated AM fungi on maize grown in the field.

Experiment 5: Verification of AM fungi showing effects on maize grown on Rhodic Kandiustox in the field and pot experiments

To find out the AM fungal species which had been found in Experiment 4 to show significant effects on growth and yields of maize grown in the field in the presence of indigenous AM fungi.

Experiment 6: A study on effects of AM fungal repetitive inoculation on maize grown in the field

To examine effects of repetitive inoculation on the efficiency of some selected AM fungi in promoting growth and increasing yields of maize grown in the field in the presence of indigenous AM fungi.

LITERATURE REVIEW

Arbuscular mycorrhizal fungi

Koide and Mosse (2004) reported that the name of vesicular-arbuscular mycorrhizal (VAM) fungi obtained the discovery of two important basic anatomical fungal organelles "vesicules" and "arbuscules" in the host plant roots by Schlicht in 1889, Janse in 1897 and Gallaud in 1905. Mosse (1953) showed the first report of VAM fungal infection of strawberry using nonsterile sporocarps of a fungus initially named *Endogone mosseae* in her honor (Nicolson and Gerdemann, 1968), which later became *Glomus mosseae*. Mosse (1956) found that inoculation with surface-sterilized sporocarps associated with mycorrhizal strawberry roots also produced mycorrhiza in apple, wheat, various grasses, tomato and lettuce in open pot experiments. Gerdemann (1955) also showed that spores from his "type B" isolate, later named *Gigaspora gigantea*, had a wide host range and could successfully form mycorrhizas with several species of plants including red clover, maize, strawberry and sweet clover. The experiments of Gerdemann and Mosse thus well established the absence of a strict host-specificity by at least some VAM fungi, and together provided evidence that mycorrhizas could be caused by more than one species of fungus. Gerdemann (1955) was careful to note that the mycorrhiza from his "type B" spores was arbuscular and that no vesicles were produced, which distinguished his fungus from the one used by Mosse. It thus became clear that there were at least two patterns of symbiotic development by VAM fungi.

Gerdemann and Trappe (1974) split the old Endogone, a wide variety of species, into seven genera including Endogone, Modicella, Glaziella (nonmycorrhizal genera), and four mycorrhizal genera including Glomus, Sclerocystis, and two new genera, Gigaspora and Acaulospora, which corresponded to the honey-colored sessile spores of Mosse and Bowen (1968). These were all placed in the Endogonaceae, Endogonales, Zygomycetes. Trappe and Schenck (1982) recognized another mycorrhizal genus, Entrophospora. Walker (1987) also recognized five VAM fungal genera and added Scutellospora. In 1990, Morton and Benny placed the genera of

Walker (1987) into three families (Glomaceae, Acaulosporaceae, Gigasporaceae) and two suborders (the Glomineae and the Gigasporineae), both of which were then placed in a new order, the Glomales. The name "VAM fungi" was established and persisted until recently. Species in the genera *Gigaspora* and *Scutellospora* in Gigasporineae do not form vesicles in root cells. The recognition that not all fungi formed vesicles led to the proposal that this symbiosis should be renamed "arbuscular mycorrhizal (AM) fungi". This change is now widely accepted, however, in some of these associations the fungi may not even produce proper arbuscules (Smith and Smith, 1997). Later, Morton and Benny (2001) recognized two other families, the Archaeosporaceae and Paraglomaceae, with two new genera, Archaeospora and Paraglomus. Schüßler *et al*. (2001) used molecular data to establish the relationships among AM fungi and between AM fungi and other fungi. The group of AM fungi was elevated to the level of phylum (Glomeromycota), which was shown to be as distinct from other fungi as the Ascomycota are from the Basidiomycota. The revised the AM fungal classification scheme based on analysis of SSU rDNA sequences is as follows (Schüßler *et al*., 2001):

Phylum: Glomeromycota
- Class: Glomeromycetes
  - Order: Glomerales
    - Family: Glomaceae
      - Genus: *Glomus*
  - Order: Paraglomerales
    - Family: Paraglomaceae
      - Genus: *Paraglomus*
  - Order: Diversisporales
    - Family: Gigasporaceae
      - Genus: *Gigaspora* and *Scutellospora*
    - Family: Acaulosporaceae
      - Genus: *Acaulospora* and *Entrophospora*
    - Family: Pacisporaceae
      - Genus: *Pacispora*
    - Family: Diversisporaceae
      - Genus: *Diversispora*
  - Order: Archaeosporales
    - Family: Archaeosporaceae
      - Genus: *Archaeospora*
    - Family: Geosiphonaceae
      - Genus: *Geosiphon*

Schenck (1985) suggested that the methods employed by taxonomists have become increasingly sophisticated. While DNA variation may be the best measure of genealogical relationships among organisms, however, anatomical and DNA-based methods have yielded similar results. Routine identification of AM fungi will probably continue to be based primarily on structural characters and thus an increased appreciation of the relationship between anatomy and DNA will be important. However, the ability to properly name the fungi, avoid duplication of names and relate the species to one another depends heavily on collections such as those held by the International Culture Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM), and the International Bank for the Glomeromycota (BEG). Schenck created INVAM in 1985. Since 1990 the collection has been curated by Morton at West Virginia University. The BEG is an international collaborative effort that provides registration of individual isolates of fungi for research purposes. This will insure a higher degree of certainty of the identity of the fungi in use by researchers around the world (Koide and Mosse, 2004).

DNA fingerprinting for identifying AM fungi

A DNA fingerprinting for identification can be achieved by using restriction fragment length polymorphism (RFLP) analysis. The technique can only be used where a large amount of DNA can be obtained from individual organisms. Because AM fungi can not be maintained in pure culture, obtaining sufficient DNA for RFLP is difficult. Therefore, all DNA techniques employed for identification of AM fungi use the polymerase chain reaction (PCR), which amplified relatively small amount of DNA. A universal molecular phylogeny has been based largely on sequences of the small subunit ribosomal RNA gene and other regions of ribosomal DNA. The 18S and 5.8S genes evolve relatively slowly and are useful for studies of distantly-related organisms. The internal transcribed spacers (ITS) region evolves faster than 18S and 5.8S genes and can differ between species. Simon *et al.* (1992, 1993) used PCR and universal primer and designed a Glomales specific primer (VANS1) for amplification of 18S rDNA gene obtained from small numbers of spores of AM fungi. The results showed that the sequence of the gene differed between species of AM fungi. Sander

et al. (1995) amplified ITS regions from single spores of AM fungi using the universal primers, ITS1 and ITS4. By cutting the ITS fragment with restriction enzymes, different fingerprints could be obtained for the different isolates. The PCR-RFLP technique was used for the rapid identification of genetically diverse spores and the technique was sensitive enough to detect genetic diversity in the community without the need to sequence the amplified products. Redecker *et al.* (1997) used PCR-RFLP technique with the universal primers ITS1 and ITS4 and three restriction enzymes to identify AM fungal spores of different genera. The results showed that ITS sequence divergence within the genus Glomus was higher than within the whole family Gigasporaceae. The ITS of the Gigasporaceae have insufficient variability to allow identification by RFLP patterns. However, the restriction patterns can be used to confirm the morphological classification.

Simon *et al.* (1992) and Di Bonito *et al.* (1995) amplified 18S DNA with the primer pair VANS1-NS21 to detect AM fungi on roots of leek, lettuce, zinnia, pepper and endive plants. However, Clapp *et al.* (1995) found difficulty with the VANS1 primer when used for the amplification of AM fungal DNA from field-collected bluebell roots. Redecker (2000) designed a set of primers for nested PCR to amplify ITS and 18S DNA from colonized roots in the absence of spores. The PCR products can be used to identify AM fungi at the genus or species level using two restriction enzymes. With exception for Gigasporaceae, RFLP patterns were not variable enough for identification. He also proved that the RFLP patterns obtained from colonized roots matched the patterns obtained from spores.

Effects of AM fungi on host plant growth

The AM fungi are biotrophic, and carbon compounds may primarily flow from host to fungus via living arbuscules (Becard and Piche, 1989). The digestion of the arbuscules by the host does appear to be a method of restricting the degree of parasitism. Mosse (1957) published a report showing that AM fungal infection led to improved growth of apple seedlings and clonal leaf bud cuttings in autoclaved soil. Clark (1963) reported an increase of growth of tulip poplar trees planted in fumigated

soil using surface-sterilized mycorrhizal roots as inoculum. Gerdemann (1964) also demonstrated improved growth in maize on steamed soil after inoculating test plants with sporocarps but the benefit of AM fungi on maize yields was less when plants were grown in unsterilized soil. Many researchers have shown, for example, that mycorrhizal fungi can inhibit phytopathogenic fungi (Baltruschat and Schoenbeck, 1972; Chou and Schmitthenner, 1974; Dehne and Schoenbeck, 1979). When AM fungi were present, the pathogen was restricted to the epidermis and exodermis. Others have shown negative effects of mycorrhizal fungi on pathogenic nematodes (Fox and Spasoff, 1972; Hussey and Roncadori, 1977; Cooper and Grandison, 1986). Other non-nutritive effects of mycorrhizal fungi may be very important. In some respects, the early emphasis placed on the role of mycorrhizal fungi in promoting plant growth may have distracted us from another very important role they play as stabilizers of soil structure (Clough and Sutton, 1976; Nicolson and Johnston, 1979, Tisdall and Oades, 1979, Miller and Jastrow, 2000) and as integral components of a very diverse soil biota (Franke-Snyder *et al*., 2001).

Further progress in understanding the effects of AM fungi on plant growth was made possible by producing large volumes of inoculum initiated from single isolates of fungal species produced in pot cultures (Nicolson, 1967; Gerdemann, 1971). The variation in the nature of mycorrhizal effects on the host has been noted recently (Janos, 1980; Johnson *et al*.,1997). Thus, there are notable cases of growth depression apparently caused by AM fungi in "non-host" species (Francis and Read, 1985) or in host species when phosphate availability is high (Mosse, 1973; Peng *et al*., 1993; Kaeppler *et al*, 2000) whereas *Glomus macrocapum* was a cause of stunt disease in tobacco (Modjo and Hendrix, 1986).

Nutrient uptake and transfer from fungi to host

Baylis (1959) suggested that the beneficial mycorrhizal effect was mediated by P uptake. Mycorrhizal Griselinia seedlings grown in a P-deficient soil took up 3–5 times as much P as nonmycorrhizal seedlings. Gerdemann (1964) also demonstrated that nonmycorrhizal plants exhibited "severe phosphorus deficiency symptoms" and

had significantly lower P concentrations and higher K and Mg concentrations than mycorrhizal plants. Gray (1964) showed that mycorrhizal plants contained more P than nonmycorrhizal plants. Holevas (1966) further showed positive effects of mycorrhizal infection in P-deficient soil but not in soil to which additional P was added. Similar findings were reported by Daft and Nicolson (1966), Murdoch *et al*. (1967), Nicolson (1967) and Hayman and Mosse (1971).

Baylis (1970, 1972b) studied the growth responses to mycorrhizal infection of five plant species at three levels of added P. The results showed that the species fell into three groups according to their requirement of a minimum value of available P, below which they grew very little. This threshold value might relate to the extent of root-soil interface, and AM fungi and root hairs were essentially alternative mechanisms for plant P uptake. Baylis (1972a) suggested that root hair length was a good predictor of benefit from mycorrhizal fungi. A refinement of this hypothesis was offered by Abbott and Robson (1984) and Koide (1991). Their results indicated that prediction of benefit from mycorrhizal fungi depended on both the supply of P, which was affected by root hair length, and the requirement for P, such as represented by the potential for plant growth. Thus, the extent to which the P requirement exceeded the P supply determines plant response. Sanders and Tinker (1973) reasoned that the hyphae took up and transferred P to the host because P inflow into mycorrhizal roots was substantially higher than in nonmycorrhizal roots, which was limited by diffusion. Therefore, the extra P in mycorrhizal plants could be due either to an indirect mycorrhizal effect on root structure or physiology, or to direct uptake by hyphae with subsequent transfer to the root, or both (Sanders and Tinker, 1973). The distinction between indirect effects on the root and direct hyphal effects was made possible by spatial separation of colonised roots and extrametrical mycelium (Hattingh *et al*., 1973; Schuepp *et al*., 1987). The basic method of Schuepp *et al*. (1987), which employed fine meshes to separate root from hyphal compartments, subsequently led to many important observations, including the discovery that some fungal species mainly explored the soil immediately adjacent to the root, while others explored it more distantly (Jakobsen *et al*. 1992a, 1992b). The existence of such functional diversity among AM fungi suggests that a combination of several species

of fungi could increase the effectiveness of phosphate extraction from the soil. The separation of fungal from root compartments also led to the discovery that the fungi could absorb the majority of P eventually acquired by the plant and, in some cases, the fungi performed virtually all of this function (Pearson and Jakobsen, 1993; Smith *et al*., 2003). The extra P in mycorrhizal roots could be due either to better soil exploration by the extramatrical mycelium, or to the ability of the fungus to utilize or mobilize sources of soil P not available to plant roots. Following ^{32}P labeling of labile soil phosphate, the specific activities of P in mycorrhizal and nonmycorrhizal plants were not significantly different. This suggested that the primary mechanism by which mycorrhizal fungi improve P uptake is through more extensive soil exploration rather than a unique capacity to mobilize sources of P not available to plants (Sanders and Tinker, 1971; Hayman and Mosse, 1972). While much of the P in the soil is inorganic, a large fraction may also be found in organic compounds. Joner *et al*. (2000) and Koide and Kabir (2000) found that AM fungi could secrete phosphatases to help hydrolyze phosphate. Bieleski (1973) calculated that with four hyphal entry points per millimeter root length and hyphae extending 20 mm from the root surface, P uptake per unit surface would be 60 times greater if P diffusion in the soil were limiting, and 10 times greater if it were not. Polyphosphate granules existed within the hyphae, and the frequently observed cytoplasmic streaming was hypothesized to be the major mechanism for long distance transport of this polyphosphate from external to internal hyphae (Cox *et al*., 1975, 1980; Callow *et al*., 1978; Cooper and Tinker, 1981). The presence of alkaline phosphatase in the vacuoles of the fungi suggested a way to hydrolyze the polyphosphate prior to transfer to the host (Gianinazzi *et al*., 1979).

Bowen and Rovira (1968) suggested that transfer of nutrients from the fungus to host occurred across functional, intact arbuscules, followed by Woolhouse's model for active transmembrane exchange (Woolhouse, 1975). Marx *et al*. (1982) and Gianinazzi-Pearson *et al*. (1991, 2000) presented a presence of active transport mechanisms in P transfer. The results showed that the host plasmalemma, which invaginates around the arbuscular hyphae, had a very high H^+-ATPase activity. Kinden and Brown (1975) suggested that breakdown of the arbuscule contributed

significantly to nutrient transfer. Nevertheless, Cox and Tinker (1976) concluded that arbuscule digestion was not necessary to account for the P transferred, based on calculated arbuscule lifespan, arbuscule volume and P concentration. The ultrastructural and physiological evidence suggests that most nutrient exchange occurs across the living host-fungus interface. Smith and Smith (1997) questioned whether arbuscules (alive or dying) were needed for P transfer, as intercellular hyphae might also be a site of P transfer (Ryan *et al*., 2003). However, plant P transporters, some of which are mycorrhiza specific, appear to be localized in cortical cells containing arbuscules (Rosewarne *et al*., 1999; Rausch *et al*., 2001; Harrison *et al*., 2002; Paszkowski *et al*., 2002). Phosphorus is not the only mineral element taken up and transported to the host by mycorrhizal fungi. Gilmore (1971) may have been the first to point out that AM fungi could increase host Zn content, and Ross and Harper (1970) demonstrated the same for Cu. Heap and Newman (1980) were perhaps the first to demonstrate the existence of hyphal linkages between roots of the same or different plant species. Ritz and Newman (1985) further showed that such linkages could transfer significant amounts of P from dying to living roots.

Factors affecting efficiency of AM fungi

An arbuscular mycorrhizal symbiosis is the result of an interaction between a plant genotype, one or several fungal genotypes and the soil environment (Hamel, 2004). Enhancing the effects of the symbiotic association on crop production should consider the AM fungi involved, the level of nutrient supply, the conditions of the environment created by cropping practices and the genetic make up of the crop plant.

Influence of AM fungi

Mosse and Hayman (1971) and Mosse (1972) noted that different strains of the fungi produced different effects on plant growth. Thus, the selection of superior strains of AM fungi that are notably effective on particular crops is an important activity for a time (Abbott and Robson, 1982). However, the ability to displace indigenous strains, even those less effective than the introduced, superior strains, is

often difficult to prove (Abbott *et al*., 1983). Moreover, what is superior for one crop may not be so for another subsequently planted crop, and what is superior under one set of environmental conditions may not be so under another (Menge, 1985). Powell *et al*. (1982) found that mycorrhizal fungi markedly stimulated growth of four onion cultivars and P uptake in sterilized soil and there were very significant fungi and onion cultivar interactions in non-sterilized soil. However, there have been some successes in at least the short-term establishment of effective strains following their inoculation in large-scale field trials (Owusu-Bennoah and Mosse, 1979; Plenchette *et al*., 1981). Suwanarit *et al.* (1997) carried out a pot experiment to test effectiveness of 14 AM fungal species with maize grown on non-sterile Pak Chong soils. The results showed that maize plants which were inoculated with T6 AM fungal species from Germany, *Scutellospora* sp. and *Acaulospora spinosa* highly significantly superior to nonmycorrhizal plants in term of plant dry weight. In a maize-groundnut intercroping system under field conditions with indigenous AM fungi, the inoculation of *Scutellospora* sp. to maize and *Glomus* no. 2 to groundnut increased grain yield of the maize, whereas, inoculation of *Acaulospora spinosa* to both maize and groundnut and inoculation of T6 German species to both maize and groundnut in 1996's late rainy season tended to increase grain yield of the maize.

Influence of crop genetics

The early observations that plant species differed in their response to mycorrhizal fungi were reported by Lohman (1927) and Baylis (1970, 1972b). Simspson and Daft (1990) found that cereals produced more spores than the legumes. Karasava *et al*. (1998) found that shoot weight and AM fungal colonization of maize grown after sunflower (host plant) was much high than those of maize grown after mustard (nonhost plant) in nine soils with low available P. The antagonistic interactions between AM fungi and some plant species may also serve to exclude these plants from mycorrhizal plant communities (Allen *et al*., 1989; Francis and Read, 1985). Youpensuk *et al.* (2006) found that AM fungi had no effect on shoot dry weight of upland rice but increased shoot dry weight of *Macaranga denticulata*.

Differences in response to AM fungi of plants can be found not only among plant species but also among cultivars of the host plant (Tawaraya 2003). Manske (1990) found that improved high yielding wheat (*Triticum aestivum*) cultivars were less responsive to AM fungi than landrace cultivars whereas Zhu *et al*. (2003) found that an improved barley (*Hordeum vulgare* L.) cultivar was less responsive to AM fungi than a landrace barley. Khalil *et al*. (1994) who studied three non-improved and three improved cultivars of maize (*Zea mays* L.) found that one of the non-improved cultivars did not respond to AM fungi whereas all of the other cultivars received benefit from AM association. These suggest that, in some cases, crop improvement reduces the response of crops to AM fungi. From experimental investigation with maize and wheat, Toth *et al*. (1990) and Hetrick *et al*. (1992) hypothesized that increasing resistance of crops to fungal pathogens by plant breeding decreased AM fungal colonization and decreased benefit from the symbiosis.

Influence of nutrient supply

The development of AM fungi has been found to be affected by fertilization. Bevege (1971) found that application of urea fertilizer in a high-P soil reduced AM fungal infection in *Aracaria* root, but increased infection in medium-P soil. Hepper (1983) reported that root colonization of *G. mosseae* decreased with increases in N:P ratio in the culture solution. Baath and Spokes (1989) reported that at low levels of soil P, nitrogen addition did not affect mycorrhizal infection rate in *Allium shoenoprasum*. Infection rate was decreased at high P and high N but at high P and low N the infection was slightly affected. Dekkers and Van der Werff (1998) found that the percentage of total mycorrhizal colonization and arbuscular colonization were reduced by the application of 52.5 kg P ha^{-1} $year^{-1}$ after 10 and 11 years without fertilizer application but was not affected by application of 17.5 kg P ha^{-1} $year^{-1}$. Kaeppler *et al*. (2000) found that increased P reduced benefit from AM fungi in maize. Some investigations on spore production of AM fungi in a long-term N and P fertilization plots under continuous maize cropping system in Thailand have been reported. Tattao (1987) found that the total spore intensity of indigenous AM fungal species tended to increase with increased rates of fertilizers up to 120 and 120 kg N

and P_2O_5 ha^{-1} $year^{-1}$ and tended to decrease with application of 180 and 180 kg N and P_2O_5 ha^{-1} $year^{-1}$. Suwanarit *et al*. (2000) found that the spore intensity tended to decrease when the rate of fertilizers were 120 and 120 kg N and P_2O_5 ha^{-1} $year^{-1}$ whereas the spore intensity was not affected by the fertilizer rate up to 60 and 60 kg N and P_2O_5 ha^{-1} $year^{-1}$. Nevertheless, Na Bhadalung *et al*. (2005) found that the spore intensity decreased when the rate of fertilizer increased from non-application to 120 and 120 kg N and P_2O_5 ha^{-1} $year^{-1}$ and decreased slightly from 120 and 120 to 180 and 180 kg N and P_2O_5 ha^{-1} $year^{-1}$.

Some research focused on the discovery of root exudates, mostly phenolics, which could stimulate growth of the fungus and its entry into the root (Gianinazzi-Pearson *et al*., 1989; Nair *et al*., 1991; Siqueira *et al*., 1991; Becard *et al*., 1992; Chabot *et al*., 1992; Kape *et al*., 1992). One of these phenolics, formononetin has now been produced commercially and field tests have been performed (Elmer, 2002). Kuwada *et al.* (2006) found that the application of algae extracts as AM fungal growth stimulators enhanced efficacy of AM fungi in papaya seedlings.

Influence of biotic factors in soil

As a natural reflection of their basic training in plant pathology, many researchers have investigated interactions among mycorrhizal fungi and phytopathogenic nematodes, viruses and fungi (Dehne, 1982; Graham, 1986). In some cases, the suppressive effect of mycorrhizal fungi on the development of disease was determined at the tissue level. For example, Dehn and Dehne (1985) showed that in the absence of mycorrhizal fungi, Cochliobolus infected all root tissues. Interactions between mycorrhizal fungi and other organisms occur and may influence the function of the fungi. While grazing of mycorrhizal hyphae by fungivorous collembola can reduce host plant P uptake (Warnock *et al*., 1982; McGonigle and Fitter, 1988) collembola may also disseminate mycorrhizal fungal propagules (Klironomos and Moutoglis, 1999). Rodents may also be agents of dispersal as Endogone spores were shown to remain viable after passage through their alimentary tracts (Godfrey, 1957). Paulitz and Menge (1985) reported that *Anguillospora*

pseudolongissima was a mycoparasite of AM fungi that reduced AM fungi infected root of onion. Boonlue (1997) found that *Chaetomium erraticum* and *Penicillium javanicum* were hyperparasites of spores of *Scutellospora* sp. Lebron *et al*. (1998) found that roots of *Frimbristylis cymosa* in the plot without earthworm showed more AM fungal infection than roots in plot with earthworm.

Influence of abiotic factors in soil

The autecology of the AM fungi has been the subject of research for many years. For example, Lohman (1927) and Porter *et al*. (1987) investigated the effects of soil pH on mycorrhization. Hayman and Travares (1985) found that all of AM fungal species infected in roots in pH range of 4-7. The efficiency of each AM fungi on enhancing plant growth was dependent on soil pH. *Glomus clarum* had a maximum effectiveness at pH 4, while *G. fasciculatum* and *Acaulospora laevis* at pH 5, *G. epigaeum* at pH 7 and *G. epigaeum, G. mosseae, G. fasciculatum, G. caledonium* and *Gigaspora heterogama* at pH 6 and 7. Clark *et al*. (1998) studied the effectiveness of eight AM fungal isolates on growth of *Panicum virgatum* in two acidic soils. The results showed that when compared with non-mycorrhizal plants, mycorrhizal plants gave 52-fold increases in dry matter in soil with pH 4 and 26-fold increases in soil with pH 5. *Glomus clarum* and *G. diaphanum* plants gave highest dry matter and *Gigaspora rosea* plants gave lowest. Youpensuk *et al.* (2006) found that AM fungi increased N, P and K content in seeds of upland rice at pH 7.8 but AM fungi had no the effects at pH 4.5 and 5.9.

Many have investigated the effects of light and temperature. Harley (1972) found that colonization of AM fungi on onion roots were reduced when photosynthetic period was less than 6 hours per day. Daniels and Trappe (1980) found that the optimum temperature for germination and spore production of *Glomus epigaeam* was in the range of 18-25 ^{0}C. Raju *et al*. (1990) found that *Glomus macrocapum* colonized on sorghum roots best and enhanced plant growth and mineral uptake more than *G. fasciculatum*, especially at 30 ^{0}C while *G. intraradices* depressed shoot growth and mineral uptake. Simpson and Daft (1990) found that spore

production of AM fungi was reduced by water-stress. The nature of spore dormancy and the environmental factors that overcome it have been investigated (Mosse, 1959; Siqueira *et al*., 1985). Some authors have noted that mycorrhiza inoculum potential varies with soil depth (Schwab and Reeves, 1981; Koide and Mooney, 1987).

Influence of cultural practices

Jackson *et al*. (1972) found that placements of inoculum in a layer 5 cm below maize seeds or mixed with a 5-cm layer of soil below the seeds enhanced growth of corn plants about 50% above that resulting from indigenous AM fungi. Powell *et al*. (1980) found that drilling of AM fungal inoculum below the barley seed in the field increased mycorrhizal infection levels, shoot dry matter and N and K uptake.

Because of the costs of inoculum production and inoculum application, and the unpredictable consequences of strain selection, attention eventually has turned to managing existing mycorrhizal fungal populations. Kruckelmann (1975) reported some important effects of various agricultural practices on the densities of mycorrhizal fungal chlamydospores. For example, the strong disturbance due to rotary hoeing significantly reduced spore density. The soil disturbance by tillage practice reduces mycorrhizal infection by disrupting the extrametrical mycelium, resulting in reduced root infection, P uptake, growth and yield in maize (O'Halloran *et al*., 1986; Evans and Miller, 1988; Gavito and Miller, 1998 and Mozafar *et al*., 1998). Thompson (1987) reported that long period of fallow was the cause of an insufficiency in mycorrhiza inoculum. The short fallow periods, particularly in combination with harsh winter conditions, may also lead to a decline in inoculum potential that can be overcome by cover cropping (Kormanik *et al*., 1980; France *et al*. 1985; Dodd and Jeffries, 1986; Galvez *et al*., 1995). Nevertheless, Johnson *et al*. (1992) found that continuous monoculture increased populations of detrimental AM fungal species and decreased that of beneficial species in the AM fungal community and mycorrhizae could cause yield depression. Miller (2000) summarized that physical disruption of the soil mycelium by tillage reduced early formation of mycorrhizae and ability of mycorrhizae to absorb P in maize grown under field

conditions. Auge (2004) found that soil hyphal colonization had larger direct and total effects on dehydration tolerance of bean than did root hyphal colonization or several other soil or plant variables. Moreover, the positive effects of AM fungi on host plant may be a result of production of glomalin protein by hyphae and the protein, in turn, increases soil aggregation, nutrient storage capacity and water-holding capacity (Rillig, 2004).

Nopamornbodi *et al.* (1984) found that groundnut plants inoculated with 100 spores of beneficial AM fungal species gave the maximum dry weight of pods when compared with inoculation with 10, 20, 30 and 50 spores. However, Suwanarit *et al.* (1997) found that the positive effect of inoculation of AM fungi in the field was observed although number of spores in soil of inoculated AM fungal species were less than ten percent of the total spores. Increasing spores density of the inoculated AM fungal species in soil may then be expected to increase competition with indigenous AM fungal species to colonize in root and, accordingly may increase the enhancing effect on maize.

The common use of pesticides in agriculture led some to determine their effects on the AM symbiosis. Depending on the crop and soil, some pesticides were found to have stimulatory, some to have depressive, and some to have essentially no significant effect on mycorrhizal fungi (Smith,1978; Menge, 1982 and Trappe *et al*., 1984).

There is still lack of knowledge concerning the potential of mycorrhiza for sustainable plant production. Mycorrhizal technology is relatively complex, as it encompasses several diverse aspects of plant production, that is, cultivation media, nutrient cycling, plant physiology, interactions with other microbes, and numerous environment factors (Gianinazzi and Vosatka, 2004). Therefore, the development of a diverse AM fungal population which can adapt to management and environmental changes is likely to be a key factor in improving the sustainability of low input cropping systems.

MATERIALS AND METHODS

Experiment 1: Identification of AM fungi by PCR technique

Preparation of spores and colonized roots

Two AM fungal species, i.e. *Glomus aggregatum* and *Scutellospora fulgida* were obtained from Department of Microbiology, Faculty of Science, Kasetsart University. The inocula were multiplied by inoculated on roots of maize (*Zea mays* L. cv. Suwan 5) and grown on 7 kg of Pak Chong soil series (Very-fine, kaolinitic, isohyperthermic, Rhodic Kandiustox: Soil Survey Division Staff, 1998) in a greenhouse for four months. Spores were collected from soil by wet-sieving and decanting method (Gerdeman and Nicolson, 1963).

Preparation of colonized maize roots was done as follows. A single spore was inoculated on maize root in plastic pot containing 110 g fired clay (Terragreen®). Each plant was grown under 16 hours photoperiod of artificial light and at 18 ^{o}C during the night and 23^{o}C during the day. The nutrient solution of Long Ashton (0.4 mM KNO_3, 0.15 mM $MgSO_4$, 0.13 mM NaH_2PO_4, 1 µM $MnSO_4$, 5 µM H_3BO_3, 0.5 µM $NH_4Mo_7O_{24}$, 0.1 µM $CuSO_4$, 0.2 µM $CoCl_2$, 10 µM NaCl, 1 µM $ZnSO_4$, 5 µM FeNaEDTA) was supplied according to plant requirement.

Genomic DNA preparation from spores of *Glomus aggregatum*

Genomic DNA was obtained from spores by using the modified protocol of Vandenkoornhuyse and Leyval (1998). The fresh spores of *G. aggregatum* (Figure 1) from soil inoculum were surface sterilized with 0.05% (v/v) triton X-100, 2% (w/v) chloramine T on ice for 10 min, and antibiotic solution containing streptomycin (250 mg l^{-1}), gentamycin (100 mg l^{-1}) and kanamycin (250 mg l^{-1}) and three times in DNAse-free distilled water (Bioprobe Systems, France), respectively. Spores were

crushed in 10 µl DNAse-free distilled water with round pasture pipette. The suspension of crushed spore were used as DNA template.

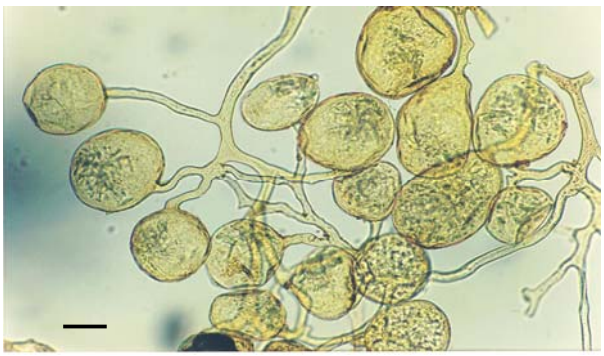

Figure 1 Sporocarp of *Glomus aggregatum*. Bar = 40 µm.

(a) (b)

Figure 2 Two morphotypes of *Scutellospora fulgida* spores presented with (a) or without (b) bulbous suspensor. Bar = 40 µm.

Genomic DNA preparation from colonized roots of *Glomus aggregatum*

The colonized roots were collected 3 months after planting, washed and cut into pieces of 0.5 cm length. About 100 mg wet weight of root pieces was ground

with a mortar and pestle in liquid nitrogen. DNA was extracted using the Dneasy™ Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's manual.

Genomic DNA preparation from spores of *Scutellospora fulgida*

The fresh spores from soil inoculum were surface sterilized with 0.05% (v/v) triton X-100, 2% (w/v) chloramine T on ice for 10 min, and antibiotic solution containing streptomycin (250 mg l^{-1}), gentamycin (100 mg l^{-1}) and kanamycin (250 mg l^{-1}) and three times in DNAse-free distilled water (Bioprobe Systems, France), respectively. Two morphotypes of one to twenty spores, which presented with or without bulbous suspensor (Figure 2) were crushed in 10 µl DNAse-free distilled water with round pasture pipette to obtain template DNA.

18S rDNA amplification using PCR technique

Genomic DNA derived from spores or colonized roots were used as templates. The MH2, 5'TTC GAT GGT AGG ATA GAG G3', and MH4, 5'GTC TCA CTA AGC CAT TC3', were use as primers in PCR reaction mixture (Vandenkoornhuyse and Leyval, 1998).

The PCR reaction mixture (100 µl) contained 10 µl of template DNA, 1.2 µM of each primer, 125µM of each dNTP, 1X PCR buffer (20 mM Tris-HCl pH 8.0, 50 mM KCl, 0.05% (v/v) of detergent Wl, 1.5 mM $MgCl_2$) and 2.5 U *Taq* DNA polymerase (Gibco BRL LifeTechnologies). PCR reaction mixture was covered with two drops of mineral oil.

The amplification conditions were 94°C for 2 minutes, then 33 cycles of 94°C for 1 minute, 48°C for 1.30 minutes minus 0.1°C per cycle and 72°C for 2 minutes. Amplification was ended at 72°C for 8 minutes in the extension step (MiniCycler™

PTC 150, U.S.A.). PCR products were detected by 1% agarose gel electrophoresis in 0.5% TAE (Tris-acetate buffer) and illuminated under UV light after staining gel in ethidium bromide solution.

The PCR products were purified using the High Pure Purification PCR product kit (Boehringer Mannheim, Meylan, France) and then cloned into pCR2.1 (TA Cloning vector; Invitrogen) and transformed in INVaF' competent cells (One shot; Invitrogen). The white colonies were picked up from Luria-Bertani (LB) medium containing 40 mg ml^{-1} X-Gal and 50 µg ml^{-1} kanamycin and then cultured in 5 ml LB broth containing kanamycin at 37°c overnight. After centrifugation, bacterial cell suspension was washed twice with 2 ml of DNAse-free distilled water and resuspened in 200 µg ml^{-1} of the water. The 10 µl of bacterial cell suspension was mixed in PCR reaction to amplify 18S rDNA.

18S rDNA fingerprinting using RFLP technique

The PCR products were digested with restriction enzymes. The mixtures of each restriction enzyme reaction contained 15 µl of PCR product, 0.5 µl of *Hin*fI or *Ban*I or *Taq*I (Gibco BRL LifeTechnologies), 3.0 µl of specific reaction buffer and 11.5 µl of DNAse-free distilled water. The reactions of *Hin*fI and *Ban*I were incubated at 37°C for 150 minutes whereas *Taq*I reaction was incubated at 65°C. The fragments of DNA were separated by 1.5% agarose gel electrophoresis in 0.5% TAE. The gels were stained with ethidium bromide and viewed under UV light. The estimated restriction fragment lengths were calculated by using the 1 kb DNA ladder (Promega).

Experiment 2: Effects of AM fungal species, nitrogen and phosphorus fertilizers on maize growth, root colonization and spore production

Experimental design

A pot experiment using a 3 x 4 x 4 factorial in randomized complete block design with three replications was carried out under a plastic roof at the Department of Soil Science, Kasetsart University, Bangkok, Thailand. The experimental factors were: (1) three AM fungal species (2) four rates of N fertilizer and (3) four rates of P fertilizer. The types of mycorrhizal inoculation were: (1) Check (CK), i.e. not inoculated with AM fungi; (2) A, inoculated with *Acaulospora spinosa* and (3) G, inoculated with *Glomus aggregatum*. The rates of N fertilizer were N_0, N_1, N_2 and N_3 representing 0, 136.2, 408.6 and 817.2 mg N pot^{-1}, respectively (equivalent to 0, 31, 94 and 188 kg N ha^{-1}) as urea (46% N). The rates of P fertilizer were P_0, P_1, P_2 and P_3 representing 0, 136.2, 272.4 and 544.8 mg P_2O_5 pot^{-1}, respectively (equivalent to 0, 31, 63 and 125 kg available P_2O_5 ha^{-1}) as triple superphosphate (46% available P_2O_5).

Soil and pot preparation

A Pak Chong soil series (Very-fine, kaolinitic, isohyperthermic, Rhodic Kandiustox: Soil Survey Staff, 1998) with a clay texture, pH 6.2 (1:1, soil:water), 3.2% organic matter and 18 ppm P (by Bray-II method) was sterilized with Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) at the rate 60 g 100 kg^{-1} soil. An aliquot of 8.5 kg dry sterilized soil was then put in each sterilized pot (25 cm diameter and 22 cm depth) mounted on saucer. One half of the N fertilizer and all of the P fertilizer were well mixed with the soil in the pot before planting.

Planting, inoculation and cultural practices

Maize (*Zea mays* L. cv. Suwan 2) seeds were surface-sterilized by soaking in 10% sodium hypochloride solution for 10 minutes followed by rinsing with sterilized water. The *A. spinosa* and *G. aggregatum* inocula were originally obtained from the

Department of Microbiology, Faculty of Science, Kasetsart Univ. (STD 3 Project CT. 39-0256) as the isolates of Boonlue (1997) and recultured in the greenhouse on maize. One hundred gram (approximately 300 spores) of soil inoculum of the desired AM fungi was put in one pitch in each pot followed by sowing four maize seeds in the inoculum. The inoculum was then covered with 2-cm soil layer. Distilled water was sprayed on the soil surface to keep the soil moist throughout the cropping period. The plants were thinned to two plants and one plant per pot at 10 and 25 days after planting, respectively. At 30 days after planting, the second half of N fertilizer was applied by banding on the soil surface around the plant followed by water spraying. No other chemical was applied to the plants.

Harvest

The plants were cut at 60 days after planting (just after tasseling). Dry weight of the plant shoot was measured after drying in oven at 105^0C to constant weight.

Examination of root colonization and spore intensity

After cutting the plant shoot, the soil was allowed to dry out within two weeks. All of the soil of each pot was then taken out of the pot, well mixed and crushed to pass a 2-mm sieve. Aliquots of 5-g soil were used for examining spore intensity using sucrose centrifugation method according to Daniels and Skipper (1982).

Preparation of roots for examination of AM fungal colonization under compound microscope was done according to Koske and Gemma (1989). The intensity of root colonization of the AM fungi was examined by a semisubjective ranking method of Trouvelot *et al.* (1986).

Statistical analysis

Data were calculated to obtain (1) tasseling age, (2) shoot dry matter, (3) root colonization and (4) spore g^{-1} soil. Analysis of variance was used to determine effects

of AM fungal inoculation x N x P rates interactions on maize growth using the IRRISTAT program of International Rice Research Institute. AM fungal root colonization and spore production were tested for each species. Duncan's Multiple Range Test ($DMRT_{0.05}$) was used to test the treatment means differences. Correlation coefficients (r) among root colonization and spore intensity of AM fungi and shoot dry matter of maize plants were calculated using IRRISTAT program and the equations of relations among root colonization and spore intensity of each AM fungal species were calculated using Excel for Window® program.

Experiment 3: Comparative responses to AM fungi of maize cultivars different in downy mildew resistance and fertilizer requirement

Experimental design

A pot experiment using a 4 x 3 factorial in randomized complete block design with 3 replications was carried out under a plastic roof at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The two factors were 4 maize cultivars and 3 AM fungal inoculation practices. The maize cultivars included two open-pollinated cultivars, namely Suwan-1 selection cycle 0 (SW1C0) and Suwan-1 selection cycle 11 (SW1C11) and two hybrids, namely Suwan 2301 (SW2301) and Suwan 3851 (SW3851). The AM fungal inoculation practices were: (1) non-inoculated with AM fungi, (2) inoculated with *Scutellospora fulgida* and (3) inoculated with *Glomus aggregatum.*

SW1C0 maize was used to represent non-downy mildew resistance (non-DMR) cultivars whereas SW1C11 to represent DMR cultivars. SW2301 and SW3851, which were DMR hybrids, were used as a cultivar requiring low fertilizer (N and P) rate and that requiring high fertilizer rate to attain their maximum yields, respectively. SW2301 was reported to require 90-90 kg N-P_2O_5 ha^{-1} to reach a maximum grain yield of 4.25 t ha^{-1} whereas other four tested cultivars required 120-120 kg N-P_2O_5 ha^{-1} or more to reach their maximum yields of 5.7 t ha^{-1} or more (Suwanarit *et al*., 1985). SW3851 was reported to require more than 180-180 kg N-P_2O_5 ha^{-1} to attain its maximum yield of 6.58 t ha^{-1} whereas the other three cultivars required 60-60 to 120-120 kg N-P_2O_5 ha^{-1} to attain their maximum yields of 4.31 t ha^{-1} or more (Suwanarit *et al*., 2000).

AM fungal preparation

The AM fungal species were collected from the field in Thailand by Boonlue (1997) and had been maintained with pot culture on Pak Chong soils. Spores were collected by wet-sieving and decanting method (Gerdemann and Nicolson, 1963). Single spore cultures were done with maize grown on fired clay (Terragreen®) at CPB-CNRS, Nancy, France. The soil inoculum production was done with maize grown on Pak Chong soils at Kasetsart University. Morphotype of spores were observed to detect contamination in the pot cultures. The soil inoculum of each AM fungal species included the infected roots, hyphae and spores (about 1 spore g^{-1} soil) without contamination of any other AM fungal species.

Soil and pot preparation

The soil used was Pak Chong soil series (Very-fine, kaolinitic, isohyperthermic, Rhodic Kandiustox) collected from the National Corn and Sorghum Research Center, Nakhon Ratchasima province. The soil (with 13 ppm P by Bray-II method) was sterilized with Dazomet (Basamid®, 60 g 100 kg^{-1} soil) according to Suwanarit *et al*. (1997). Plastic pots and saucers were sterilized by spraying with 70% ethanol. Then, 7 kg of the sterilized soil was weighed out into each plastic pot.

Planting, inoculation and cultural practices

Eight seeds were buried 1 cm below the surface of 300 g soil inoculum contained in a pitch in each pot (Suwanarit *et al*., 1997). The inoculum was then covered with 1-cm layer of sterilized soil and expanded clay (Hydroton®), respectively. Three hundred gram of sterilized soil was applied instead of soil inoculum in the pots for the non-inoculated AM fungal treatment. The seedlings were thinned to 3 plants per pot at 10 days after planting (DAP). The temperature during the experiment in plastic house fluctuated between 18 and 45^{o}C. The plants were

liberally supplied with distilled water throughout the growing period by spraying water on the soil surface. No chemical was applied to the plants. The positions of pots in each replication were rerandomized weekly until tasseling. After silking of 50% of the plants in all treatments, watering was stopped. The plants were cut just above the soil surface at 90 DAP and the shoots were ovened at 70^{o}C until constant weight before weighing.

Data collection and calculation

The height of plants were measured at 30, 45, and 62 DAP. The tasseling and silking ages and dry weight of plant shoots at 90 DAP were recorded. Ground samples of shoot were digested with H_2SO_4 + Na_2SO_4 + Se (100: 10: 1) digestion mixture. N, P and K in the digest were measured with micro Kjeldhal distillation (Bremner, 1965), the vanadomolybdophosphoric yellow colorimetric method using a Spectronic-21 colorimeter (Jackson, 1958), and a frame emission spectrophotometer, respectively. After cutting the plant shoot, the soil was allowed to dry out within two weeks. All of the soil of each pot was then taken out of the pot, well mixed and malleted to pass a 2-mm sieve. One hundred gram of soil was used for examining number of spore of the inoculated AM fungal species 100 g^{-1} soil using wet sieving and decanting method (Gerdemann and Nicolson, 1963). The enhancing effects of AM fungal inoculation on maize plants were presented as percentage increase in the case of positive response and percentage decrease in the case of negative response. It was calculated as:

$$\text{Increase or decrease (\%)} = \left[\frac{(M_i - M_{ni})}{M_{ni}}\right] \times 100 \qquad (1)$$

where M_i and M_{ni} were values of parameter of maize plants with and without AM fungal inoculation, respectively.

The nutrient efficiencies of non-inoculated maize plants were calculated as:

$$\text{Nutrient efficiency} = \frac{\text{Shoot dry weight}}{\text{Nutrient uptake in shoot}} \qquad (2)$$

Statistical analysis

Analysis of variance was used to determine effects of AM fungal inoculation x maize cultivar interactions on maize growth using the IRRISTAT program of International Rice Research Institute. Duncan's Multiple Range Test ($DMRT_{0.05}$) was used for comparison on the effects between various AM fungal inoculation within a maize cultivar. The shoot dry weight, N, P and K efficiencies of non-inoculated plants and spore intensity in soil were compared using standard errors.

Experiment 4: A study on time courses of the effects on maize of AM fungi applied to maize in the field

Soil characteristics and sites

A field experiment was conducted on non-sterilized Pak Chong series at the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima Province, Thailand. The soil before the first cropping had pH 6.9 (1:1 soil: water), 3.0% OM, 15 ppm available P (Bray II method), 500 ppm exchangeable K.

Experimental design and treatments

A Randomized Complete Block design with 4 treatments and 4 replications was employed. The treatments were (1) non-inoculation with AM fungi (Control), (2) inoculation with T6 AM fungal species from Germany, (3) inoculation with *A. spinosa* and (4) inoculation with *S. fulgida* to maize and *G. aggregatum* to groundnut (*Arachis hypogaea* L.). These treatments were applied only in the first cropping. After the first cropping, nine successive cropping with sole maize (*Z. mays* L. cv. Suwan 5) were conducted without reinoculation with AM fungi. The cropping season included the 1996's late rainy season, the 1997's early rainy season, 1998's dry season, the 1998's late rainy season, the 1999's dry season, the 1999's late rainy season, the 2000's early rainy season, the 2000's late rainy season, the 2001's early rainy season and the 2001's late rainy season (Figure 3). Dry season was from December to March, early rainy season from April to July and late rainy season from August to November.

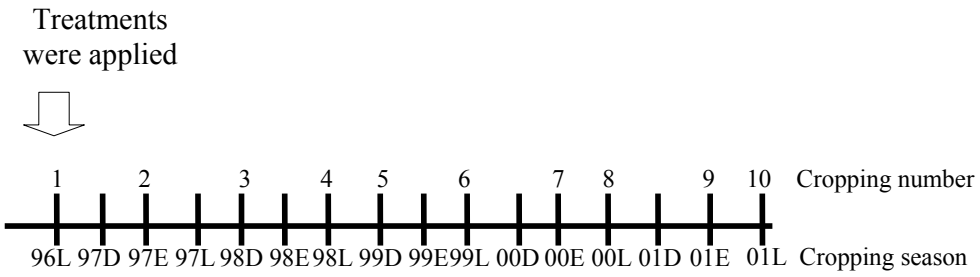

Figure 3 Cropping seasons in which sequential cropping were done (Experiment 4). 96-01 = year 1996-2001; D = dry season (December to March); E = early rainy season (April to July); L = late rainy season (August to November).

Plants and plant spacing

In the first cropping, the maize plants grown with double row spacing was intercropped with groundnut. Each double row of maize consisted of two rows of plants, with spacing of 40 cm between the rows of each double row. The distance between the center of the adjacent double rows was 150 cm. In the space between the adjacent maize double rows, two rows of groundnut plants were grown with spacing of 40 cm between the two groundnut rows. Plant rows were arranged so that the plot border lines were at the center of the outmost maize double rows. Each plot accordingly accommodated two double maize rows and one single maize row on each side of the plot and six rows of groundnut plants. Hill spacing were 25 cm for both maize and groundnut. In each hill, one plant was grown in the case of maize and three plants were grown in the case of groundnut. The harvest area was 3.5 x 3.0 m and only the four central rows were accounted for data collection.

Plots of individual treatments measured 4.5m x 7m in all of the subsequent cropping which were done with sole maize. Plant spacing was 75 cm between rows and 25 cm between hills. In the 1998's late rainy season, each plot consisted of six rows of maize plants. Only the four central rows, discarding 1 meter at each end of the rows were accounted for data collection. After the 1998's late rainy season, each plot

will consisted of five rows of maize plants. Only the three central rows, discarding 1 meter at each end of the rows, were accounted for in data collection.

Inoculum production

The AM fungal species under study were collected from the field in Thailand by Boonlue (1997). The AM fungal inocula were originally obtained from the Department of Microbiology, Faculty of Science, Kasetsart Univ. (STD 3 Project CT. 39-0256) and maintained with pure pot culture on Pak Chong soils at Kasetsart University and in fired-clay at CPB-CNRS, Nancy, France. Mass inoculum was done with pot culture in plastic house at Kasetsart University. The soil used was Pak Chong series collected from the National Corn and Sorghum Research Center, Nakhon Ratchasima Province. The soil, with 13 ppm available P (by Bray II method) was sterilized with Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) at the rate 60 g 100 kg^{-1} soil (Suwanarit *et al.*, 1997). An aliquot of 7-kg dry sterilized soil was then put in each sterilized pot (25 cm diameter and 22 cm depth) mounted on saucer.

For inoculum production, T6 AM fungal species from Germany, *A. spinosa* and *S. fulgida* were propagated on maize plants whereas *G. aggregatum* was propagated on groundnut. Five holes per pot were made on the surface of soil. Soil inoculum was then added into the holes, 20 g per hole. One seed was then buried in the soil inoculum in each hole. Four weeks after planting, the plants were thinned so that 2 plants were left to grow per pot. The plants were given with distilled water throughout the growing period by spraying water on the soil surface. After tasseling, watering was stopped and the soils and plants were left to dry out. After drying, the soil and roots were crushed and mixed for using as inoculum. Spore intensity was examined under stereomicroscope. One gram of soil inoculum of *A. spinosa*, *S. fulgida* and *G. aggregatum* contained 3, 5 and 17 spores, respectively. Shortly before application, the inoculum were kept at 4 ^{o}C for 3 days to break dormancy of spores (Boonlue, 1997).

Inoculation with AM fungi, planting and cultural practices

The inoculation with AM fungi of the field experiment were done in maize-groundnut intercropping under field conditions with the presence of indigenous AM fungi in the 1996's late rainy season. Inoculation of AM fungi was done by making pitches, of 2-3 inches in diameter and depth, in the soil and approximately 100 g of the inoculum was placed in each of the pitches. The seeds were then placed in the inoculum at 1-3 cm depth. The pitches were then covered with the soil with 1-2 cm thickness. In addition to AM fungal inoculation, groundnut was inoculated with appropriate rhizobium by coating the seeds with inoculum of rhizobium (in peat-carrying form) using boiled tapioca paste as sticker. Only N fertilizer at the rate of 20 kg N ha^{-1} as urea was applied to all treatments. The crops were essentially rain-fed. However, supplemental irrigation, with sprinklers, was given in the case of inadequate rain. Weed control was done by hands at about 2 weeks and 4 weeks after planting. Harvest of maize was done at physiological maturity. The residual effects of inoculation in the first field experiment were studied with sole maize. No chemicals or fertilizer were applied in the following cropping with the exception for the eighth following cropping, in which N fertilizer at the rate of 20 kg N ha^{-1} as urea was applied to all treatments at 30 DAP in the following cropping. Normally, harvests were done after the physiological maturity. However, harvest was done at 90 DAP in the 2001's late rainy season after plant lodging caused by rainstorm. The straw of maize plant was incorporated into the soil after harvest.

Chemical analyses of plant samples

Ground samples of grain and stubble were digested with the $H_2SO_4 + Na_2SO_4 +$ Se (100:10:1) digestion mixture. N in the digest was then measured with micro Kjeldhal distillation (Bremner, 1965). The P content was measured with the vanadomolybdophosphoric yellow colorimetric method using a Spectronic-21 colorimeter (Jackson, 1958). The K content was measured with a frame emission spectrophotometer.

Mycorrhizal spore counts

Spores of the inoculated AM fungi in the soil were counted after plant harvest. Composite samples of the soil to the 15 cm depth in the rectangular area of 75 cm (across the plant row direction) by 25 cm (along the plant row direction), with the plant being at the center were collected. Fifty to one hundred gram of sample soil was used for examining spore intensity by wet-sieving and decanting method (Gerdemann and Nicolson, 1963). The abundance of inoculated AM fungi was calculated from the percentage proportion between number of spore of inoculated AM fungi and total spore in 100 g soil.

Statistical analysis

Analysis of variance was used to determine effects of AM fungal inoculation on maize growth using the IRRISTAT program of International Rice Research Institute. Duncan's Multiple Range Test ($DMRT_{0.05}$) was used for comparison on the effects between various AM fungal inoculation. The relative yields and trend lines were calculated using Excel program on Microsoft Windows XP.

The response to AM fungal inoculation of maize plant were calculated as:

$$\text{Relative yield} = \frac{\text{Mi}}{\text{Mni}} \times 100 \qquad (3)$$

where M_i and M_{ni} were values of parameter of maize plants with and without AM fungal inoculation, respectively.

Experiment 5: Verification of AM fungi showing effects on maize grown on Rhodic Kandiustox in the field and pot experiments

Soil characteristics and sites

The field experiment was conducted at the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima Province, Thailand. The Pak Chong soils had pH (1:1 soil: water) 7.0, 2.5% OM, 15 ppm available P (Bray II method), 270 ppm exchangeable K (with NH_4OAc, pH 7).

The soil used in the pot experiment was from the surface layer (0-15 cm depth) in the area before the field experiment was conducted. The pot experiment was carried out at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

Experimental design and treatments

The field experiment was carried out with five subsequent cropping of maize on non-sterilized Pak Chong soils. The experimental treatments were applied only in the first cropping. A Randomized Complete Block design with 4 treatments and 4 replications was employed. The experimental treatments were: (1) Control (without AM fungal inoculation), (2) inoculation with *S. fulgida*, (3) inoculation with *G. aggregatum* and (4) dual inoculation with *G. aggregatum* and *S. fulgida*.

The pot experiment was carried out with only one cropping. A Randomized Complete Block design with the 4 treatments and 5 replications was employed. The experimental treatments were the same as those of the preceding field experiment (Experiment 4).

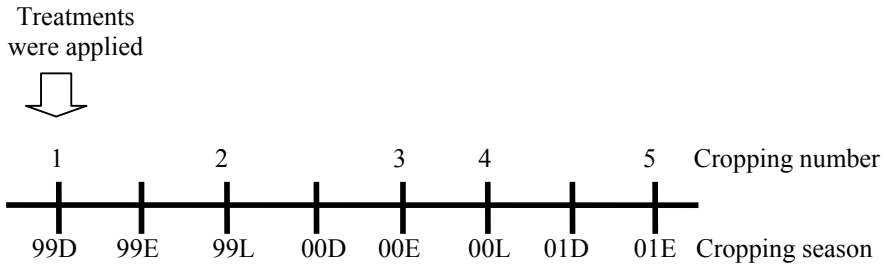

Figure 4 Cropping seasons in which sequential cropping were done (Experiment 5). 99-01 = year 1999-2001; D = dry season; E = early rainy season; L = late rainy season. Refer to Figure 3 for further captions.

These treatments were applied only in the first cropping. After the first cropping, four successive cropping with sole maize (*Z. mays* L. cv. Suwan 5) were conducted without reinoculation with AM fungi. The cropping seasons included the 1999's dry season, the 1999's late rainy season, the 2000's early rainy season, the 2000's late rainy season and the 2001's early rainy season (Figure 4).

Plants and plant spacing

In the field experiment, plots of individual treatments measured 4.5m x 7m. Plant spacing was 75 cm between rows and 25 cm between hills of one plant. Each plot consisted of five rows of maize plants. Only the three central rows, discarding 1 meter at each end of the rows, were accounted for data collection.

In the pot experiment, each pot, mounted on saucer, containing 25 kg of the soil. In each replication all pots were placed in one line along the north-south direction with 75 cm spacing between pots. No space was left between pots adjacent replications.

Inoculum production

The AM fungal species under study were collected from the field in Thailand by Boonlue (1997). The AM fungal inoculum were originally obtained from the Department of Microbiology, Faculty of Science, Kasetsart Univ. (STD 3 Project CT. 39-0256) and maintained with pure pot culture on Pak Chong soils at Kasetsart University and in fired-clay at CPB-CNRS, Nancy, France. Mass inoculum was prepared with pot culture in plastic house at Kasetsart University. The soil used was Pak Chong series, which was collected from the National Corn and Sorghum Research Center, Nakhon Ratchasima Province. The soil with 13 ppm available P level was sterilized with Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) at the rate 60 g 100 kg^{-1} soil (Suwanarit *et al.*, 1997). An aliquot of 7 kg dry sterilized soil was then put in each sterilized pot (25 cm diameter and 22 cm depth) mounted on saucer.

For inoculum production of the two experiments, twenty seeds of pearl millet (*Pennisetum americanum* L.) were buried 1 cm below the surface of 300 g soil inoculum contained in a pitch in each pot. Then, the inoculum was covered with 1 cm layers of sterilized soil and expanded clay, respectively. Sterilized Pak Chong soil series was used instead of soil inoculum in the pots of non-inoculated with AM fungal treatments. The seedlings were thinned to eight plants per pot at 10 days after planting (DAP). The temperature under the plastic roof fluctuated between 18 and 45^{o}C. The plants were supplied with distilled water. No chemical substance was applied. At 80 DAP soil moisture stress in the soil was imposed. At 90 DAP, shoot of plants were cut and the soil was left to dry out in the pot. After drying, the soil was crushed and roots were cut into 1 cm length. The soil and cut roots in each pot were mixed and spore intensity was examined under stereomicroscope. Soil inoculum of each AM fungi including infected roots, hyphae and spores (about 1 spore g^{-1} soil) were kept at 4 ^{o}C for 3 days before using as inoculum in the experiment (Boonlue, 1997).

Inoculation with AM fungi, planting and cultural practices

The inoculation with AM fungi of the field experiment were performed in sole maize cropping under field conditions with the presence of the indigenous AM fungi in the 1999's dry season. Control was applied with sterilized Pak Chong soils without AM fungi. The inoculation with AM fungi was done by putting about 300 g of inoculum in one pitch. Five maize seeds (Suwan-5 cultivar) were buried in the inoculum. The inoculum was then covered with 2 cm soil layer. The seedlings were thinned to one plant per hill at two weeks after planting. Supplemental irrigation was applied with sprinklers in the case of inadequate rainfall. Weed control was done by hands at about 2 weeks and 4 weeks after planting. No chemicals or fertilizer was applied in the first to fourth cropping whereas N fertilizer at the rate of 20 kg N ha^{-1} as urea was applied to all treatments at 30 DAP of plants in the fifth cropping. Harvest was performed after the physiological maturity. The stubble of maize plant was incorporated into the soil after harvest.

In the pot experiment, five maize seeds were sown in each pot. The seedlings were thinned to one plant per pot at two weeks after planting. The inoculation and fertilization of the pot experiment were similar to those described for the field experiment. Weed control was done by hands. Tap water was given liberally on soil surface. Harvest was done after the physiological maturity. The stubble was taken away at harvest.

Chemical analyses of plant samples

Ground samples of grain and stubble were digested with the H_2SO_4 + Na_2SO_4 + Se (100:10:1) digestion mixture. N in the digest was then measured with micro Kjeldhal distillation (Bremner, 1965). The P content was measured with the vanadomolybdophosphoric yellow colorimetric method using a Spectronic-21

colorimeter (Jackson, 1958). The K content was measured with a frame emission spectrophotometer.

Mycorrhizal spore counts

Spores of the inoculated AM fungi in the soil were counted after plant harvest. In the field experiment, composite samples of the soil to the 15 cm depth in the rectangular area of 75 cm (across the plant row direction) by 25 cm (along the plant row direction), with the plant being at the center were collected. In the pot experiment, composite samples of the soil to the 15 cm depth in the circular area around the plant with 20 cm in radius were collected. Fifty to one hundred gram of soil sample was used for examining spore intensity by wet-sieving and decanting methods (Gerdemann and Nicolson, 1963). The abundance of inoculated AM fungi was calculated from the percentage proportion between number of spore of inoculated AM fungi and total spore in 100 g soil.

Statistical analysis

Analysis of variance was used to determine effects of AM fungal inoculation on maize growth using the IRRISTAT program of International Rice Research Institute. Duncan's Multiple Range Test ($DMRT_{0.05}$) was used for comparison on the effects between various AM fungal inoculation. The relative yields (Equation 3 in Experiment 4) and trend lines were calculated using Excel program on Microsoft Windows XP.

Experiment 6: A study on effects of AM fungal repetitive inoculation on maize grown in the field

Soil characteristics and sites

This experiment was imposed on the plots of the Experiment 5 after the fifth cropping.

Experimental design and treatments

The experimental treatments of the Experiment 5 were repeated twice in two successive cropping after the fifth cropping. In Experiment 6, the fifth cropping of the Experiment 5 was regarded as cropping with non-repetitive application of the treatments. The experiment was therefore conducted with a Randomized Complete Block design with 4 treatments and 4 replications. The experimental treatments were: (1) Control (without AM fungal inoculation), (2) inoculation with *S. fulgida*, (3) inoculation with *G. aggregatum* and (4) dual inoculation with *G. aggregatum* and *S. fulgida*. These experimental treatments were repeated twice in two cropping seasons (August to November, 2001 and April to July, 2002) (Figure 5).

Inoculum production

The AM fungal species under study were collected from the field in Thailand by Boonlue (1997). The AM fungal inoculum were originally obtained from the Department of Microbiology, Faculty of Science, Kasetsart Univ. (STD 3 Project CT. 39-0256) and maintained with pure pot culture on Pak Chong soils at Kasetsart University and in fired-clay at CPB-CNRS, Nancy, France. Mass inoculum was performed with pot culture in plastic house at Kasetsart University. The soil used was Pak Chong series, which was collected from the National Corn and Sorghum Research Center, Nakhon Ratchasima Province. The soil with 13 ppm available P

level was sterilized with Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) at the rate 60 g 100 kg^{-1} soil (Suwanarit *et al.*, 1997). An aliquot of 7 kg dry sterilized soil was then put in each sterilized pot (25 cm diameter and 22 cm depth) mounted on saucer.

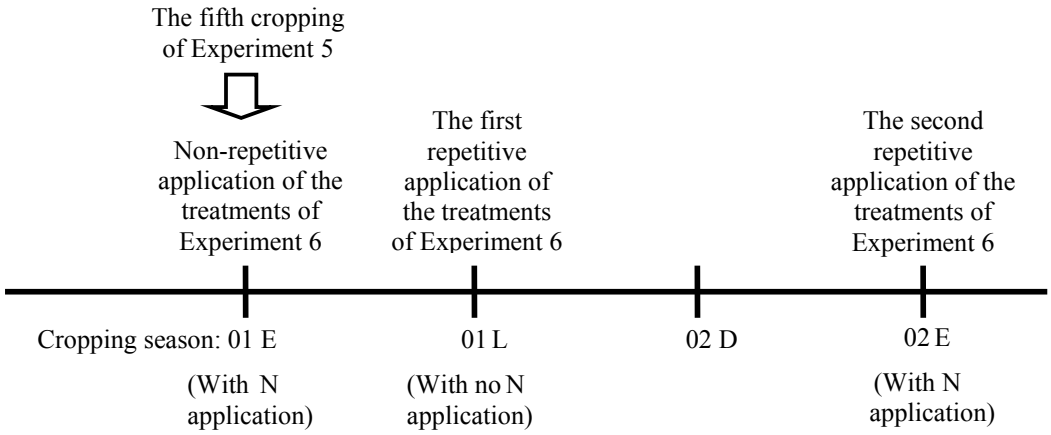

Figure 5 Cropping seasons in which the first and the second repetitive application of the treatment were applied (Experiment 6). 01-02 = year 2001-2002; D = dry season; E = early rainy season; L = late rainy season.

For inoculum production of the study of the first repetitive inoculation, maize (*Z. mays* L. cv. Suwan 5) seeds were surface-sterilized by soaking in 10% sodium hypochloride solution for 10 minutes followed by rinsing with sterilized water. Eight maize seeds were buried 1 cm below the surface of 300 g soil inoculum contained in a pitch in each pot. Then, the inoculum was covered with 1 cm layers of sterilized soil and expanded clay, respectively. Sterilized Pak Chong soil series was used instead of soil inoculum in the pots of non-inoculated with AM fungal treatments. The seedlings were thinned to 3 plants per pot at 10 days after planting (DAP). The temperature under the plastic roof fluctuated between 18 and 45 ^{o}C. The plants were supplied with distilled water. No chemical substance was applied. At 80 DAP soil moisture stress in the soil was imposed. At 90 DAP, shoot of plants were cut and the soil was left to dry out in the pot. After drying, the soil was crushed and roots were cut into 1 cm

length. The soil and cut roots in each pot were mixed and spore intensity was examined under stereomicroscope. Soil inoculum of each AM fungi including infected roots, hyphae and spores (about 1 spore g^{-1} soil) were kept at 4 ^{o}C for 3 days before using as inoculum in the experiment (Boonlue, 1997).

For inoculum production of the study of the second repetitive inoculation, maize (*Z. mays* L. cv. Suwan 1 cycle 0 and cycle 11, Suwan 2301 and Suwan 3851) were inoculated with each AM fungal species. The other procedures of inoculum production and spore intensity in the inoculum were described in proceeding paragraph.

Plant and plant spacing

Plant and plant spacing were the same as those described for Experiment 5.

Inoculation with AM fungi, planting and cultural practices

In the first repetitive inoculation cropping, Control was applied with sterilized soil by the amount equivalent to the amount of inoculum applied in the other treatments. In the second repetitive inoculation cropping, Control was applied with soil and roots of maize plants grown without AM fungi. The inoculation with AM fungi was done by putting about 300 g of inoculum in the first repetitive inoculation cropping and 100 g in the second repetitive inoculation cropping in one pitch. Five maize seeds (Suwan-5 cultivar) were buried in the inoculum. The inoculum was then covered with 2 cm soil layer. The seedlings were thinned to one plant per hill at two weeks after planting. Supplemental irrigation was applied with sprinklers in the case of inadequate rainfall. No chemicals or fertilizer were applied in the first repetitive inoculation cropping whereas N fertilizer at the rate of 30 kg N ha^{-1} as urea and 2, 4 - D for weed control were applied to all treatments at 30 DAP of plants in the second repetitive inoculation cropping. Harvest was performed at 90 DAP in the first

repetitive inoculation cropping after plant lodging caused by rainstorm and after the physiological maturity in the second repetitive inoculation cropping. The stubble of maize plants was incorporated into the soil after harvest.

Chemical analyses of plant samples

Ground samples of grain and stubble were digested with the H_2SO_4 + Na_2SO_4 + Se (100:10:1) digestion mixture. N in the digest was then measured with micro Kjeldhal distillation (Bremner, 1965). The P content was measured with the vanadomolybdophosphoric yellow colorimetric method using a Spectronic-21 colorimeter (Jackson, 1958). The K content was measured with a frame emission spectrophotometer.

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Statistical analysis

Analysis of variance was used to determine effects of AM fungal inoculation on maize growth using the IRRISTAT program of International Rice Research Institute. Duncan's Multiple Range Test ($DMRT_{0.05}$) was used for comparison on the effects between various AM fungal inoculation. The relative yields (Equation 3 in Experiment 4) were calculated using Excel program on Microsoft Windows XP.

Duration and places

Experiment 1

The study was done in laboratories and in growth chamber since October 1999 to March 2000 at Centre de Pedologie Biologique, CNRS, F-54501 Vandoeuvre-les Nancy, France.

Experiment 2

The experiment was conducted in greenhouse and laboratories at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok.

Experiment 3

The experiment was conducted in greenhouse and laboratories during November 2001 to October 2002 at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok.

Experiment 4

Ten planting seasons were carried out during August 1996 to November 2001 at the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima.

Experiment 5

The field experiment was carried out at the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima in 5 cropping seasons (the 1999's dry season, the 1999's late rainy season, the 2000's early rainy season, the 2000's late rainy season and the 2001's early rainy season). The pot experiment was carried out at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok in the 1999's dry season.

Experiment 6

The field experiment was carried out at the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima in the 2001's late rainy season and the 2002's early rainy season.

RESULTS AND DISCUSSION

Experiment 1: Identification of AM fungi by PCR technique

In this study, *G. aggregatum* spores and in colonized roots and *S. fulgida* spores were identified by using molecular techniques. Amplification of 18S rDNA genes were characterized by selection of suitable restriction enzymes for RFLP patterns.

18S rDNA patterns of *Glomus aggregatum*

The sizes of PCR products of *G. aggregatum* amplified with MH2 and MH4 primers were approximately 1.4 Kb (Figure 6). The colonized root with *G. aggregatum* from pot culture gave the same 18S rDNA patterns as DNA derived from spores (Figure 7). The estimated sizes of *G. aggregatum* from apparent bands on gels were shown in Table 1.

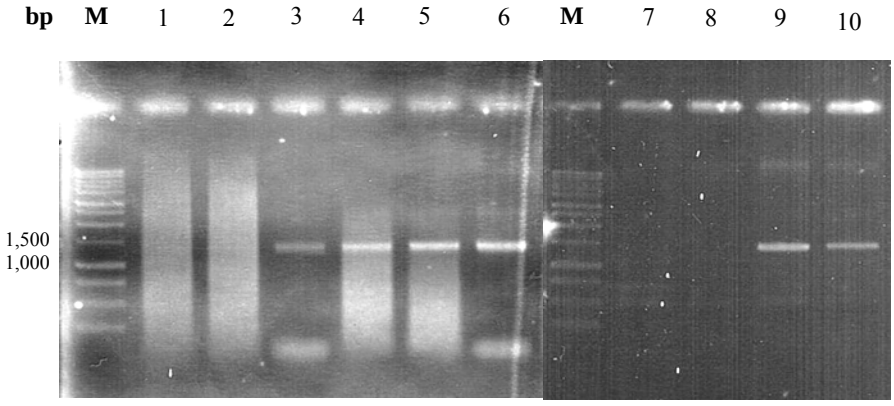


Figure 6 1.4 Kb PCR products of 18S rDNA of *Glomus aggregatum* derived from spores (1-6) and colonized roots (7-10) (M : 1 Kb DNA ladders).

The remained roots from single spore culture stained with glycerol trypan blue (Koske and Gemma, 1989) had about 40% colonization of *G. aggregatum* in the stage of vesicle development. Therefore, the PCR-RFLP could be used to identify the fungus in the hyphal growth stage in plant roots.

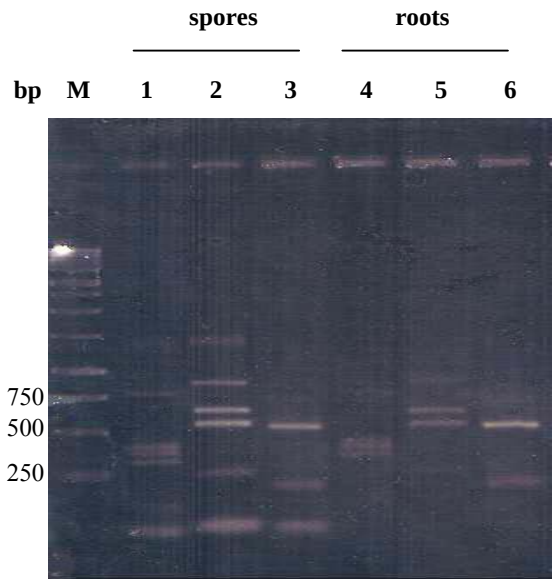


Figure 7 RFLP patterns of PCR products amplified from 18S rDNA of *Glomus aggregatum* derived from spores and colonized roots with MH2 and MH4 primers. *Hin*fI (lane 1 and 4), *Ban*I (lane 2 and 5) and *Taq*I (lane 3 and 6) M = 1 Kb DNA ladder.

Table 1 *Hin*fI, *Ban*I and *Taq*I digested fragment sizes (bp) of PCR products amplified from 18S rDNA of *G. aggregatum* with MH2 and MH4 primers.

Restriction enzymes	Fragment sizes (bp)
*Hin*fI	435, 369, 323
*Ban*I	610, 512, 243
*Taq*I	502, 184

18S rDNA patterns of *Scutellospora fulgida*

Genomic DNA from two morphotypes of one spore, two spores and 20 spores, which presented with or without bulbous suspensor were used as templates to amplify the 18S rDNA region by using MH2 and MH4 primers. Approximately 1.4 Kb of PCR products were obtained from both morphotypes (Figure 8) and they gave similar RFLP patterns (Figure 9). In many cases, the PCR products were not visible when stained by ethidium bromide, and their concentrations were not sufficient for restriction analysis. The amplification after cloning was done to increase concentration of PCR products. Clearly apparent bands of restriction fragments of *S. fulgida* clones that correspond to the weak bands of digestion without cloning were shown in Figure 10. One spore, two spores and 20 spores gave the similar fragment size of RFLP patterns (Figure 10). The estimated sizes of the fungus from apparent bands on gels were showed in Table 2.

These results confirmed morphological identification after crushing both morphotypes of spores under microscope to observe spore wall layers (Figure 11) and Melzer's staining reaction (Figure 12).

Table 2 Restriction fragment lengths (bp) of PCR products amplified from 18S rDNA of *S. fulgida* by MH2 and MH4 primers.

Restriction enzymes	Fragment lengths (bp)
*Hin*fI	490, 330, 250, 140, 90
*Ban*I	600, 520, 260
*Taq*I	610, 400, 200, 120, 50

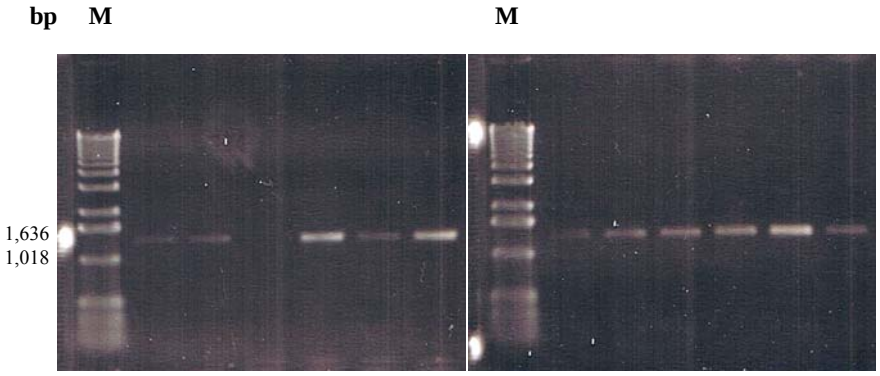


Figure 8 1.4 Kb PCR products of 18S rDNA of *Scutellospora fulgida* derived from spores without bulbous suspensor (a) and with bulbous suspensor (b) (M: 1 Kb DNA ladder).

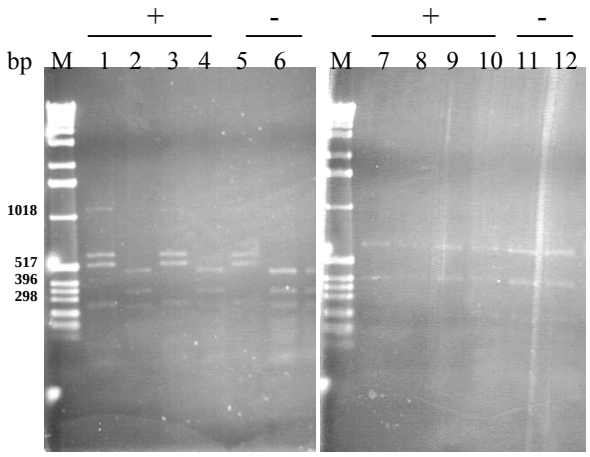


Figure 9 RFLP patterns derived from *Ban*I (lane 1, 3 and 5), *Hin*fI (lane 2, 4 and 6) and *Taq*I (lanes 7 to 12) digestion of the 18S rDNA region of *Scutellospora fulgida* spore with (+) and without (-) bulbous suspensor. M : 1 Kb DNA ladder.

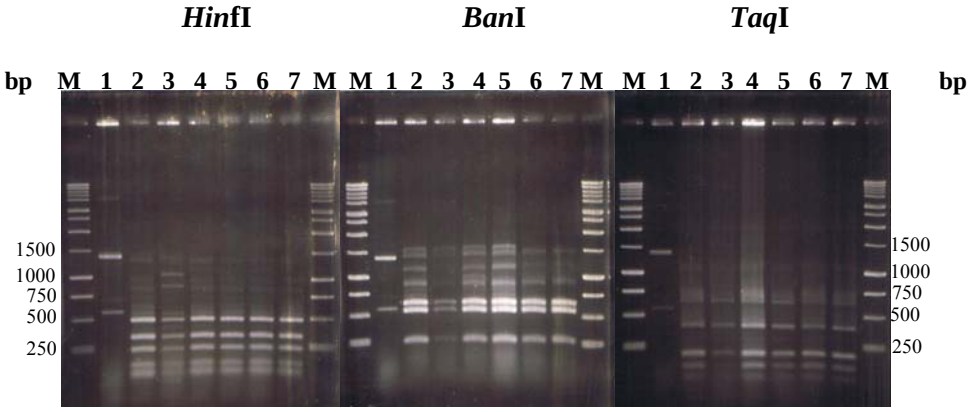
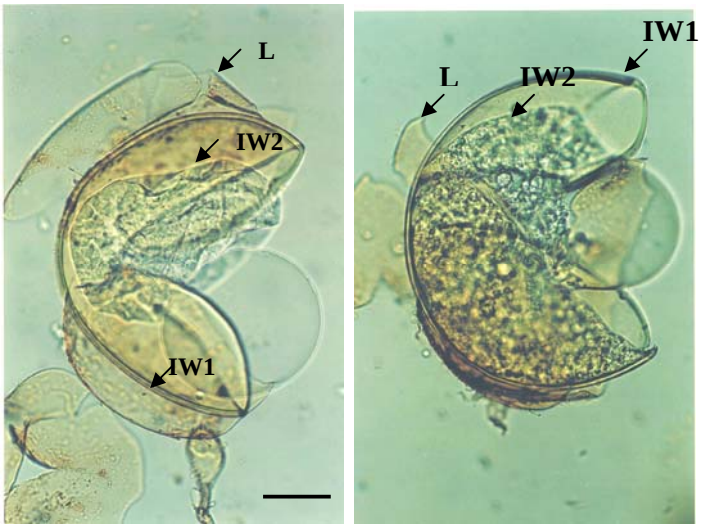


Figure 10 RFLP patterns derived from *Hin*fI, *Ban*I and *Taq*I digestion of the 18S rDNA region of *Scutellospora fulgida* clones. M : 1 Kb DNA ladder; lane 1 : uncut fragment; lane 2, 3 and 7 : single spore crushing; lane 4 : two spores crushing; lane 5 and 6 : twenty spores crushing.



(a) (b)

Figure 11 Three spore wall layers of crushed spores presented with (a) or without (b) bulbous suspensor. L : spore wall; IW1 : inner wall; IW2 : innermost wall. Bar = 40 µm.

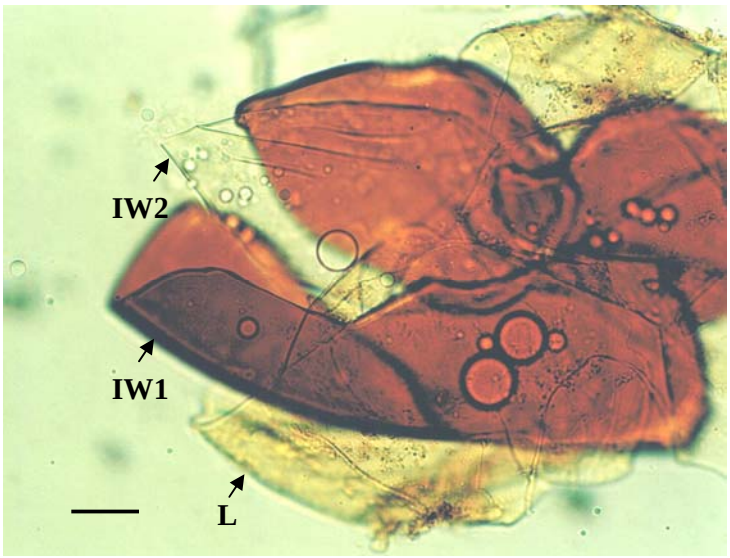


Figure 12 Squashed spore of *Scutellospora fulgida* stained with Melzer's reagent. L : spore wall; IW1 : inner wall; IW2 : innermost wall. Bar = 20 µm.

The present results were supported by Redecker (2000), who designed a set of primers for nested PCR. ITS and 18S rDNA were amplified from colonized roots in the absence of spores. The PCR products could be used to identify AM fungi at the genus and species levels using *Dpn*II and *Hin*fI with the exception for members within the Gigasporaceae. In this study, *Hin*fI and *Taq*I were selected as proper restriction enzymes for PCR-RFLP and could verify morphological identification of *G. aggregatum* and *S. fulgida*. However, study on more restriction patterns of the members of the both genus is neccessary for identifying different members of the two AM fungal genera. In addition, purification, cloning and re-amplification of PCR products should be done to produce clearer results of PCR-RFLP patterns.

Experiment 2: Effects of AM fungal species, nitrogen and phosphorus fertilizers on maize growth, root colonization and spore production

Tasseling age

N rate, N rate x P rate interactions, AM fungal species x N rate interactions and AM fungal species x N rate x P rate interactions showed no significant differences whereas different AM fungal species, P rate and AM fungal species x P rate interactions showed significant differences in tasseling age, i.e. number of days from planting to tasseling (Figure 13). Inoculation with *G. aggregatum* gave shorter tasseling ages than *A. spinosa* while Check gave the longest. Inoculation with *A. spinosa* and with *G. aggregatum* shortened the tasseling age. Inoculation with *G. aggregatum* gave shorter tasseling age than inoculation with *A. spinosa*. In the case of inoculation with *G. aggregatum*, P fertilization did not affect the tasseling age. In the case of inoculation with *A. spinosa* and Check, the P fertilization shortened the tasseling age. The largest effects of P fertilization was obtained when no AM fungi was applied whereas no effect of added P was obtained with *G. aggregatum* inoculation.

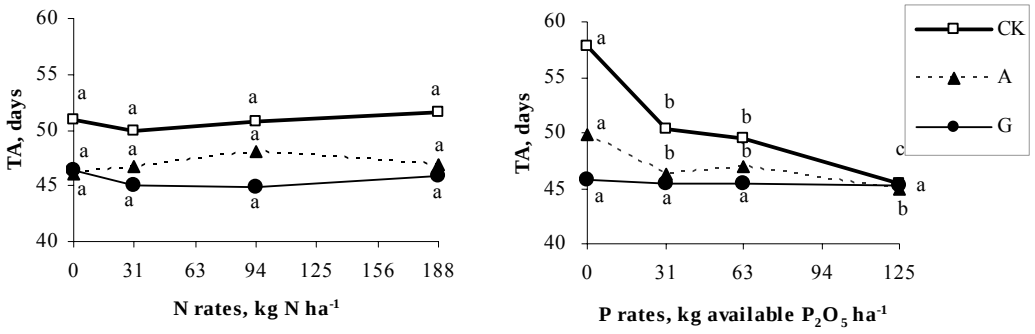


Figure 13 Effects of AM fungal species and N rates, AM fungal species and P rates on tasseling age (TA) of maize. CV=5.1%. On the same line, points with a common letter were not significantly different by DMRT at $P < 0.05$. CK = Check; A = inoculated with *Acaulospora spinosa*; G = inoculated with *Glomus aggregatum*.

Shoot dry matter

There were no significant differences in the effects of different N rates, N rate x P rate interactions, AM fungal species x N rate interactions and AM fungal species x N rate x P rate interactions but significant differences were evident in the effect of AM fungal species, P rate and AM fungal species x P rate interactions on shoot dry matter of the maize (Figure 14). At all of the N rates, the inoculation with *G. aggregatum* gave higher shoot dry weight than *A. spinosa* while Check gave the lowest.

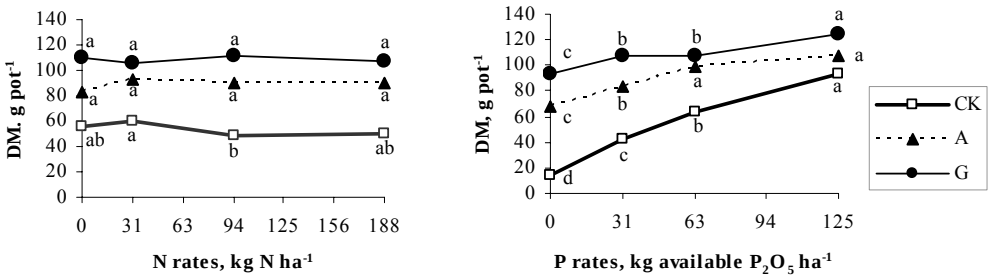


Figure 14 Effects of AM fungal species and N rates, AM fungal species and P rates on shoot dry matter (DM) of maize. CV = 14.4%. Refer to Figure 13 for further captions.

The effects of P application were most pronounced in the case of Check and least pronounced in the case of *G. aggregatum*. On average, *G. aggregatum* and *A. spinosa* increased shoot dry weight by 102% and 66%, respectively, when compared with Check. *G. aggregatum* increased shoot dry weight by 570%, 151%, 69% and 32% whereas *A. spinosa* increased shoot dry weight by 380%, 97%, 55% and 14%, when compared with Check at the rate 0, 31, 63 and 125 kg available P_2O_5 ha^{-1}, respectively. When inoculation with each AM fungi at the rate 31, 63 and 125 kg available P_2O_5 ha^{-1} was compared with that without P fertilizer, *G. aggregatum* increased shoot dry weight by 14%, 15% and 32% whereas *A. spinosa* increased shoot dry weight by 25%, 46% and 59%, respectively.

These results showed that plants inoculated with *G. aggregatum* gave lower positive response to P fertilization than inoculation with *A. spinosa* while Check gave the largest response. The AM fungal inoculation did not show any effects on the response to N fertilizer of the plants. The AM fungal inoculation showed the highest ability to increase shoot dry weight when P fertilizer was not applied. The positive effects of the inoculation with AM fungi on shoot dry weight were observed even at the highest P rate.

The responses in tasseling age and shoot dry matter of maize to P fertilization showed that *G. aggregatum* was more effective than *A. spinosa* in promoting growth and development of maize. In addition, the plants inoculated with *G. aggregatum* were less responsive to the P fertilizer than those inoculated with *A. spinosa*. The difference in efficacy of the AM fungi might be a result from different acquisition of P by the mycorrhizal plants with different AM fungal species. The growth responses of maize to AM fungi were highest when soil was most seriously deficient in P. The effect of AM fungal inoculation on tasseling age was supported by the finding of Daft and Okusanya (1973) who found that AM fungal colonization stimulated tassel development of maize. The tasseling age shortening might be related to the increase in plant weight or P uptake early in the growth of maize inoculated with AM fungi. The decrease in the growth promotion effect with increase in P fertilizer was supported by the finding of Posta and Fuleky (1997) who also found that the positive effects of AM fungal inoculation to maize also decreased with increase in rate of P fertilizer. The lack of response of maize to N fertilization might be attributed to the soil sterilization that in turn released N from the dead soil microorganisms to meet the demand of plants (Thingstrup *et al*., 1998). The current results which showed that plants inoculated with AM fungi gave higher efficacy of the AM fungi at lower rates of P fertilizer (or higher N:P ratios) than at higher rates of P fertilizer (or lower N:P ratios) were supported by the results of Sylvia and Neal (1990) who found that plants inoculated with AM fungi gave higher efficacy of AM fungi at high N:P ratio than at low N:P ratio when the plants were sufficiently supplied with N.

Root colonization of AM fungi

A. spinosa showed no significant effect of P fertilization on percent root colonization when N fertilization was applied at the rate N_1, N_2 and N_3 but showed significant effects of P fertilization when no N fertilizer was applied (Figure 15). Without N fertilizer, application of P at the rate of 31 kg available P_2O_5 ha^{-1} resulted in highest root colonization whereas higher P rates reduced the root colonization.

G. aggregatum showed significant effects of P application on percent root colonization at all of the rates of N application (Figure 15). Without N fertilizer (N_0), percent root colonization was not affected by P application at the rates up to 63 kg available P_2O_5 ha^{-1} whereas application of P at the highest rate reduced the root colonization. With N_1, percent root colonization was reduced with increased P fertilizer rates up to the rate of 63 kg available P_2O_5 ha^{-1}. With N_2, percent root colonization was reduced by the application of 63 and 125 kg available P_2O_5 ha^{-1}. With N_3, percent root colonization tended to be increased by P application at the rates up to 63 kg available P_2O_5 ha^{-1} whereas application of P at the highest rate reduced the root colonization.

These results suggested that with 31 and 94 kg N ha^{-1}, root colonization by *G. aggregatum* decreased with increase rate of P application. Without N and with 188 kg N ha^{-1}, only very high rate of P fertilizer reduced the root colonization.

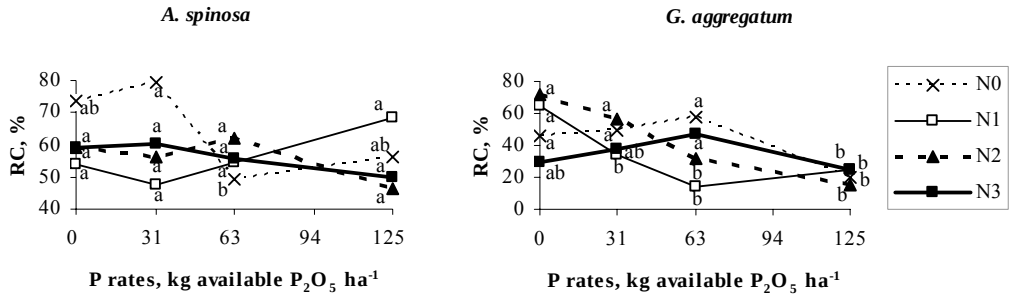


Figure 15 Effects of different rates of N and P fertilizers on root colonization (RC) of *A. spinosa* and *G. aggregatum*. CV=26.8% and 32.3%, respectively. On the same line, points with a common letter were not significantly different by DMRT at $P < 0.05$. N_0, N_1, N_2 and N_3 = rates of N fertilizer were 0, 31, 94 and 188 kg N ha^{-1}, respectively.

Effects of P fertilizer on root colonization of the two AM fungi varied with rate of N fertilizer. The patterns of responses to P and N fertilizers of the two AM fungal species were also different. The maximum root colonization was obtained with 31 kg available P_2O_5 ha^{-1} and no N fertilizer in the case of *A. spinosa* and with 94 kg N ha^{-1} and no P fertilizer in the case of *G. aggregatum*. These results demonstrated that the two AM fungal species required different N:P ratios to attain their maximum root colonization. This finding accordingly explained the discrepancy among finding of different researchers. Mosse (1973) found that increased N tended to reduce the inhibitory effect of high P. Hepper (1983), for example, found that root colonization was increased with higher N:P ratios. Thomson *et al*. (1986) found that P addition decreased root colonization by AM fungi. Baath and Spokes (1989) found that combinations of high rates of N and P fertilization decreased colonization in plant roots. Sylvia and Neal (1990) found that root colonization was decreased with P fertilization when N was sufficient whereas the colonization was not affected by P fertilization when N was deficient.

Spore production

In the case of *A. spinosa*, the P fertilization did not affect spore intensity when no N (N_0) and N_1 were applied (Figure 16). With N_2, spore intensity tended to decrease with increased P rates up to 63 kg available P_2O_5 ha^{-1}, after which the spore intensity decreased with P rate. With N_3, the P fertilization decreased the spore intensity. These results suggested that P fertilization did not affect spore production of *A. spinosa* at N_0 and N_1 but decreased the spore production when N_2 and N_3 were applied.

In the case of *G. aggregatum*, there was no significant difference in the effects, on spore intensity, of N rate but there were significant differences in effects of P rate and N rate x P rate interactions. Effects of P rates at different N rates were shown in Figure 16. With N_0 fertilizer, spore intensity was not affected by P fertilization. With N_1, spore intensity decreased with increased P rates. With N_2, spore intensity tended to decrease with increased P rates up to 63 kg available P_2O_5 ha^{-1}, after which the spore intensity decreased with P rate. With N_3, spore intensity increased with increased P rates up to 63 kg available P_2O_5 ha^{-1}, after which spore intensity tended to decrease with P rate. These results suggested that, for soil with very high N status, a moderate rate of P fertilizer was required to maximize the spore production. The highest spore production was obtained with 31 kg N ha^{-1} without P application.

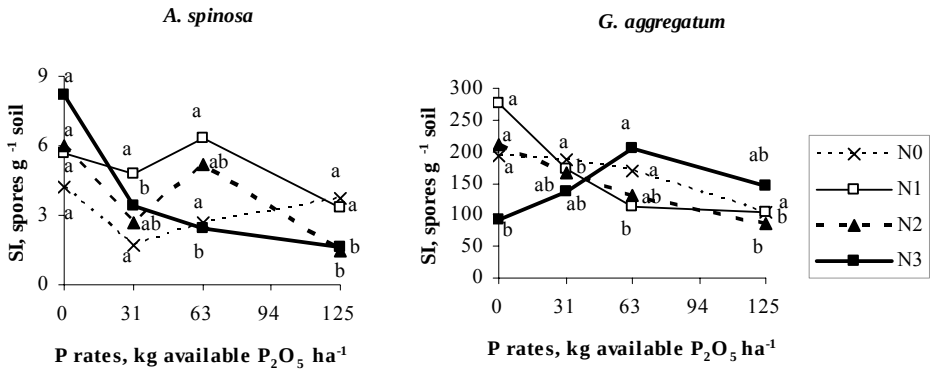


Figure 16 Effects of different rates of N and P fertilizers on spore intensity (SI) of *A. spinosa* and *G. aggregatum*. CV=66.4% and 33.1%, respectively. Refer to Figure 15 for further captions.

The results on spore intensity also demonstrated that the two AM fungal species required different N:P ratios to attain their maximum spore production. Moreover, for each AM fungal species N:P ratio to attain its maximum root colonization and that to attain its spore intensity were different. To produce maximum root colonization, *A. spinosa* required lower N:P ratio than *G. aggregatum* but to produce maximum spore production the reverse was true.

Relationship between root colonization and spore intensity

Relationship between root colonization and spore intensity was analyzed by linear regression. *A. spinosa* showed no relation between root colonization and spore intensity whereas *G. aggregatum* showed highly significant positive correlation (Figure 17).

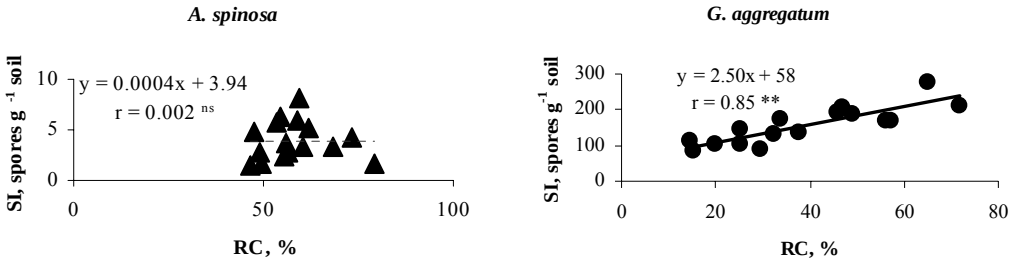


Figure 17 Relations among root colonization (RC) and spore intensity (SI) of *A. spinosa* and *G. aggregatum*. ns = not significant at $P < 0.05$; ** = significant at $P < 0.01$. n = 16.

Relationship between root colonization and spore intensity of AM fungi and shoot dry weight of maize plant

Root colonization and spore intensity of both AM fungi did not show relationship with shoot dry weight of maize plant (Table 3). These results showed that the degree of root colonization did not affect shoot dry weight of maize and shoot dry weight of maize did not affect spore intensity of both AM fungi.

Table 3 Correlation coefficients (r) among root colonization (RC) and spore intensity (SI) of AM fungi and shoot dry matter (DM) of maize plants.[1/]

Relation	*A. spinosa*	*G. aggregatum*
RC vs DM	-0.32 ns	-0.44 ns
SI vs DM	-0.47 ns	-0.25 ns

[1/] ns = not significant at $P < 0.05$. n = 16.

The current study demonstrated that spore intensity in the soil might or might not correlate with root colonization due to difference in growth development and

nutrient demand of each AM fungal species. The present results on the relationship between root colonization and intensity of spores in the soil observed from *G. aggregatum* which produced small spores was supported by the results of Jensen and Jakobsen (1980) who observed close relationship between the variables resulted from AM fungal species that produced small spores.

The results of the experiment indicated that shoot dry weight increased with increased P rate but was not affected by increased N rate. On the other hand, each AM fungal species required different specific N and P rates (N:P ratio) to attain maximum intensity of colonization within roots and of spores in the soil. These resulted in the absence of relationship between the shoot dry weight of maize and root colonization and spore intensity of both AM fungal species. However, the results were not supported by the finding of Menge *et al.* (1978) and Khaliq and Sanders (1997) who found that increased P application increased plant growth but decreased hyphal growth and spore production, suggesting negative relationship among plant growth and root colonization as well as spore production.

Experiment 3: Comparative responses to AM fungi of maize cultivars different in downy mildew resistance and fertilizer requirement

Plant height

At 30 and 45 DAP, the plants inoculated with AM fungi were taller than non-inoculated plants (Figure 18 and 19). However, the height of plants inoculated with *G. aggregatum* was comparable to that of plants inoculated with *S. fulgida* except the height at 30 DAP in which SW3851 plants inoculated with *G. aggregatum* was greater than that of plants inoculated with *S. fulgida*. Percentage increases in height in response to the two AM fungi were comparable in all maize cultivars (Figure 18 and 19)

At 62 DAP, there was no significant difference in height between inoculation and non-inoculation in maize cultivar SW2301 and SW3851. However, the AM fungus inoculated plants were taller than the non-inoculated plants in the case of SW1C11. The SW1C0 plants inoculated with *S. fulgida* were taller than non-inoculated plants but tended to be taller than the plants inoculated with *G. aggregatum* (Figure 20). Percentage increases in height in response to the two AM fungi were comparable in all maize cultivars (Figure 20).

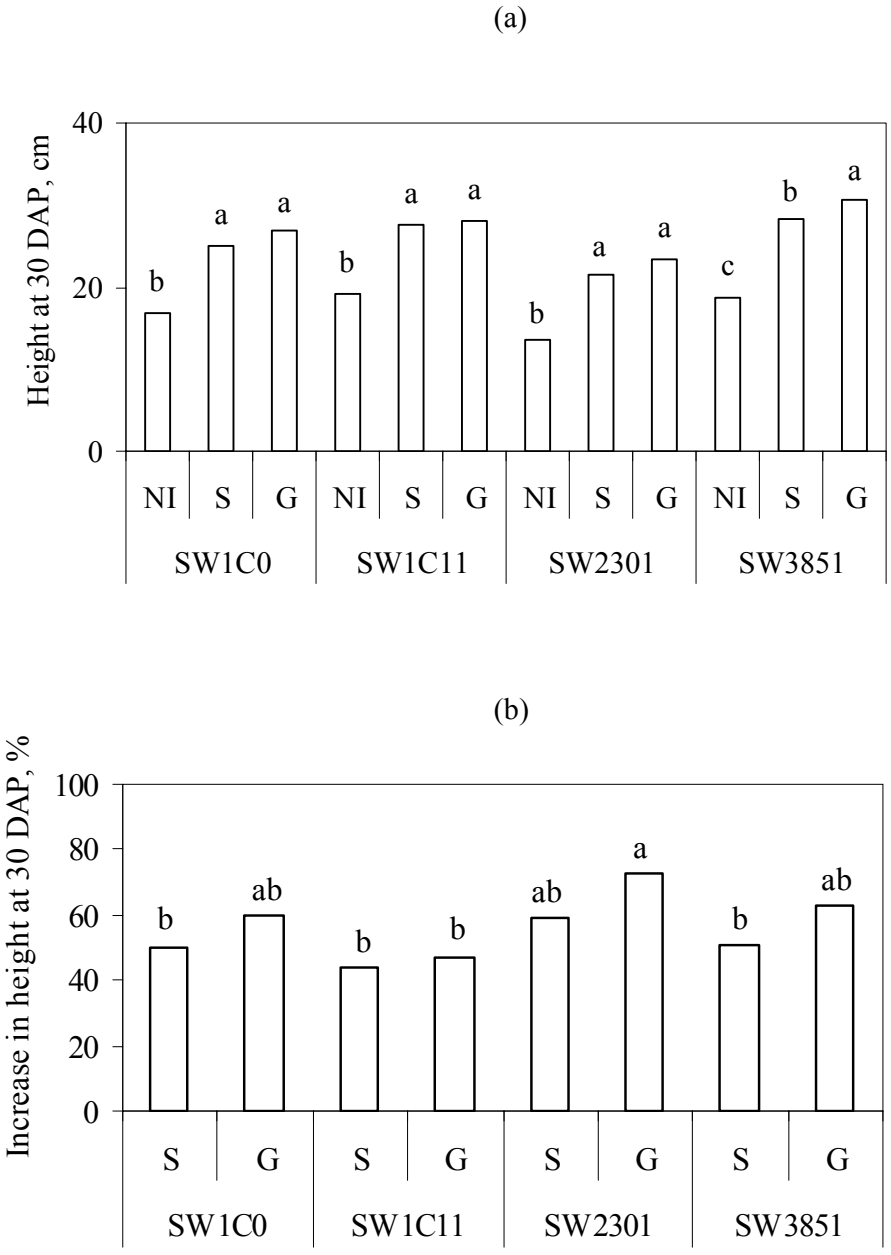
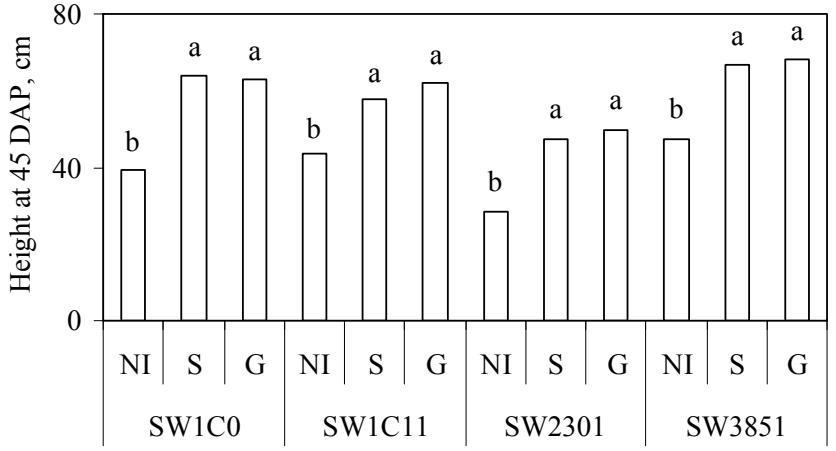


Figure 18 Height (a) and increase in height, as compared with that of NI, (b) of maize at 30 DAP as affected by maize cultivar and AM fungal species. Within the same maize cultivars, means with a common letter are not significantly different at $P \leq 0.05$. NI = non-inoculated with AM fungi; S = inoculated with *Scutellospora fulgida* ; G = inoculated with *Glomus aggregatum*.

(a)



(b)

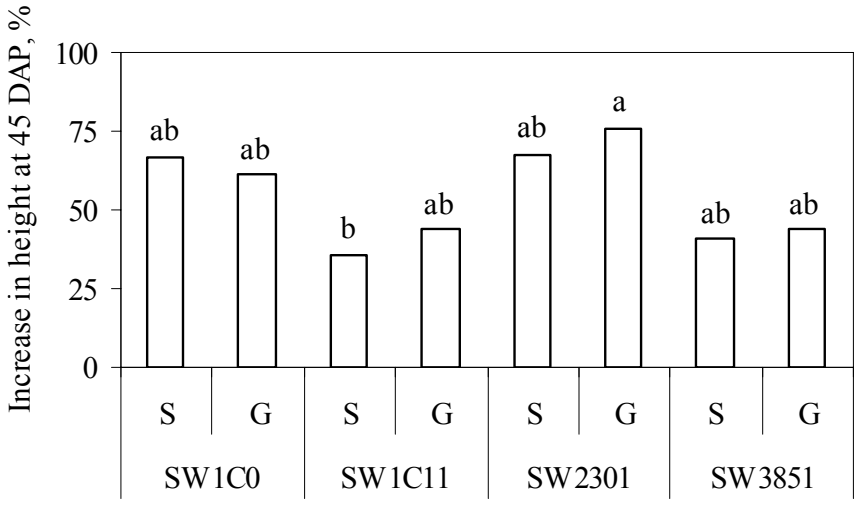
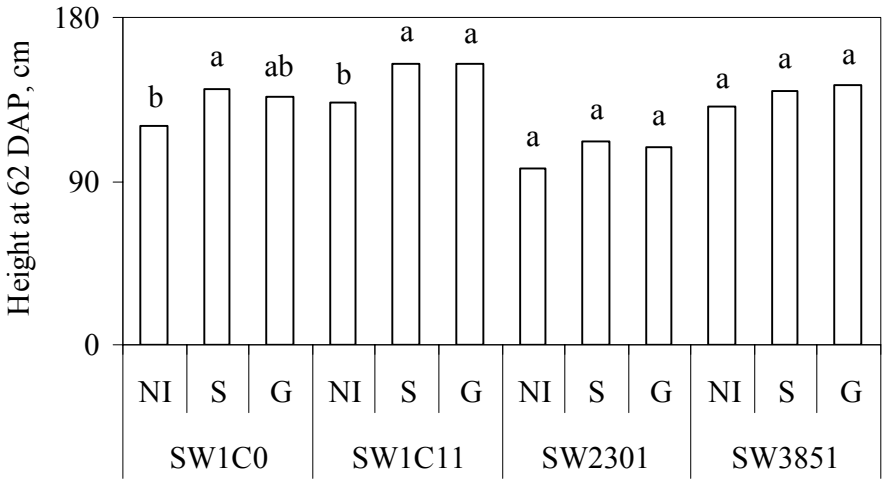


Figure 19 Height (a) and increase in height, as compared with that of NI, (b) of maize at 45 DAP as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

(a)



(b)

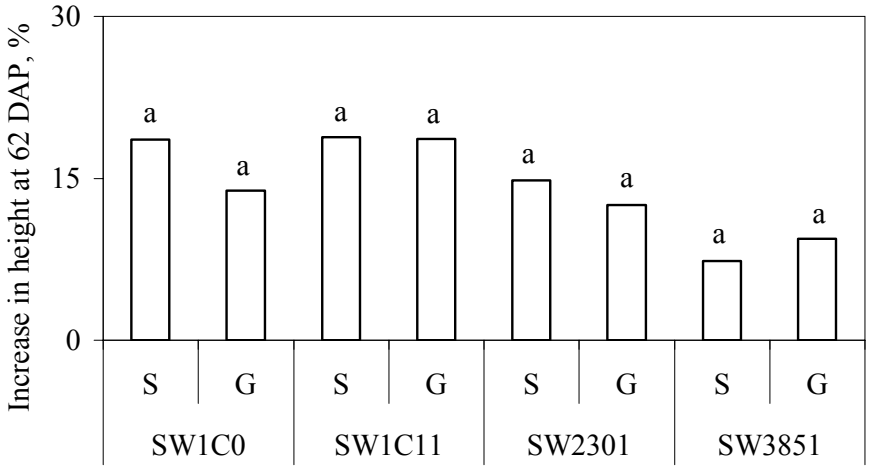


Figure 20 Height (a) and increase in height, as compared with that of NI, (b) of maize at 62 DAP as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

Tasseling and silking ages

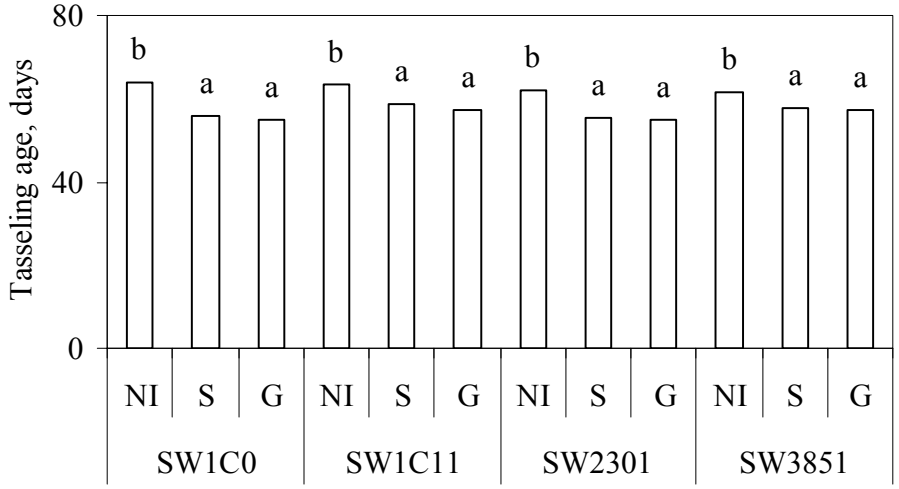
The tasseling and silking ages of the AM fungus inoculated plants were shorter than those of the non-inoculated plants (Figure 21 and 22, respectively). Results of the present study were similar to previous findings of Subramanian and Charest (1997) that the emergence of tassels and silks were earlier in AM fungus inoculated plants than in non-inoculated plants.

Percentage decreases in the flowering ages in response to the two AM fungi were comparable in all maize cultivars (Figure 21 and 22).

Shoot dry weight

The plants inoculated with AM fungal species gave greater shoot dry weight than non-inoculated plants (Figure 23). Percentage increases in shoot dry weight in response to the two AM fungi were comparable in all maize cultivars (Figure 23).

(a)



(b)

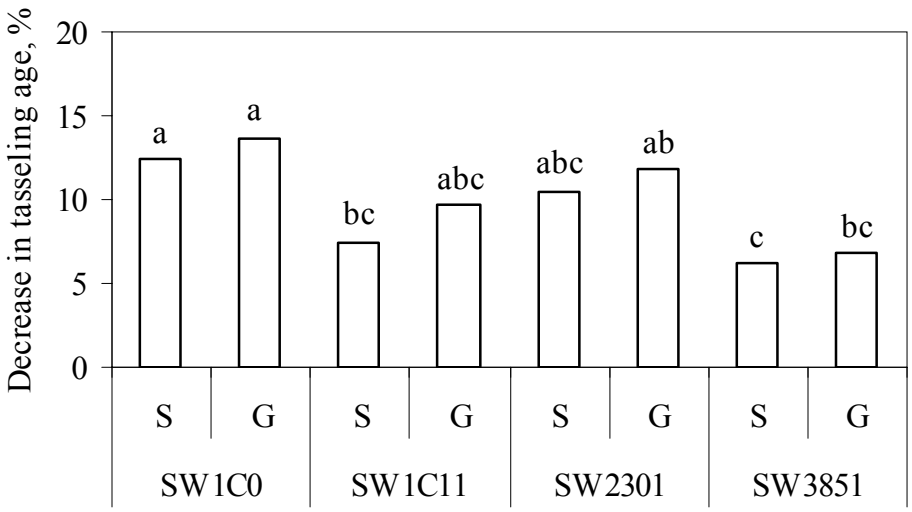
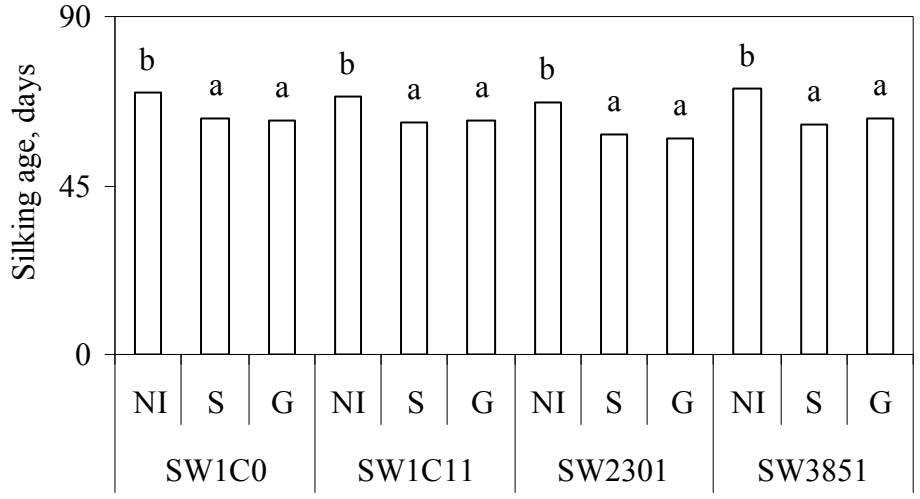


Figure 21 Tasseling age (a) and decrease in tasseling age, as compared with that of NI, (b) of maize as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

(a)



(b)

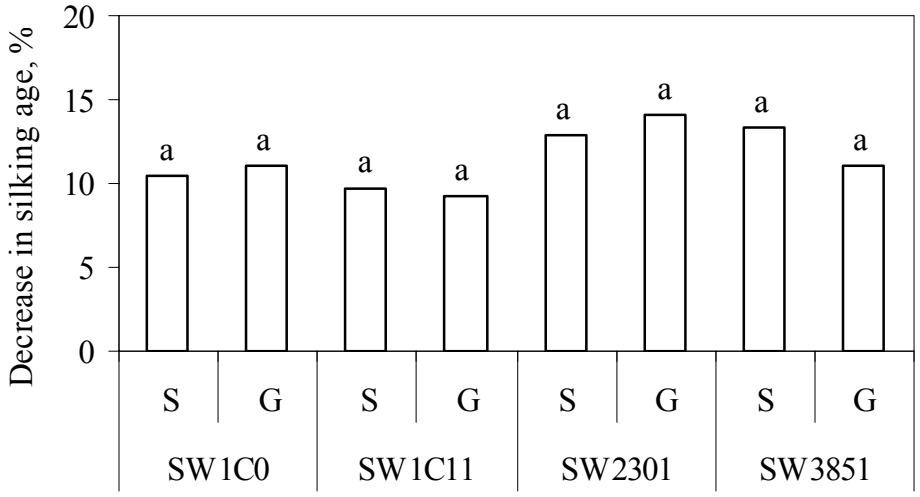
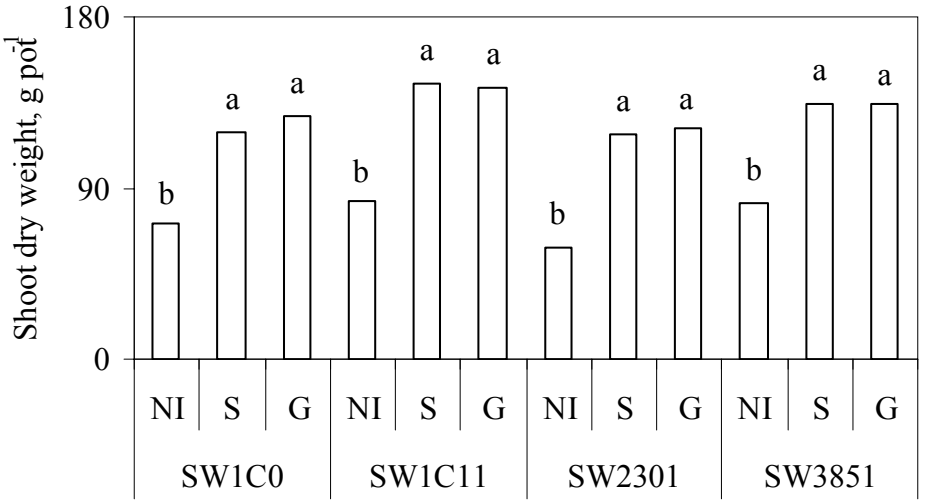


Figure 22 Silking age (a) and decrease in silking age, as compared with that of NI, (b) of maize as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

(a)



(b)

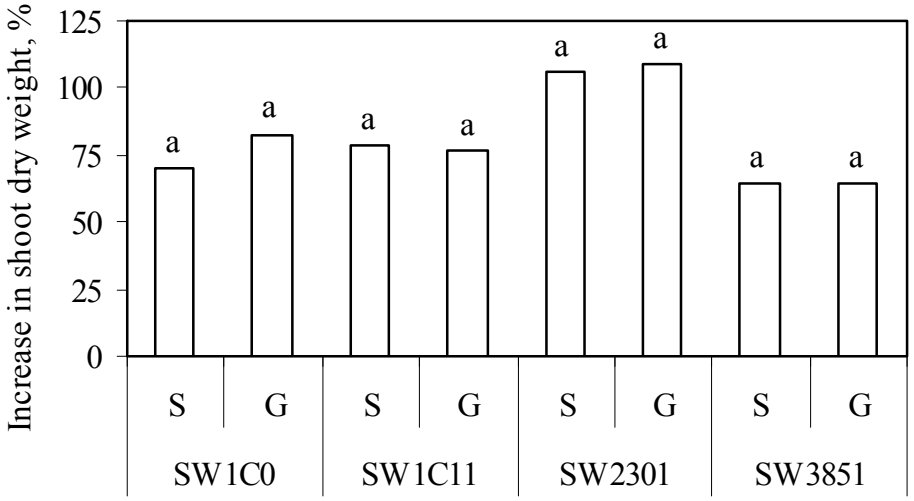


Figure 23 Shoot dry weight (a) and increase in shoot dry weight, as compared with that of NI, (b) of maize as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

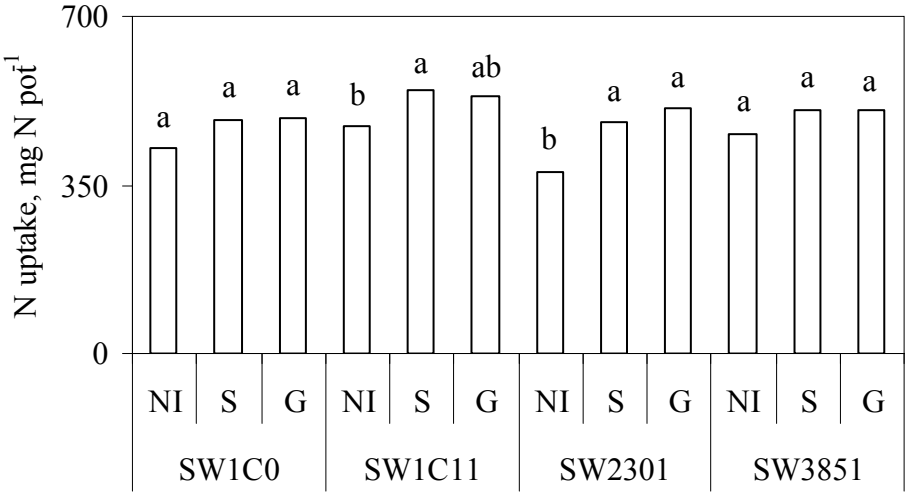
N uptake

The inoculation with *S. fulgida* or *G. aggregatum* on SW2301 gave greater N uptake in shoot of maize than non-inoculation. The inoculation with *S. fulgida* on SW1C11 gave greater N uptake than non-inoculation whereas the inoculation with *G. aggregatum* tended to increase N uptake. The inoculation with AM fungi on SW1C0 and SW3851 cultivars did not show significant enhancing effects (Figure 24). Percentage increases in N uptake in response to the two AM fungi were comparable in all maize cultivars (Figure 24).

P uptake

Inoculation with *S. fulgida* showed significant enhancing effect on P uptake in the case of SW2301 but showed no significant effect in the cases of SW1C0, SW1C11 and SW3851. Inoculation with *G. aggregatum* showed significant enhancing effects in the cases of SW2301 and SW3851 but showed no significant effect in the case of SW1C0 and SW1C11 (Figure 25). Percentage increases in P uptake in response to AM fungi were comparable between the two AM fungi and between SW1C0 and SW1C11 (Figure 25). However, percentage increases in P uptake in response to each of the two AM fungi in the case of SW2301 were higher than those in the case of SW3851. With the same maize cultivar, the two AM fungi showed comparable percentage increases.

(a)



(b)

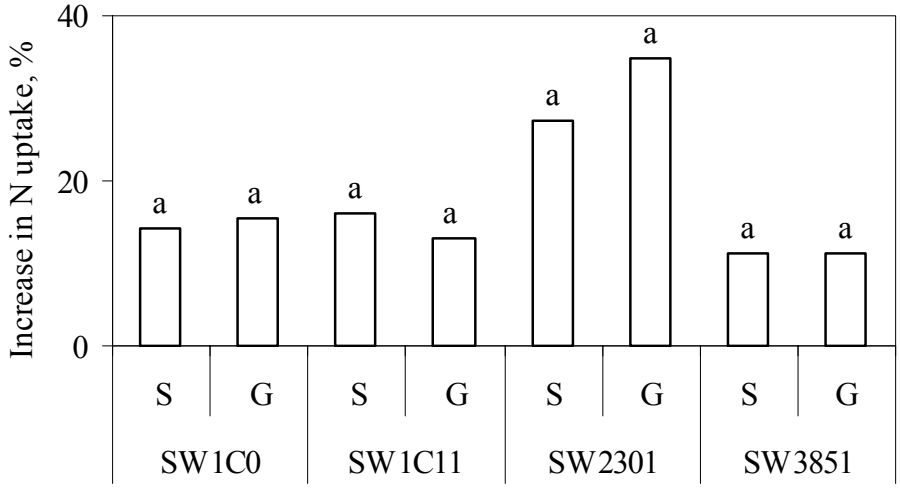
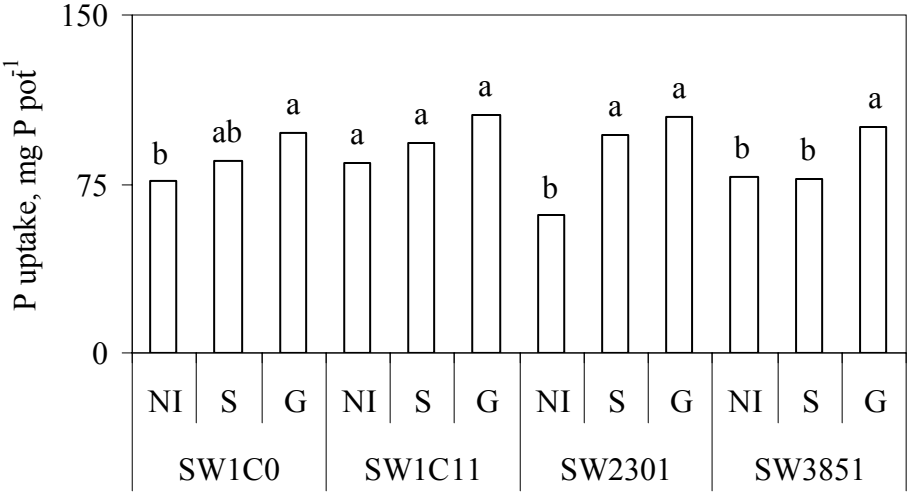


Figure 24 N uptake in shoot (a) and increase in N uptake in shoot, as compared with that of NI, (b) of maize as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

(a)



(b)

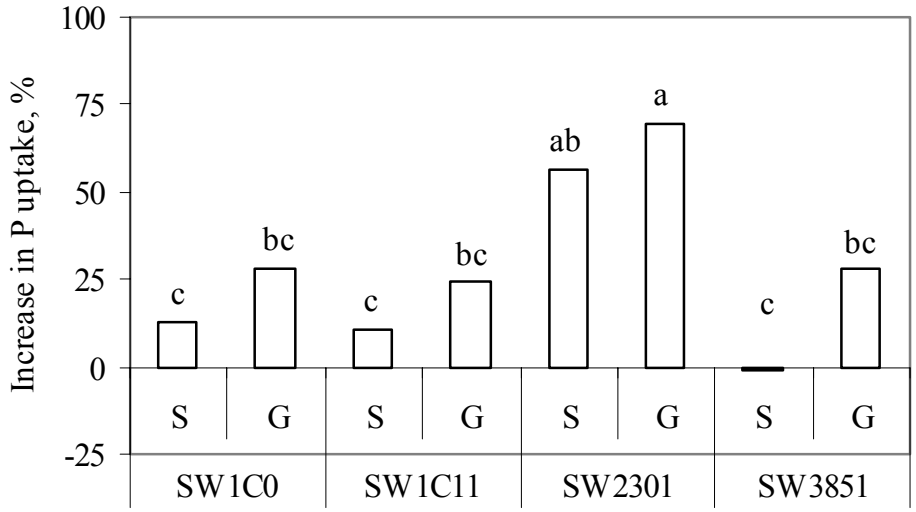


Figure 25 P uptake in shoot (a) and increase in P uptake in shoot, as compared with that of NI, (b) of maize as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

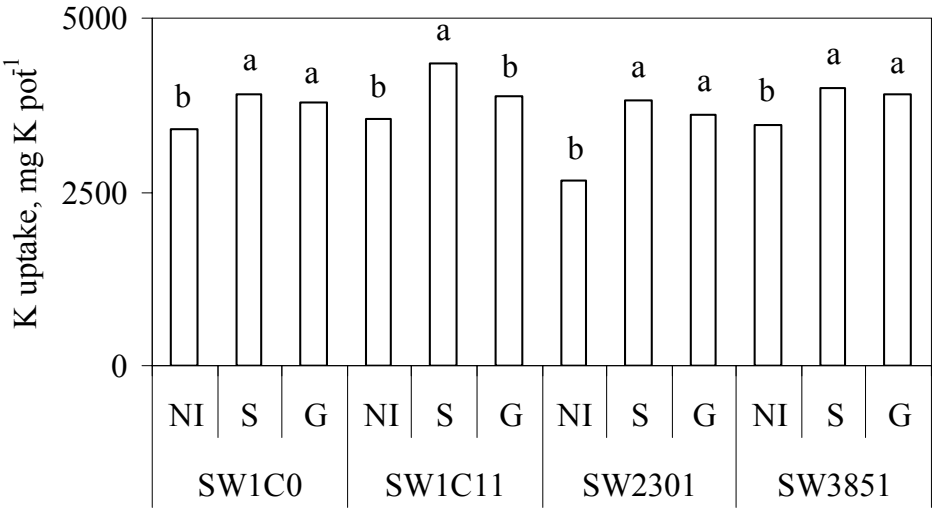
K uptake

Inoculation with either of the two AM fungi gave greater K uptake than non-inoculation, with an exception for the SW1C11 plants inoculated with *G. aggregatum* that did not show significant effect on K uptake (Figure 26). Percentage increase in K uptake in response to AM fungi were comparable between the two AM fungi and between SW1C0 and SW1C11 (Figure 26). However, responses to each of the two AM fungi in the case of SW2301 were higher than those in the case of SW3851. With the same maize cultivar, the two AM fungi showed comparable responses.

AM fungi spore intensity in soils

There was no difference between intensity of spores of each AM fungal species in the inoculation treatments whereas the studied AM fungal species was not found in the non-inoculation treatment (Figure 27). This showed that the inoculation was affective. Furthermore, no contamination of AM fungal species between treatments of AM fungal inoculation was detected.

(a)



(b)

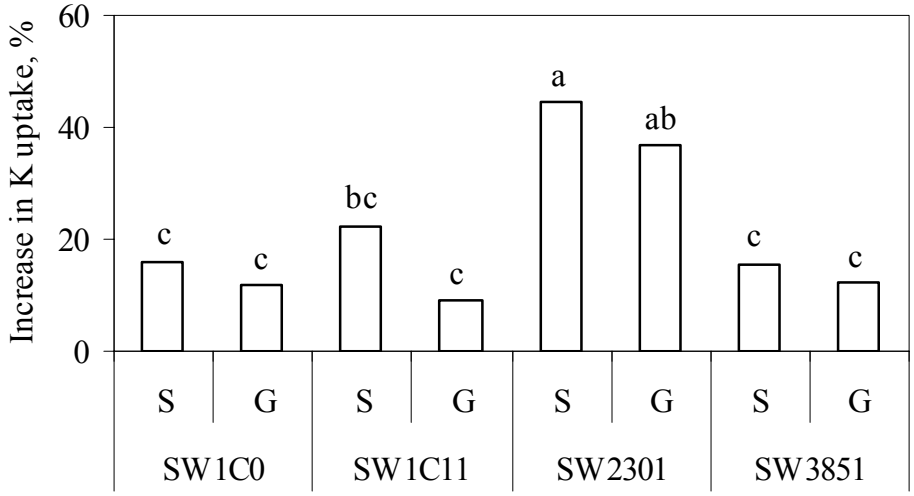


Figure 26 K uptake in shoot (a) and increase in K uptake in shoot, as compared with that of NI, (b) of maize as affected by maize cultivar and AM fungal species. Refer to Figure 18 for captions.

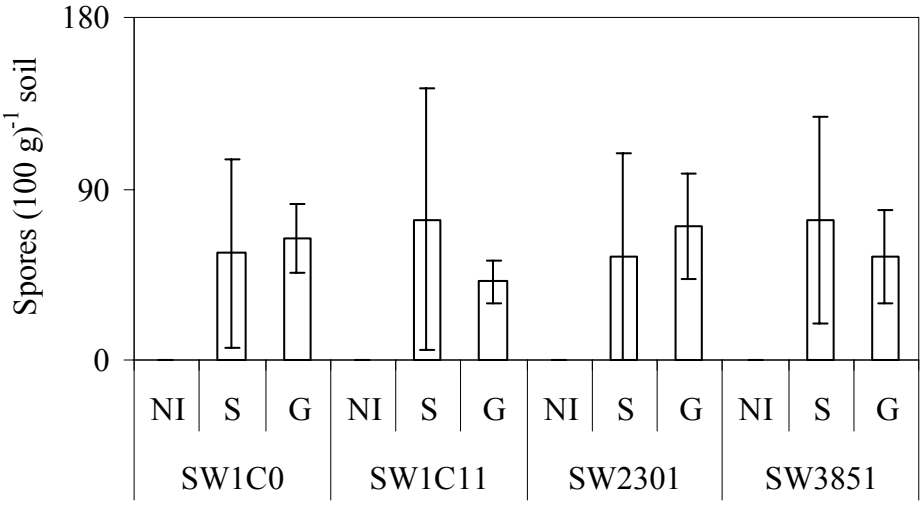


Figure 27 Spore intensity of the inoculated AM fungal species in soil after harvest. **I** on the top of each bar is the standard error. Refer to Figure 18 for captions.

Mycorrhizal response of non-DMR and DMR maize

SW1C0 and SW1C11 gave comparable responses to *G. aggregatum* and *S. fulgida* in all plant parameters (Figure 18, 19, 20, 22, 23, 24, 25 and 26), with an exception for the tasseling age in which SW1C0 gave higher response to *S. fulgida* than SW1C11 (Figure 21). This suggested that the non-DMR and DMR maize cultivars were comparable in their responses to AM fungi. However, the lower response to AM fungi in tasseling age did not seem to be a result of DMR because SW2301, a DMR hybrid, also showed a similar result as SW1C0 when inoculated with *S. fulgida*.

Comparison between the non-DMR (SW1C0) and DMR (SW1C11) cultivars in plant height at different ages, flowering ages, shoot dry weight and nutrient uptake indicated that there was no significant effect of improvement of maize cultivar at high soil fertility for high yield and DMR on response to AM fungi. Furthermore,

SW2301, which was a DMR hybrid, gave greater response to AM fungi in P and K uptake than the non-DMR cultivar (Figure 25 and 26). This finding disagreed with Toth *et al*. (1990) and Hetrick *et al*. (1992) who hypothesized that increasing resistance of crops to fungal pathogens and plant breeding at high soil fertility decreased benefit from the symbiosis. The present results showed that resistance to downy mildew did not affect benefit from mycorrhiza in maize.

Mycorrhizal responses of maize cultivars different in fertilizer requirement

SW2301 showed greater positive responses to *S. fulgida* and *G. aggregatum* in P and K uptake than SW3851 did (Figure 25 and 26), though, these two maize cultivars gave comparable responses to the AM fungi in other plant parameters (Figure 18, 19, 20, 21, 22, 23 and 24). This was supported by Linderman and Davis (2004) who reported effects of different combinations between plant cultivars and AM fungal species on the advantages from AM fungal inoculation. The results showed that different AM fungi affected various cultivars of *Tagetes* spp. differently, in terms of degree of plant growth enhancement.

The shoot dry weight and P utilization efficiency of maize plant without AM fungal inoculation (Table 4) may be used for predicting the response to AM fungi, in shoot dry weight, of each maize cultivar (Kaeppler *et al*., 2000 and Baon *et al*., 1993). The responses to AM fungi in shoot dry weight observed in the present study were highly negative correlated to shoot dry weight of non-inoculated plants (Figure 28). The lowest shoot dry weight of SW2301, as compared with SW3851, might explain the greatest response to AM fungi of this cultivar. This suggested that the lower shoot dry weight of the maize plant might be a cause of the higher response to AM fungi. This finding was supported by the results of Baon *et al*. (1993) and Kaeppler *et al*. (2000).

Table 4 Shoot dry weight (SDW) and N, P and K efficiencies of the maize cultivars obtained without AM fungal inoculation. Values are the means of three replicates with standard errors of the means in brackets.

Cultivars	SDW (g pot^{-1})	Nutrient efficiencies (g SDW mg^{-1} nutrient)		
		N	P	K
SW1C0	71.7 (14.7)	0.167 (0.021)	0.933 (0.089)	0.0210 (0.0026)
SW1C11	82.7 (15.6)	0.173 (0.019)	0.985 (0.231)	0.0233 (0.0043)
SW2301	58.1 (6.4)	0.154 (0.022)	0.940 (0.058)	0.0219 (0.0018)
SW3851	81.9 (8.7)	0.180 (0.005)	1.055 (0.025)	0.0236 (0.0020)

In addition, the responses to AM fungi in shoot dry weight were highly negatively correlated to N utilization efficiency of non-inoculated plants (Figure 29). The trend of lower N efficiency in shoot of the hybrid requiring low rate of fertilizers (Table 4) might be a cause of the higher response to AM fungi. Tanaka and Yano (2005) reported that *G. aggregatum* enhanced N uptake of maize. In the present study, the response of maize to *G. aggregatum* in N uptake was highly positively correlated with spore intensity of the fungus (Figure 30). Since Pitakdantham *et al*. (2004) found that the spore intensity of the fungus correlated with colonization in maize roots, these results suggested that N uptake of maize plant inoculated with *G. aggregatum* increased with increased colonization in the roots. However, there was no correlation between the response in N uptake of maize plant inoculated with *S. fulgida* and spore intensity (Figure 30). This latter finding supported the results of Kaeppler *et al*. (2000) which showed that colonization was not correlated with response to AM fungi. This present results and the finding of Kaeppler *et al*. (2000) suggested that the enhancing effect on N uptake depended upon AM fungal species.

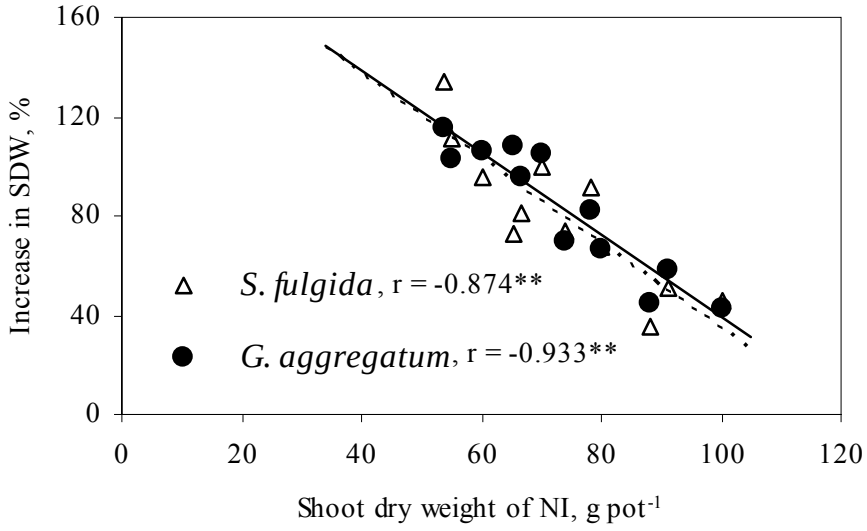


Figure 28 Relationship between shoot dry weight (SDW) of non-AM fungus inoculated plants (NI) and their responses in SDW to the two AM fungal species, *S. fulgida* (---) and *G. aggregatum* (—). ** Significant at $P \leq 0.01$.

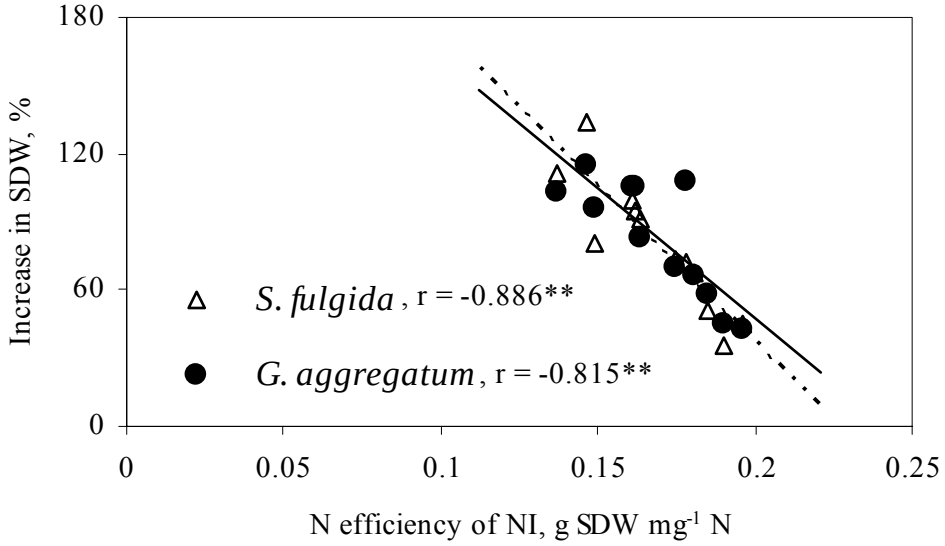


Figure 29 Relationship between N efficiency of non-AM fungus inoculated plants (NI) and their responses in SDW to the two AM fungal species. Refer to Figure 28 for captions.

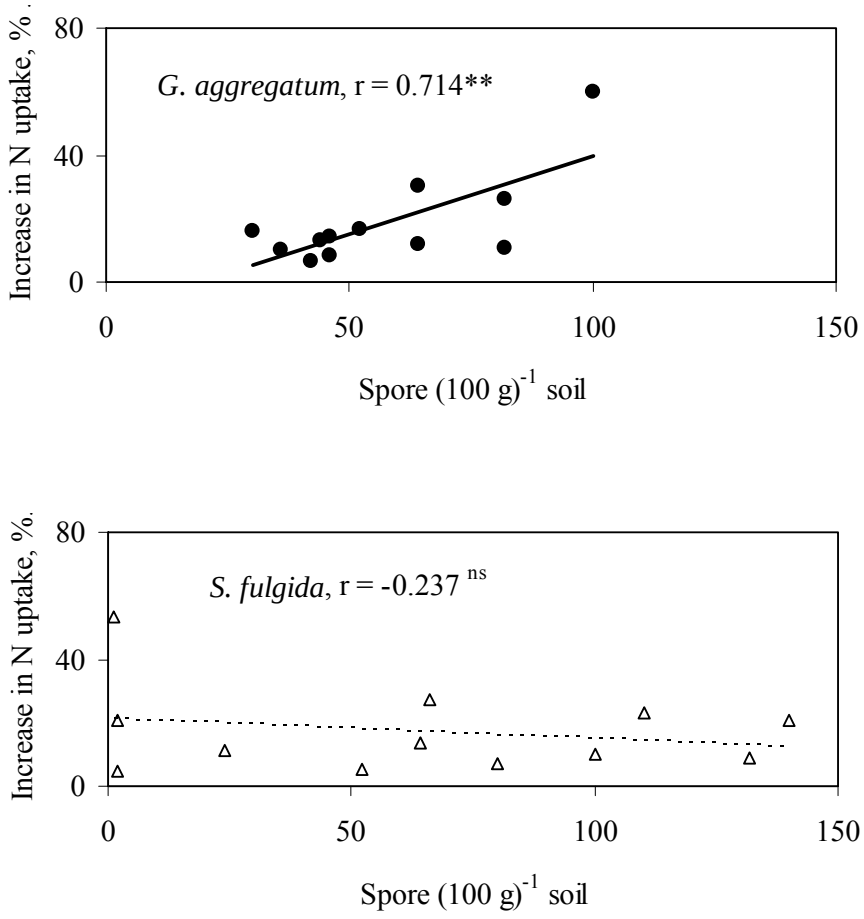


Figure 30 Relationship between spore intensity and responses in N uptake of the two AM fungal species. ^{ns} not significant at $P \leq 0.05$. Refer to Figure 28 for captions.

The responses to AM fungi in shoot dry weight were highly negative correlated to P utilization efficiency of non-inoculated plants (Figure 31). The higher P efficiency in shoot of maize plant of the hybrid requiring high rate of fertilizers (Table 4) might be a cause of the lower response to AM fungi. The present result supported those of Yao *et al*. (2001), Zhu *et al*. (2001) and Baon *et al*. (1993) who found that the host plants with high P utilization efficiency gave lower mycorrhizal response than those with lower P efficiency.

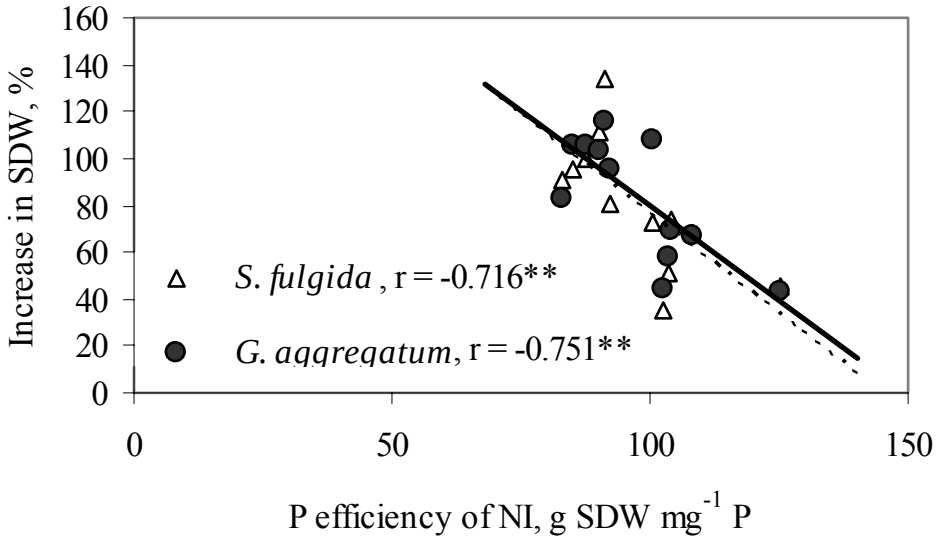


Figure 31 Relationship between P efficiency of non-AM fungus inoculated plants (NI) and their responses in SDW to the two AM fungal species. Refer to Figure 28 for captions.

Responses to *G. aggregatum* in shoot dry weight observed in the present experiment showed a negative correlation to K utilization efficiency of non-inoculated plants. But K utilization efficiency of the non-inoculated plants was not correlated with the response to *S. fulgida* (Figure 32). Accordingly, the K utilization efficiency of the non-inoculated plants (Table 4) might be used for predicting the responses to *G. aggregatum* in shoot dry weight but might not be for that of *S. fulgida*. However, the two cultivars different in fertilizer requirement were not different in K utilization efficiency.

Therefore, lower shoot dry weight and lower nutrient such as N and P efficiency of maize cultivar enhanced greater responses to AM fungi of maize. Higher response to AM fungi in nutrient uptake might be a cause of the requirement of low rate of fertilizer to attain maximum yield and drought tolerance of SW2301. Sylvia *et al*. (1993) found that the proportional response of maize grain and biomass

yield to inoculation with AM fungi increased with increasing drought stress. Improved nutrient status of AM plants may enable the host to absorb water more efficiently under drought conditions. The findings of Nelson and Safir (1982), Osonubi (1994) and Subramanian *et al*. (1997) that AM fungi enhance drought tolerance and P uptake of the host plants suggested that a maize cultivar requires lower soil fertility or lower rate of P fertilizer, such as SW2301, gave greater response to AM fungi than cultivars requiring high rate of P fertilizer. SW2301 has been a well-known drought tolerant cultivar and has been used as a source of drought tolerance in breeding program (Sriwatanapongse *et al*., 1993).

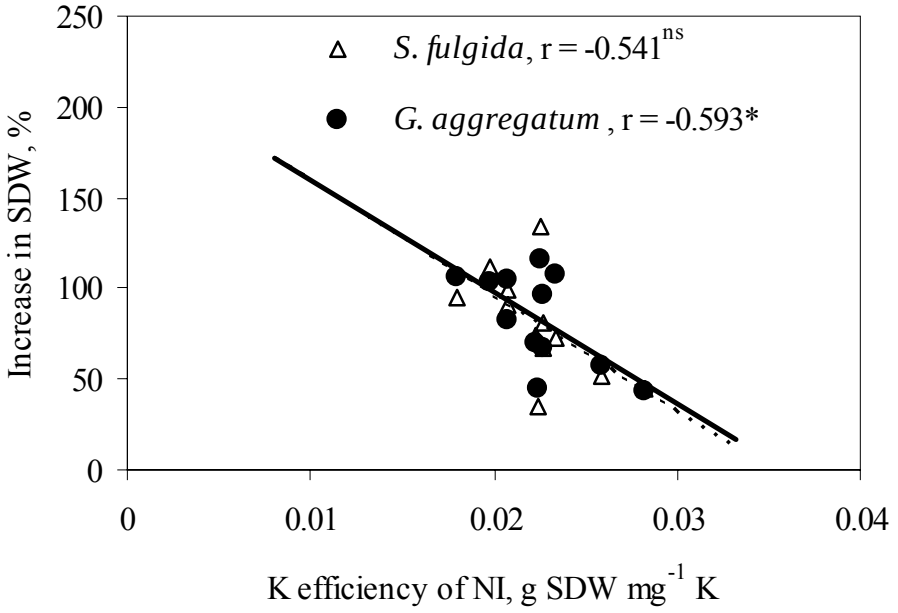


Figure 32 Relationship between K efficiency of non-AM fungus inoculated plants (NI) and their responses in SDW to the two AM fungal species. * Significant at $P \leq 0.05$. ^{ns} not significant at $P \leq 0.05$. Refer to Figure 28 for captions.

In addition, the ability to acquire nutrients in the absence of AM fungi affected the degree of response to AM fungi, however, breeding for receiving the benefit from mycorrhiza to increase fertilizer efficiency and selection of AM fungal species to show persist enhancing effects should be studied if the cost of fertilization reduce.

Experiment 4: A study on time courses of the effects on maize of AM fungi applied to maize in the field

Grain yield

In individual cropping seasons, significant enhancing effect on grain yield was obtained only from the dual inoculation treatment (S+G) of the first cropping (Figure 33). However, trends of enhancing effect on grain were obtained from all of the other individual cropping. In addition, significant enhancing effect was obtained from the dual inoculation treatment when statistical analysis was made by pooling all data in which the replicates of all cropping were treated as replicates of one experiment (10C in Figure 33). These suggested that the enhancing effect existed in all cropping but the effects were mostly too small to overrun the experimental errors.

Shoot dry weight

In all individual cropping, the dual inoculation treatment gave significant increases in shoot dry weight in the fifth (99D) and the tenth (01L) cropping and trends of increase in shoot dry weight in all of the other cropping (Figure 34). All of the other AM fungal inoculation treatments gave no statistically significant effect on shoot dry weight. Among all of the inoculation treatments the dual inoculation treatment gave the highest trend of enhancing effect when statistical analysis was made by pooling all data (10C in Figure 34). These suggested that the enhancing effects existed in all cropping but the effects were mostly too small to overrun the experimental errors.

N, P and K uptake

N uptake by maize shoot in some cropping are shown in Figure 35. Among all of the AM fungus inoculated treatments, only the dual inoculation treatment either showed significant enhancing effect or consistently showed positive trends of enhancing effect on N uptake in individual cropping. In addition, this treatment

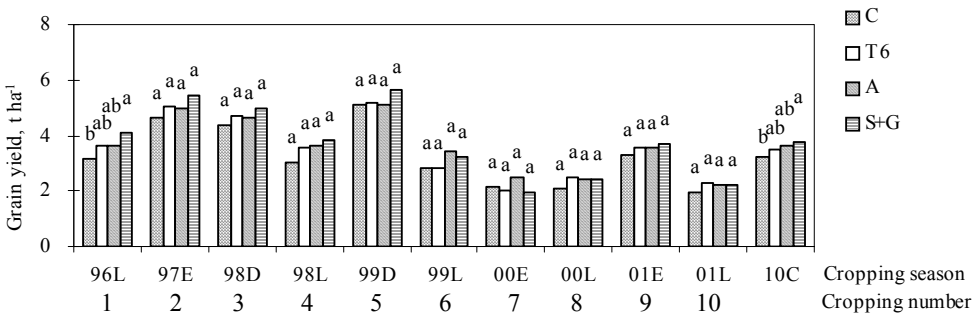


Figure 33 Grain yields of maize grown in the ten successive cropping seasons as affected by AM fungal inoculation applied in the first cropping (96L cropping season). Means within the same cropping season with a common letter were not different by $DMRT_{.05}$. %CV : 14.3%, 9.8%, 8.5%, 14.8%, 7.7%, 15.6%, 17.3%, 20.2%, 17.8%, 14.9% and 7.1% of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E, 01L and 10C, respectively. Cropping season : 96-01 = years 1996-2001; 10C = means for all cropping seasons; D = dry seasons (December – March); E = early rainy seasons (April – July); L = late rainy seasons (August – November). Treatment : C = non-inoculated with AM fungi; T6 = inoculated with T6-AM fungal species from Germany; A = inoculated with *Acaulospora spinosa*; S+G = inoculated with *Scutellospora fulgida* and *Glomus aggregatum*. (Experiment 4).

showed statistically significant enhancing effect when statistical analysis was made by pooling all of the data in which the replicates of all cropping was treated as replicates of one experiment. These suggested that the enhancing effects existed in all cropping but the effects were mostly too small to overrun the experimental errors.

Neither statistically significant enhancing effect nor consistent trends of effect on P and K uptake by maize shoots was observed (Figures 36 and 37). These suggested that the AM fungal inoculation in the first cropping did not have any effect on P and K uptake by maize in the following cropping.

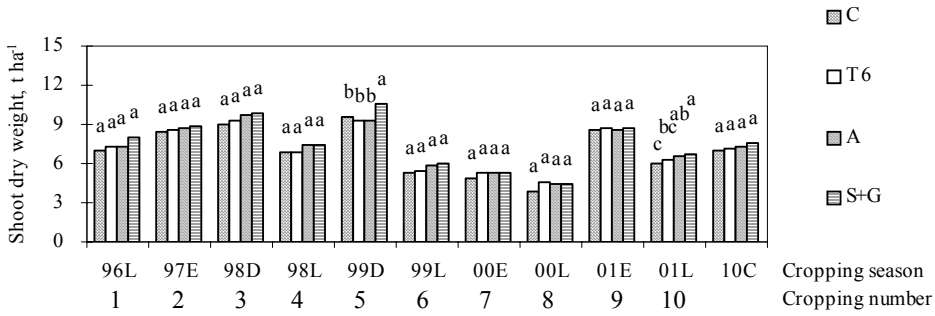


Figure 34 Shoot dry weight of maize grown in the ten successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV : 8.7%, 6.2%, 7.4%, 9.7%, 6.3%, 13.3%, 11.9%, 14.8%, 11.1%, 4.5% and 5.7% of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E, 01L and 10C, respectively. Refer to Figure 33 for further captions (Experiment 4).

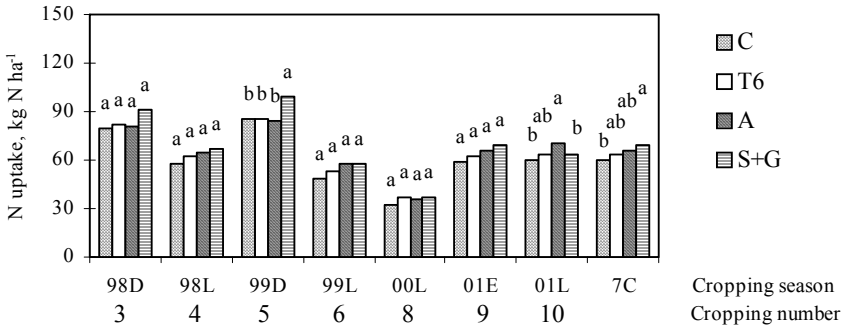


Figure 35 N uptake of maize grown in seven of the the ten successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV : 8.7%, 16.1%, 8.3%, 14.5%, 15.1%, 10.7%, 6.3% and 7.4% of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E, 01L and 10C, respectively. 7C = means for the seven cropping. Refer to Figure 33 for further captions (Experiment 4).

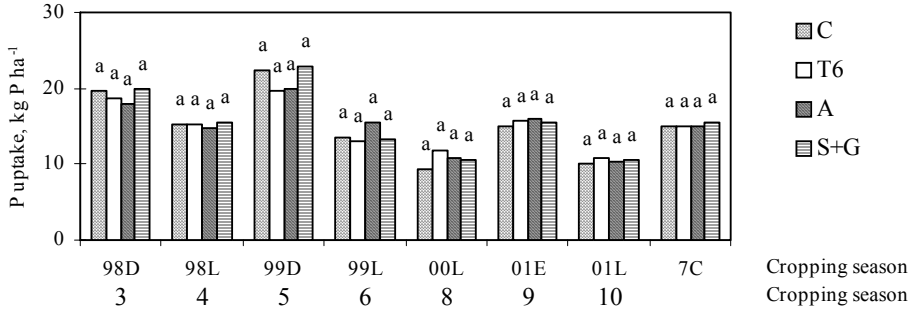


Figure 36 P uptake of maize grown in seven of the the ten successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV : 9.3%, 18.1%, 10.4%, 11.6%, 17.6%, 10.8%, 7.9% and 6.6% of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E, 01L and 10C, respectively. Refer to Figure 34 for further captions (Experiment 4).

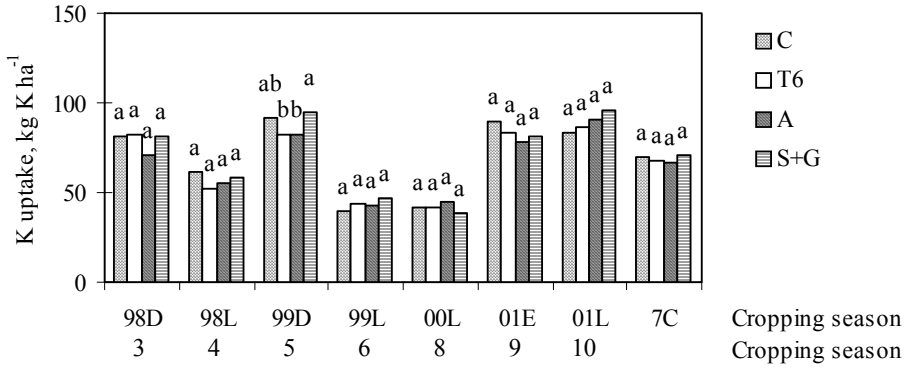


Figure 37 K uptake of maize grown in seven of the the ten successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV : 16.3%, 13.1%, 8.3%, 13.6%, 15.9%, 13.3%, 14.0% and 9.8% of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E, 01L and 10C, respectively. Refer to Figure 34 for further captions (Experiment 4).

Time courses of effects of the inoculated AM fungi

Effects of dual inoculation done in the first cropping on grain yield decreased as repeating cropping proceeded (Figure 38); the enhancing effect decreased from 23.7%, over control, in the first cropping to 7.6% in the tenth cropping. Enhancing effects of this treatment on shoot dry matter of maize also decreased as repeating cropping proceeded but with very low rate (Figure 39); the effect decreased from 10.2% in the first cropping to 9.5% in the tenth cropping. The enhancing effect on N uptake by maize decreased from 17.8% in the third cropping to 12.1% in the tenth cropping (Figure 40).

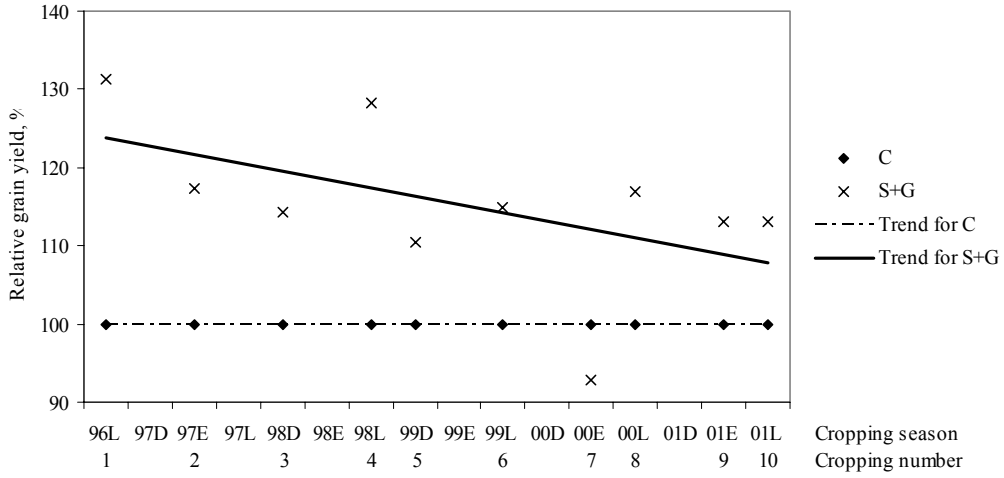


Figure 38 Time course of relative grain yields of maize grown observed in the ten successive cropping as affected by AM fungal inoculation applied in the first cropping. Refer to Figure 33 for further captions (Experiment 4).

Time course of each experimental treatment was a results of effects of many factors which were in turn affected by the experimental treatments differently. It was perceivable that N supply in soil of the dual inoculation treatment was more and more exhausted than N in the soil of Control as number of cropping increased because N uptake in the dual inoculation treatment was higher than in Control. These would result in lower growth and yields of maize in the dual inoculation treatment than those in Control. Accordingly, the actual low rate of the decrease in the enhancing effect

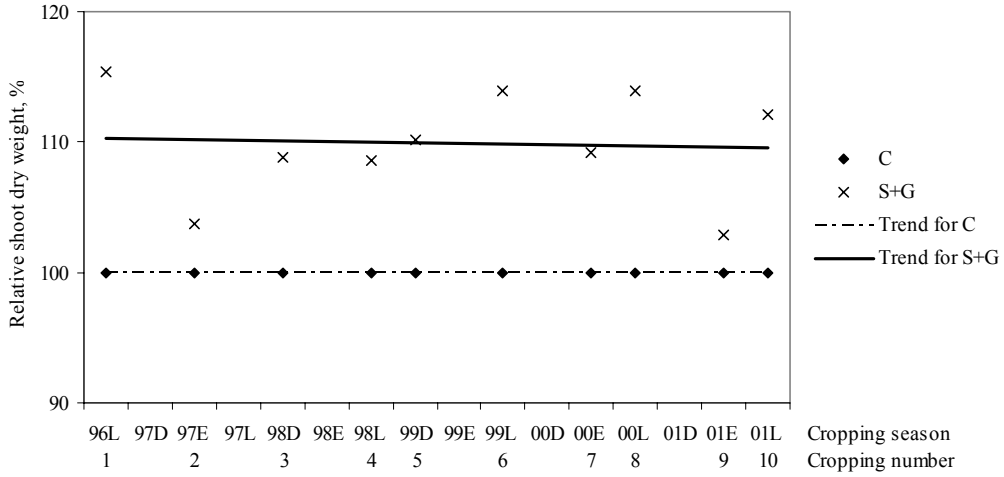


Figure 39 Time course of relative shoot dry weight of maize grown observed in the ten successive cropping as affected by AM fungal inoculation applied in the first cropping. Refer to Figure 33 for further captions (Experiment 4).

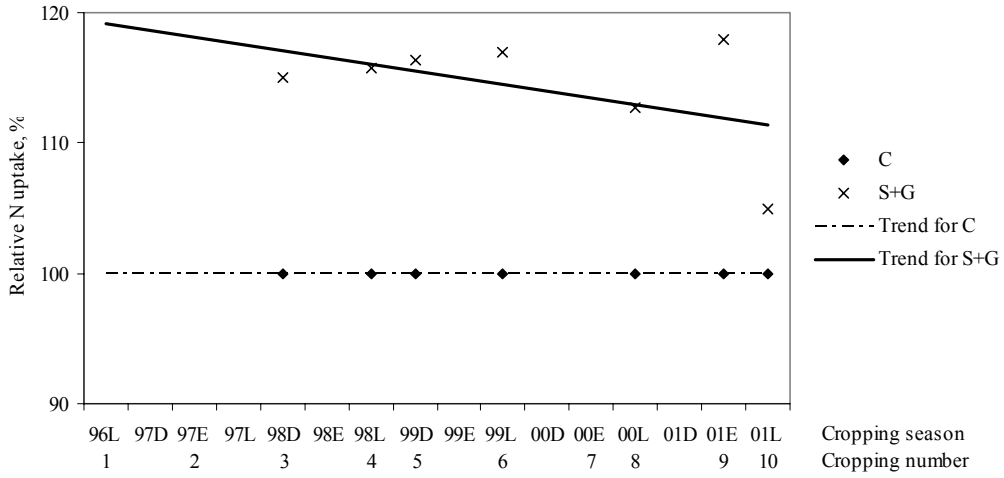


Figure 40 Time course of relative N uptake of maize grown observed in seven successive cropping as affected by AM fungal inoculation applied in the first cropping. Refer to Figure 33 for further captions (Experiment 4).

observed in maize shoot dry matter in the case of the dual inoculation treatment suggested that the enhancing effect of the dual inoculation treatment was either constant or increased as the number of cropping increased.

Abundance of the inoculated AM fungi

Percentage abundance of spore (i.e., number of spores of the specified species per 100 total number of spores found) of *G. aggregatum* and *A. spinosa* in soil around maize roots observed after harvests of the fifth, sixth, eighth, ninth and tenth cropping) are shown in Figures 41 and 42. No spore of *S. fulgida* was found in any of the cropping studied. Percentage abundance of spores for T6 could not be observed because the inoculated AM fungi were of mixed species.

Percentage abundance of spores of *A. spinosa* was statistically higher in the treatment inoculated with this AM fungus than that of Control in the fifth cropping. However, no spore of this AM fungus was detected in any of the treatment in the sixth, eight, ninth and tenth cropping (Figure 42). These suggested that the AM fungus did not exist after the fifth cropping.

Percentage abundance of spores of *G. aggregatum* were statistically higher in the dual inoculation treatment than in the Control in the fifth and the tenth cropping (Figure 41). Comparison of the percentage abundance observed in the fifth and the tenth cropping suggested that the percentage abundance of this inoculated AM fungus did not decrease as cropping was repeated. This supported the suggestion mentioned in the proceeding section that the enhancing effect of the dual inoculation treatment was constant as the number of cropping increased. However, the disappearance of the inoculated *A. spinosa* after the fifth cropping suggested that the durability of the enhancing effect varied with AM fungal species and the constant enhancing effect of the dual inoculation treatment was of *G. aggregatum*.

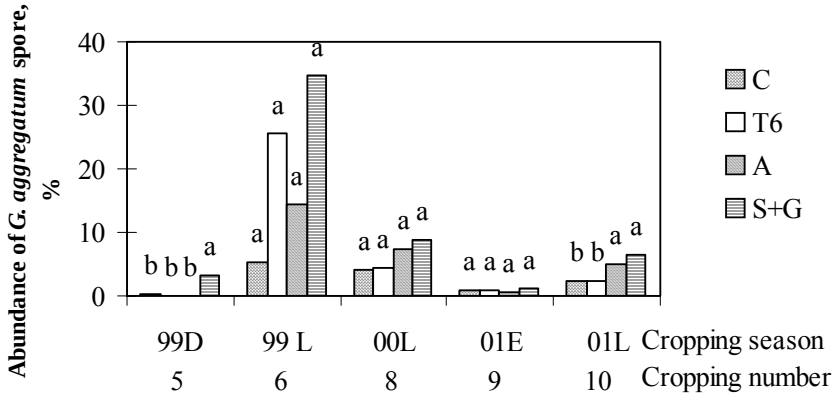


Figure 41 Abundance of *G. aggregatum* in the soils after harvest of the specified cropping seasons as affected by inoculation with different AM fungi in the first cropping season (Experiment 4).

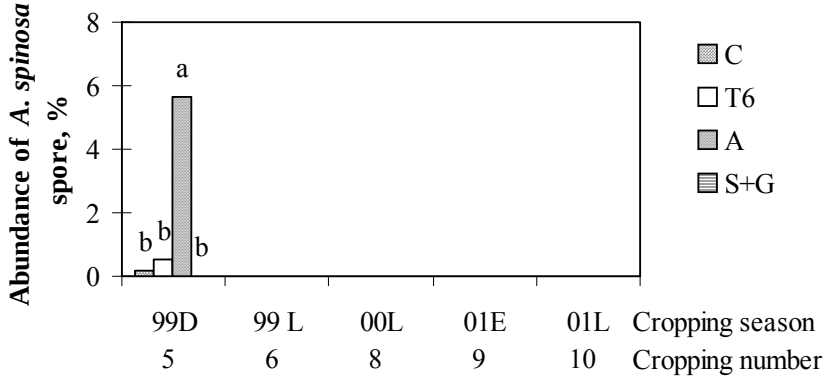


Figure 42 Abundance of *A. spinosa* in the soils after harvest of the specified cropping seasons as affected by inoculation with different AM fungi in the first cropping season (Experiment 4).

Experiment 5: Verification of AM fungi showing effects on maize grown on Rhodic Kandiustox in the field and pot experiments

Field experiment

Grain yield In individual cropping seasons, significant enhancing effect on grain yield was obtained from AM fungal inoculation treatments only in the fifth (01E) cropping (Figure 43). Nitrogen fertilizer was applied only in the fifth cropping season. This results in the fifth cropping supported results of Pugh *et al*. (1981) who found that *Gigaspora margarita* increased plant growth when N fertilizer was added in a soil deficient in N and P. Among all of the inoculation treatments, the inoculation with *G. aggregatum* and the dual inoculation treatment gave trends of enhancing effect when statistical analysis was made by pooling all data (5C in Figure 43).

Shoot dry weight Trends of enhancing effect on shoot dry weight were obtained from inoculation with *G. aggregatum* in all of the individual cropping. In addition, significant enhancing effect was obtained from the inoculation treatment when statistical analysis was made by pooling all data in which the replicates of all cropping were treated as replicates of one experiment (5C in Figure 44). These suggested that the enhancing effect of inoculation with *G. aggregatum* existed in all cropping but the effects were too small to overrun the experimental errors.

N uptake N uptake by maize shoot in some cropping were shown in Figure 45. Among all of the AM fungus inoculated treatment, only the inoculation with *G. aggregatum* treatment either showed significant enhancing effect or consistently showed positive trends of enhancing effect on N uptake in individual cropping. In addition, this treatment showed statistically significant enhancing effect when statistical analysis was made by pooling all of the data in which the replicates of all cropping was treated as replicates of one experiment (4C in Figure 45). These suggested that the enhancing effects existed in all cropping but the effects were mostly too small to overrun the experimental errors.

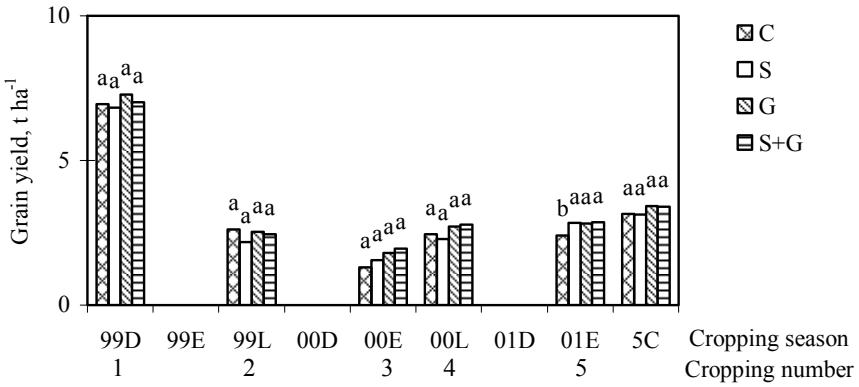


Figure 43 Grain yield of maize grown in the five successive cropping seasons as affected by AM fungal inoculation applied in the first cropping (99D cropping season). Within the same cropping season, means with a common letter were not different by $DMRT_{.05}$. %CV: 4.9%, 13.3%, 26.2%, 21.4%, 8.6% and 5.6% of each cropping in 99D, 99L, 00E, 00L, 01E and 5C, respectively. Cropping season : 99-01 = years 1999-2001; 5C = means for all cropping seasons; D = dry seasons (December-March); E = early rainy seasons (April-July); L = late rainy seasons (August-November). Treatment : C = non-inoculated with AM fungi; S = inoculated with *Scutellospora fulgida*; G = inoculated with *Glomus aggregatum*; S+G = inoculated with both AM fungi (Experiment 5).

P uptake P uptake by maize shoot in some cropping were shown in Figure 46. In individual cropping seasons, significant enhancing effects were obtained from the inoculation with *G. aggregatum* treatment of the first cropping, second and fifth cropping. In addition, a trend of enhancing effect was obtained from the treatment in the forth cropping. Significant enhancing effect was obtained from the inoculation treatment when statistical analysis was made by pooling all data in which the replicates of all cropping were treated as replicates of one experiment (4C in Figure 46). These suggested that the enhancing effect existed in all cropping but in the fourth cropping the effect was too small to overrun the experimental errors.

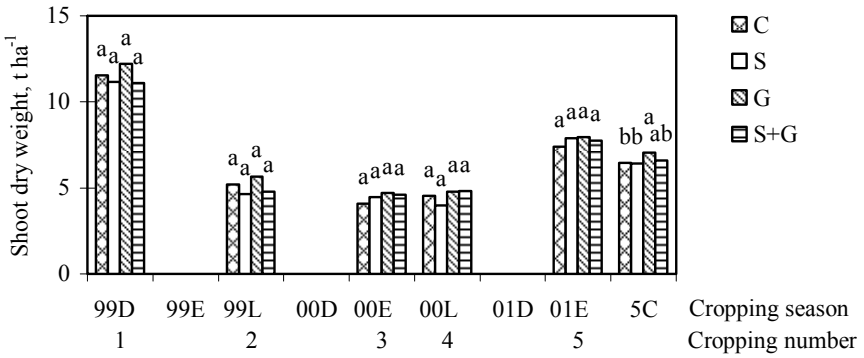


Figure 44 Shoot dry weight of maize grown in the five successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV: 6.5%, 15.0%, 10.5%, 17.3%, 9.0% and 5.2% of each cropping in 99D, 99L, 00E, 00L, 01E and 5C, respectively. Refer to Figure 43 for further captions (Experiment 5).

K uptake Neither statistically significant enhancing effect nor consistent trends of effect on K uptake by maize shoots was observed in individual cropping (Figure 47). Moreover, no significant effect was observed from any of the inoculation treatment when statistical analysis was made by pooling all data (4C in Figure 47). These suggested that the AM fungus inoculated in the first cropping did not have any effect on K uptake by maize in the following cropping.

Time courses of effects of the inoculated AM fungus Effects of inoculation with *G. aggregatum* done in the first cropping on shoot dry weight slightly increase as repeating cropping proceeded (Figure 48); the enhancing effect increased from 8.0%, over control, in the first cropping to 9.3% in the fifth cropping. Enhancing effects of this treatment on N uptake of maize also increased as repeating cropping proceeded but with higher rate (Figure 49); the effect increased from 8.0% in the first cropping to 16.0% in the fifth cropping. The enhancing effect on P uptake by maize decreased from 20.5% in the first cropping to 12.5% in the fifth cropping (Figure 50).

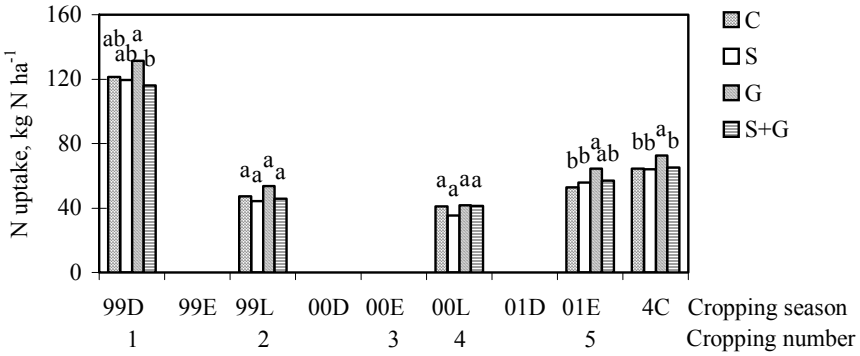


Figure 45 N uptake of maize grown in the four successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV: 6.2%, 13.1%, 18.8%, 8.3% and 7.0% for each cropping in 99D, 99L, 00L, 01E and 4C, respectively. 4C = means for the four cropping seasons. Refer to Figure 43 for further captions (Experiment 5).

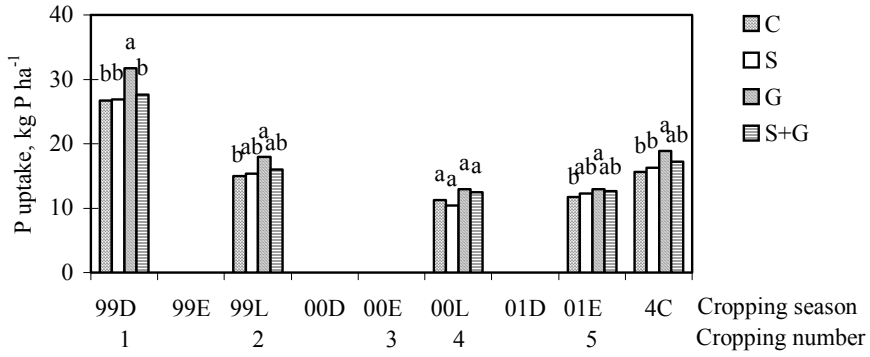


Figure 46 P uptake of maize grown in the four successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV:8.0%, 10.9%, 21.0%, 5.6% and 7.3% for each cropping in 99D, 99L, 00L, 01E and 4C, respectively. Refer to Figure 45 for further captions (Experiment 5).

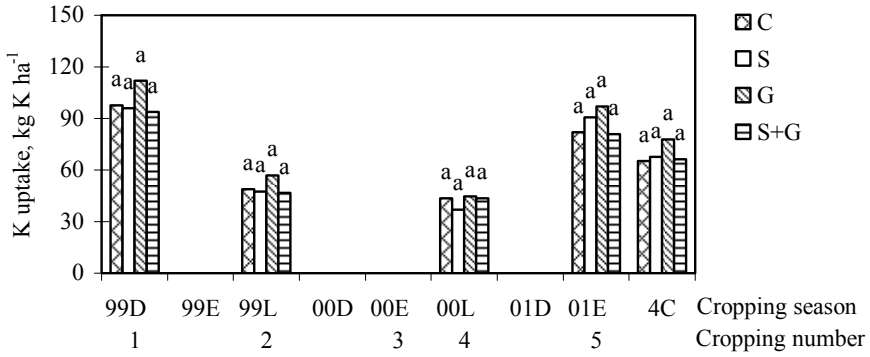


Figure 47 K uptake of maize grown in the four successive cropping seasons as affected by AM fungal inoculation applied in the first cropping. %CV: 14.1%, 15.2%, 20.4%, 16.3% and 12.0% for each cropping in 99D, 99L, 00L, 01E and 4C, respectively. Refer to Figure 45 for further captions (Experiment 5).

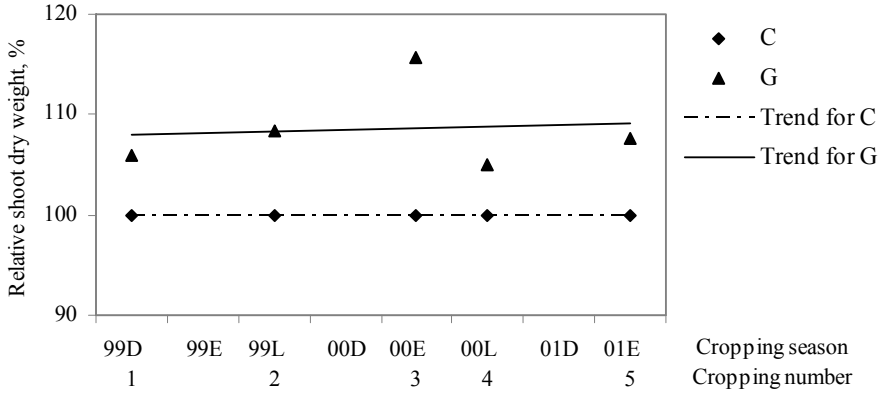


Figure 48 Time courses of relative shoot dry weight of maize grown in five successive cropping as affected by AM fungal inoculation applied in the first cropping. Refer to Figure 43 for further captions (Experiment 5).

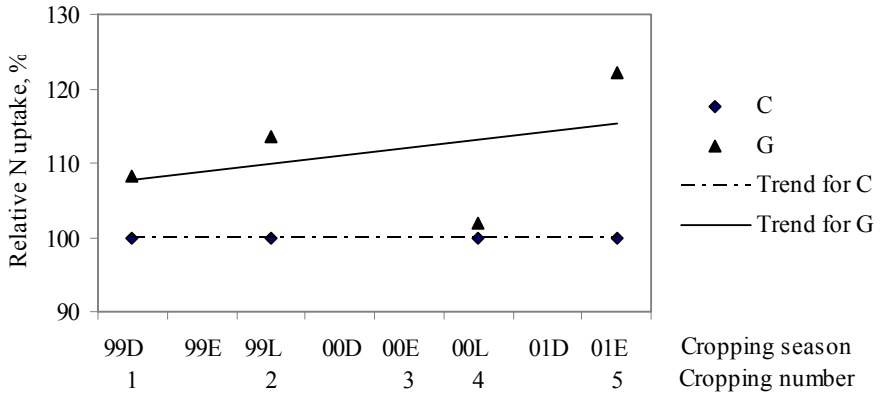


Figure 49 Time course of relative N uptake of maize grown in four successive cropping as affected by AM fungal inoculation applied in the first cropping. Refer to Figure 43 for further captions (Experiment 5).

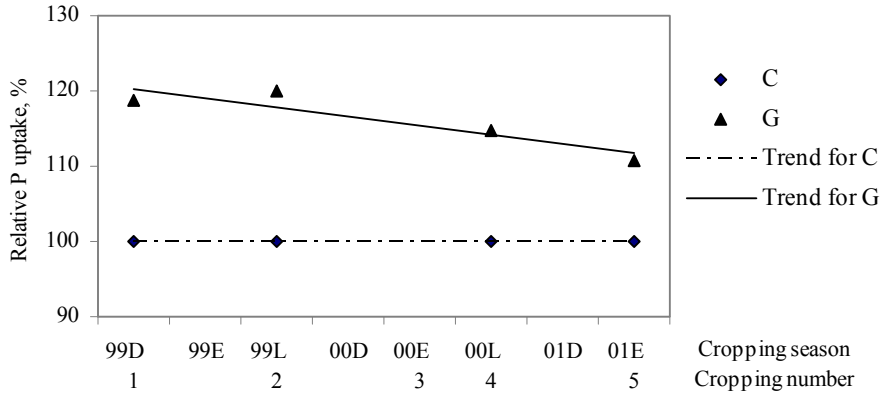


Figure 50 Time course of relative P uptake of maize grown in four successive cropping as affected by AM fungal inoculation applied in the first cropping. Refer to Figure 43 for further captions (Experiment 5).

Time course of each experimental treatment was a result of effects of many factors which were in turn affected by the experimental treatments differently. It was perceivable that N and P supply in soil of the effective inoculation treatment was more and more exhausted than N and P in the soil of Control as number of cropping increased because N and P uptake in the inoculation with *G. aggregatum* treatment was higher than in Control. Accordingly, the actual low rate of increase in the enhancing effect observed in maize shoot dry matter in the case of the inoculation with *G. aggregatum* treatment suggested that the enhancing effect observed in the inoculation treatment confirmed increase in the enhancing effect as the number of cropping increased.

Abundance of the inoculated AM fungi Percentage abundance of spore (i.e., number of spores of the specified species per 100 total number of spores found) of *G. aggregatum* in soil around maize roots observed after harvests of the first, second, fourth and fifth cropping are shown in Figure 51. No spore of *S. fulgida* was found in any of the cropping studied.

Percentage abundance of spores of *G. aggregatum* was statistically higher in the inoculation with *G. aggregatum* than in the Control in the fourth and the fifth cropping (Figure 51). This supported the consistent trends of increase in maize yields in response to *G. aggregatum* inoculation.

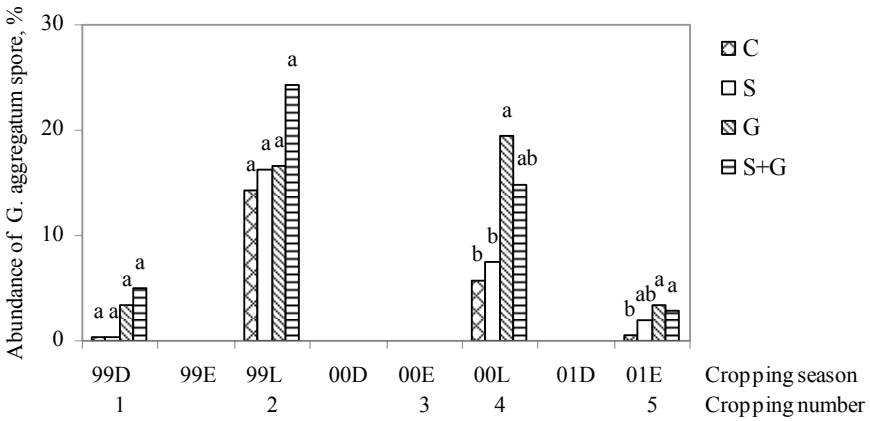


Figure 51 Abundance of *G. aggregatum* spores in soils after harvest of the specified cropping seasons as affected by inoculation with different AM fungi in the first cropping season. %CV: 134.4%, 57.4%, 53.9% and 46.2% of each cropping in 99D, 99L, 00L and 01E ,respectively. Refer to Figure 43 for further captions (Experiment 5).

Pot experiment

Shoot dry weight of maize is shown in Figure 52. The immediate effects of inoculation with *G. aggregatum* alone and inoculation with both AM fungi significantly increased shoot dry weight of maize, whereas, the inoculation with *S. fulgida* alone only showed trend to increase shoot dry weight. N uptake of maize is shown in Figure 53. The immediate effects of inoculation with both AM fungi significantly increased N uptake of maize, whereas, the inoculation with *S. fulgida* alone and inoculation with *G. aggregatum* alone only showed trend to increase N uptake. P uptake of maize is shown in Figure 54. The immediate effects of inoculation with both AM fungi and inoculation with *G. aggregatum* alone significantly increased P uptake of maize, whereas, the inoculation with *S. fulgida* alone did not show the positive effect. K uptake of maize is shown in Figure 55. No positive effect on K uptake was found in AM fungal inoculation treatment.

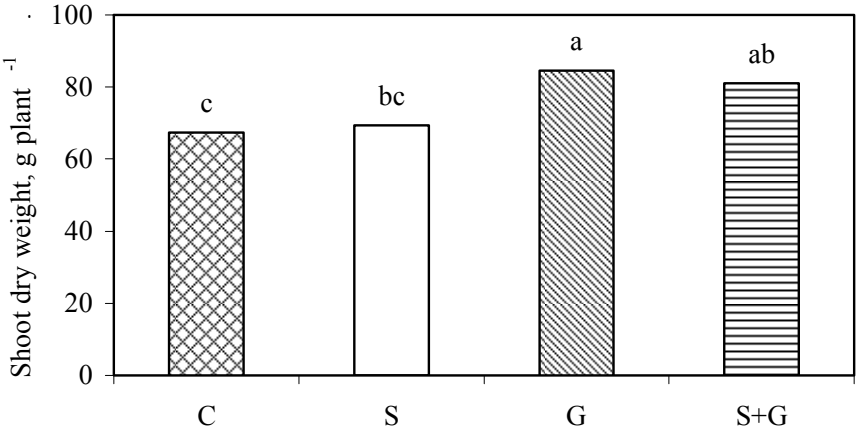


Figure 52 Shoot dry weight of maize as affected by AM fungal inoculation in the pot experiment. %CV: 12.2%. Refer to Figure 43 for further captions (Experiment 5).

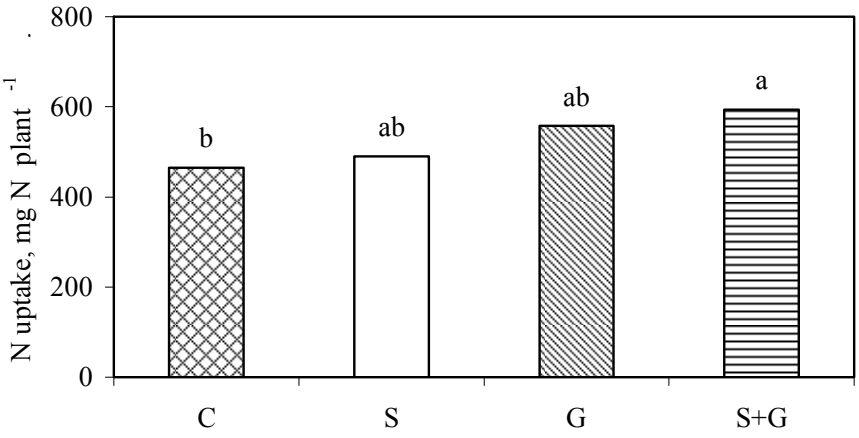


Figure 53 N uptake of maize as affected by AM fungal inoculation in the pot experiment. %CV: 15.0%. Refer to Figure 43 for further captions (Experiment 5).

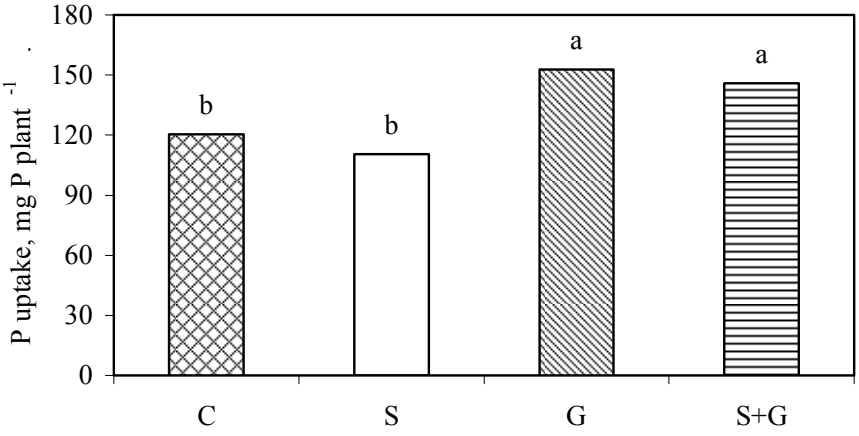


Figure 54 P uptake of maize as affected by AM fungal inoculation in the pot experiment. %CV: 9.9%. Refer to Figure 43 for further captions (Experiment 5).

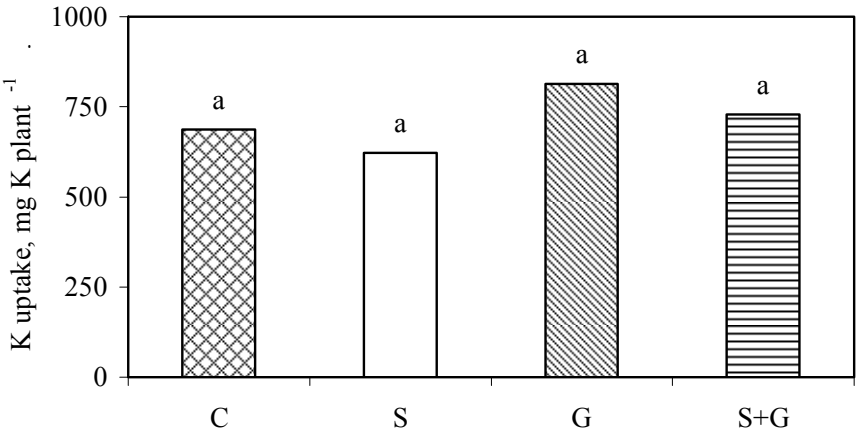


Figure 55 K uptake of maize as affected by AM fungal inoculation in the pot experiment. %CV: 18.1%. Refer to Figure 43 for further captions (Experiment 5).

The enhancement in shoot dry matter and N and P uptake of maize plants by the AM fungal inoculation in this experiment confirmed the positive effects of *G. aggregatum* observed in the field experiment described above.

Percentage abundance of spores of *G. aggregatum* was statistically higher in the inoculation with *G. aggregatum* and dual inoculation treatments than in the Control (Figure 56). However, spore of *S. fulgida* was not found in all of the treatments inoculated with this AM fungus. This might be because the species was destroyed by hyperparasitic fungi (Ross and Ruttencutter, 1977; Boonlue, 1997). The percentage abundance of *G. aggregatum* supported the effects of AM fungus in the treatment inoculated with *G. aggregatum* and that with dual inoculation.

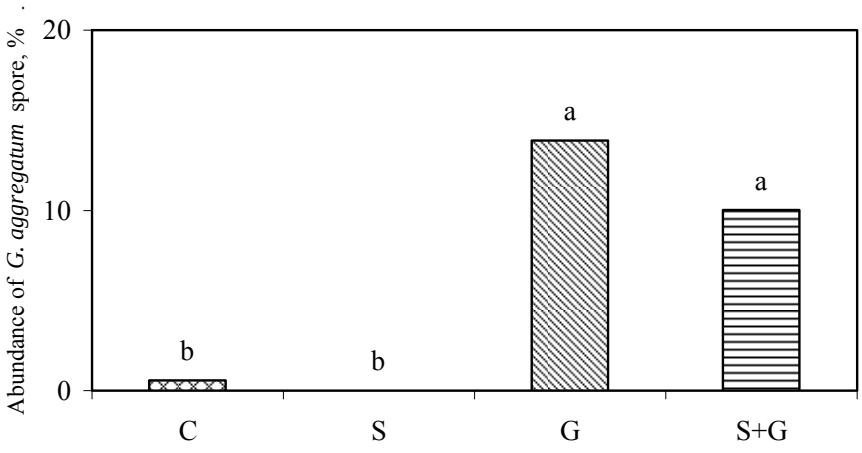


Figure 56 Abundance of *G. aggregatum* spore in soils collected from the pot after harvest of the specified cropping seasons as affected by inoculation with different AM fungi. %CV: 85.0%. Refer to Figure 43 for further captions (Experiment 5).

Experiment 6: A study on effects of AM fungi repetitive inoculation on maize grown in the field

Relative grain yield

Relative grain yields, as compared to those of the corresponding non-AM fungus inoculated treatments, of maize obtained with one, two successive, and three successive AM fungal inoculation are shown in Figure 57. In the first repetitive inoculation cropping (01L), negative effects on grain yield were obtained from all of AM fungal inoculation treatments (Figure 57). The relative grain yields in all of treatments of the first repetitive AM fungal inoculation cropping were significantly lower than that of cropping without repetitive inoculation (01E). The inoculation with *S. fulgida* in the second repetitive AM fungal inoculation cropping (02E) and that of the first repetitive inoculation and the cropping without inoculation gave comparable relative grain yields. The inoculation with *G. aggregatum* in the second repetitive inoculation cropping gave significantly higher relative grain yield than that of the first repetitive inoculation cropping but gave relative grain yield comparable to that of cropping without repetitive inoculation (Figure 57).

Relative shoot dry weight

Relative shoot dry weight, as compared to those of the corresponding non-AM fungus inoculated treatments, of maize obtained with one, two successive, and three successive AM fungal inoculation are shown in Figure 58. In the first repetitive inoculation cropping (01L), negative effects on shoot dry weight were obtained from all of AM fungal inoculation treatments. The relative shoot dry weight in the inoculation with both AM fungi and the inoculation with *G. aggregatum* treatments of the first repetitive AM fungal inoculation cropping were significantly lower than that of cropping without inoculation (01E). The inoculation with *S. fulgida* and inoculation with *G. aggregatum* of the second repetitive AM fungal inoculation cropping (02E) gave significantly higher relative shoot dry weight than that of the first repetitive inoculation and the cropping without inoculation. The inoculation with

both AM fungi in the second repetitive inoculation cropping gave significantly higher relative shoot dry weight than that of the first repetitive inoculation cropping but gave comparable relative shoot dry weight with that of cropping without repetitive inoculation.

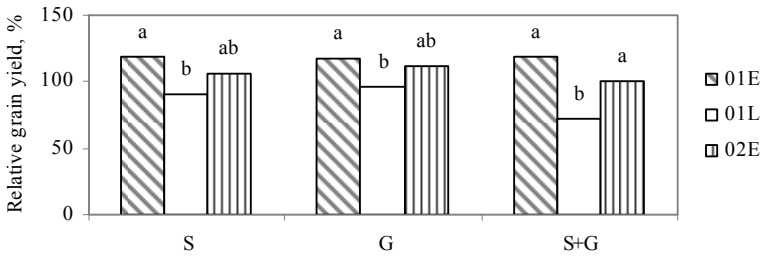


Figure 57 Relative grain yields of maize as affected by repetitive AM fungal inoculation applied in the two following cropping seasons (01L and 02E) compared to that of the previous cropping season (01E). Within the same cropping season, means with a common letter were not different by $DMRT_{.05}$. Cropping season : 01-02 = years 2001-2002; E = early rainy season; L = late rainy season. Treatment : C = non-inoculated with AM fungi; S = inoculated with *Scutellospora fulgida*; G = inoculated with *Glomus aggregatum*; S+G = inoculated with both AM fungi (Experiment 6).

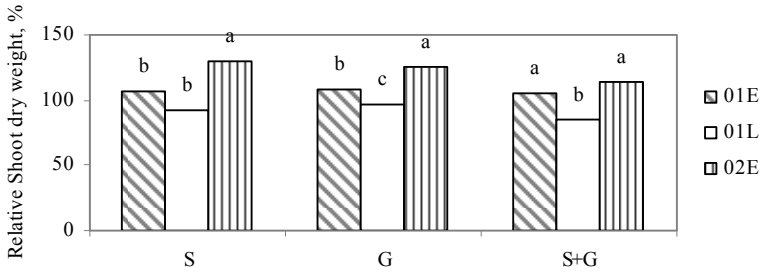


Figure 58 Relative shoot dry weight of maize as affected by repetitive AM fungal inoculation applied in the two following cropping seasons compared to that of the previous cropping season. Refer to Figure 57 for further captions (Experiment 6).

Relative N uptake

Relative N uptake, as compared to those of the corresponding non-AM fungus inoculated treatments, of maize obtained with one, two successive, and three successive AM fungal inoculation are shown in Figure 59. In the first repetitive inoculation cropping (01L), negative significant effects on N uptake were obtained from all of AM fungal inoculation treatments. The relative N uptake in all of treatments with the first repetitive AM fungal inoculation cropping were significantly lower than that of cropping without repetitive inoculation (01E). The inoculation with *S. fulgida* in the second repetitive inoculation cropping gave significantly higher relative N uptake than that of the first repetitive inoculation cropping and that of cropping without repetitive inoculation. The inoculation with *G. aggregatum* of the second repetitive AM fungal inoculation cropping (02E) and that of the first repetitive inoculation and the cropping without repetitive inoculation gave comparable relative N uptake. The inoculation with both AM fungi in the second repetitive inoculation cropping gave significantly higher relative N uptake than that of the first repetitive inoculation cropping but gave relative N uptake that was comparable to that of cropping without repetitive inoculation.

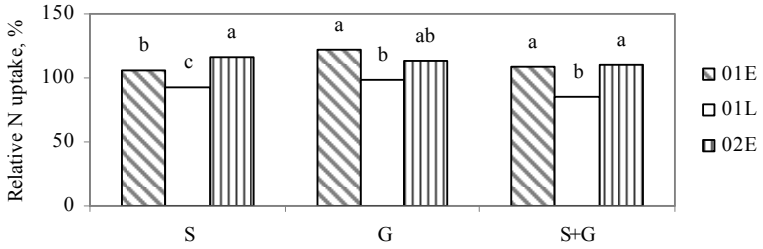


Figure 59 Relative N uptake of maize as affected by repetitive AM fungal inoculation applied in the two following cropping seasons compared to that of the previous cropping season. Refer to Figure 57 for further captions (Experiment 6).

Relative P uptake

Relative P uptake, as compared to those of the corresponding non-AM fungus inoculated treatments, of maize obtained with one, two successive, and three successive AM fungal inoculation are shown in Figure 60. The first and the second repetitive inoculation cropping with *S. fulgida* or with *G. aggregatum* and the cropping without repetitive inoculation gave comparable relative P uptake (Figure 60). The second repetitive inoculation cropping with both AM fungi gave significantly higher relative P uptake than that of the first repetitive inoculation cropping but gave comparable relative P uptake with that of cropping without repetitive inoculation (Figure 60).

Relative K uptake

Relative K uptake, as compared to those of the corresponding non-AM fungus inoculated treatments, of maize obtained with one, two successive, and three successive AM fungal inoculation are shown in Figure 61. The inoculation with *S. fulgida* and inoculation with both AM fungi of the first repetitive AM fungal inoculation cropping (01L) and the cropping without repetitive inoculation gave comparable relative K uptake whereas the inoculation with *G. aggregatum* gave significantly lower relative shoot dry weight than that of the cropping without

repetitive inoculation (Figure 61). The relative K uptake in all of treatments of the second repetitive AM fungal inoculation cropping were significantly higher than that of cropping without repetitive inoculation and that of the first repetitive AM fungal inoculation cropping (Figure 61).

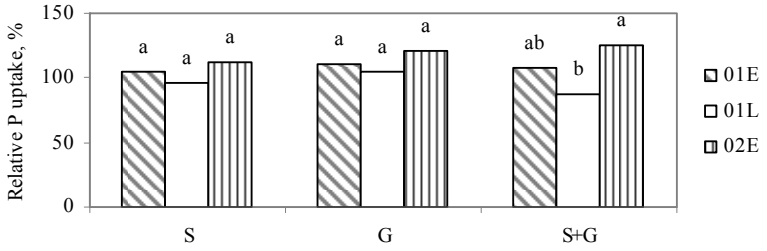


Figure 60 Relative P uptake of maize as affected by repetitive AM fungal inoculation applied in the two following cropping seasons compared to that of the previous cropping season. Refer to Figure 57 for further captions (Experiment 6).

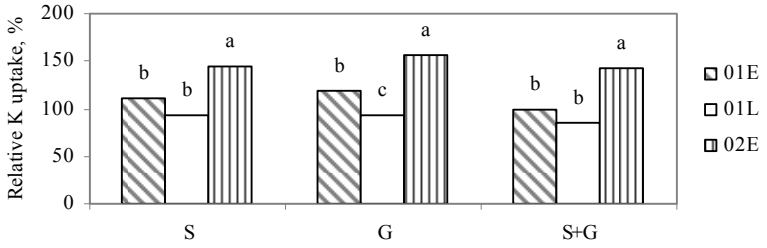


Figure 61 Relative K uptake of maize as affected by repetitive AM fungal inoculation applied in the two following cropping seasons compared to that of the previous cropping season. Refer to Figure 57 for further captions (Experiment 6).

Abundance of the inoculated AM fungi

Abundance of spore of *G. aggregatum* in soil around maize roots observed after harvests are shown in Figure 62. The first repetitive AM fungal inoculation

cropping and the cropping without inoculation gave comparable abundance of spore of *G. aggregatum*. The second repetitive AM fungal inoculation cropping gave higher abundance of spore of *G. aggregatum* than the two previous cropping within inoculation with one AM fungus.

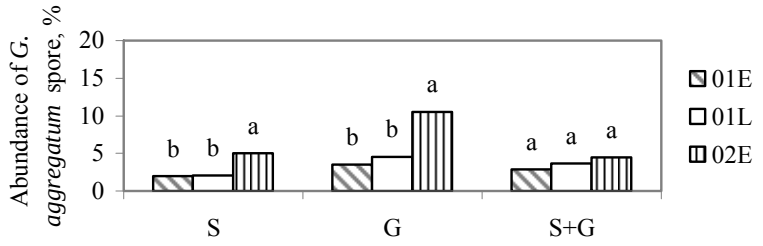


Figure 62 Abundance of *G. aggregatum* spore in soil after harvest as affected by repetitive AM fungal inoculation applied in the two following cropping seasons compared to that of the previous cropping season. Refer to Figure 57 for further captions (Experiment 6).

No spore of *S. fulgida* was found in the cropping without inoculation and the first repetitive AM fungal inoculation cropping. Abundance of spore number of *S. fulgida* in soil around maize roots observed after harvests are shown in Figure 63. The second repetitive AM fungal inoculation cropping gave higher abundance of spore number of *S. fulgida* than the both previous cropping.

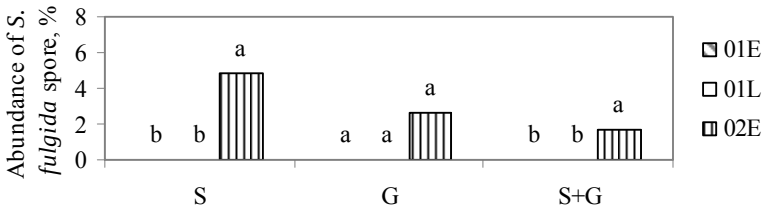


Figure 63 Abundance of *S. fulgida* spore in soil after harvest as affected by repetitive AM fungal inoculation applied in the two following cropping seasons compared to that of the previous cropping season. Refer to Figure 57 for further captions (Experiment 6).

The results of this experiment suggested that negative effects on maize were observed from the first repetitive inoculation whereas positive effects were observed from the second repetitive inoculation. In the first repetitive inoculation no fertilizer was applied whereas N fertilizer was applied to all experimental treatments. These suggested that repetitive inoculation might give positive effects when maize was not under severe N stress but might give negative effects on maize grown under severe N stress.

The presence of the negative effects might be explained as follows. If N was a limiting factor of plant during vegetative growth period, the protein synthesis is restricted. The growth rate of leaves was decreased before photosynthesis was decreased. Increased carbohydrate in leaves derived from photosynthesis was then allocated to roots. As mycorrhizal fungi needed fixed carbon from host plant for hyphal growth and respiration (Smith and Read, 1997), the roots of inoculated plants were accordingly infected with higher number of AM fungi. The accumulated carbohydrate in root used by AM fungi may be greater in the inoculated plant than in Control. Govindarajulu *et al.* (2005) and Tanaka and Yano (2005) found that inorganic N taken up by AM fungi outside the roots is incorporated into amino acids, translocated from extraradical to the intraradical hyphae as arginine, ammonium-N was transferred to the plants after arginine breakdown. The introduced AM fungi might be unable compensate fixed carbon of host with enhancement of nutrient uptake because it might accumulate N and other nutrients for its own growth (George, 2000). Then, the growth of the inoculated plant was decreased. If sufficient amount of N for plant and fungi requirement was supplied, the growth of the host plant and AM fungi were not restricted with protein synthesis. Then, the introduced AM fungi could help uptake more water and nutrient from soil to compensate carbon derived from host photosynthesis. The inoculation with both AM fungi might increase more carbon demand from host and growth depression than inoculation with one species since higher numbers of AM fungal spores were introduced.

CONCLUSION AND RECOMMENDATION

Conclusion

Experiment 1: Identification of AM fungi by PCR technique

PCR-RFLP technique could be used to identify *G. aggregatum* in colonized roots in pure pot culure. In addition, this technique confirmed the morphological identification of *S. fulgida*, even when single spore was used. *Hin*fI and *Taq*I were selected as proper restriction enzymes for PCR-RFLP patterns and could verify morphological identification of *G. aggregatum* and *S. fulgida*.

Experiment 2: Effects of AM fungal species, nitrogen and phosphorus fertilizers on maize growth, root colonization and spore production

G. aggregatum was more effective than *A. spinosa* in enhancing growth and development of maize. The growth promotion effect decreased with increased rates of P fertilizer but was not affected by N fertilizer. Different AM fungal species required different N:P ratios to attain their maximum root colonization and spore production. For each AM fungal species, different N:P ratios were required to attain its maximum root colonization and to attain its maximum spore production. To produce maximum root colonization, *A. spinosa* required lower N:P ratio than *G. aggregatum* but to produce maximum spore production it required higher N:P ratio than *G. aggregatum* did. *G. aggregatum* showed high positive correlation between root colonization and spore production whereas *A. spinosa* did not show such relationship. No significant relationship was observed between degree of root colonization and maize dry matter yield and between maize dry matter yield and spore intensity in the soil.

Experiment 3: Comparative responses to AM fungi of maize cultivars different in downy mildew resistance and fertilizer requirement

The DMR and non-DMR maize cultivars gave comparable response to AM fungi. The cultivar requiring high rate of fertilizers was lower in its response to AM fungi in P and K uptake than the cultivar requiring low rate of fertilizers did. The lower response in P uptake was explained by higher P utilization efficiency of the cultivar whereas the lower response in K uptake was not related to K utilization efficiency of the cultivar. Maize cultivars lower in nutrient (N, P and K) efficiencies or in shoot dry weight gave greater responses to AM fungi in shoot dry weight.

Experiment 4: A study on time courses of the effects on maize of AM fungi applied to maize in the field

The actual relative increases in growth and yields of maize grown on nonsterile Pak Chong soil series due to the inoculated *G. aggregatum* decreased with increase in repeating cropping. It was however elaborated that the enhancing effects of *G. aggregatum* on maize were eventually either constant or increased during ten successive cropping in the period of sixteen cropping seasons.

Experiment 5: Verification of AM fungi showing effects on maize grown on Rhodic Kandiustox in the field and pot experiments

The effects on maize growth and the time course of the enhancing effect of AM fungal inoculation observed in the present field and pot experiments confirmed the positive effects of *G. aggregatum* and the time course of the effects observed in the Experiment 4.

Experiment 6: A study on effects of AM fungal repetitive inoculation on maize grown in the field

Repeating inoculation with AM fungi might have positive effects when maize was not under nitrogen stress but might have negative effect on maize grown under severe nitrogen stress.

Recommendation

From the results of the present experiments, the following recommendations could be made.

1. To extend the use of PCR-RFLP patterns to identify various AM fungal species, more restriction enzymes should be selected in order to find the differences among AM fungal species.

2. To produce an AM fungal inoculum containing high number of spores, required ratio of N and P supplies must be determined for each AM fungal species.

3. Degree of root colonization and spore production of AM fungi should not be used to assess the efficacy of AM fungi in promoting maize growth because they showed no relationship with maize dry matter yield.

4. Maize cultivars requiring low rate of N and P fertilizers to attain their maximum yields should be used instead of cultivars requiring high rates of N and P fertilizers if high response of maize to AM fungi are aimed at.

5. *G. aggregatum* should be considered in looking for AM fungi that give significant enhancing effects on growth of maize grown on Pak Chong soil series in the presence of indigenous AM fungi.

6. The long-lasting enhancing effect of AM fungi should be taken into account in considering cost and return of AM fungal application in maize production because the effect lasted for more than sixteen cropping seasons (in 5 years and 4 months).

7. To avoid negative effects of AM fungi on maize plants, adequate rate of N fertilizer must be applied if the soil is severely deficient in N.

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APPENDIX

Appendix Table 1 Tasseling age (days) of maize as affected by AM fungal treatments, N rates and P rates (Experiment 2).

AM fungi	N rate	P rate	Rep. 1	Rep. 2	Rep. 3	Average
CK	N0	P0	60.0	56.0	56.0	57.3
		P1	50.0	50.0	53.0	51.0
		P2	48.0	48.0	52.0	49.3
		P3	46.0	46.0	47.0	46.3
	N1	P0	60.0	60.0	60.0	60.0
		P1	46.0	51.0	48.0	48.3
		P2	47.0	48.0	45.0	46.7
		P3	44.0	44.0	46.0	44.7
	N2	P0	52.0	53.0	56.0	53.7
		P1	46.0	60.0	50.0	52.0
		P2	42.0	60.0	54.0	52.0
		P3	44.0	45.0	46.0	45.0
	N3	P0	60.0	69.0	60.0	63.0
		P1	46.0	54.0	51.0	50.3
		P2	49.0	50.0	51.0	50.0
		P3	47.0	46.0	45.0	46.0
A	N0	P0	46.0	48.0	47.0	47.0
		P1	44.0	46.0	45.0	45.0
		P2	47.0	58.0	47.0	50.7
		P3	48.0	43.0	43.0	44.7
	N1	P0	51.0	51.0	48.0	50.0
		P1	46.0	48.0	46.0	46.7
		P2	46.0	49.0	44.0	46.3
		P3	45.0	42.0	46.0	44.3
	N2	P0	53.0	51.0	51.0	51.7
		P1	46.0	45.0	48.0	46.3
		P2	48.0	47.0	47.0	47.3
		P3	45.0	46.0	50.0	47.0
	N3	P0	47.0	53.0	51.0	50.3
		P1	47.0	50.0	44.0	47.0
		P2	47.0	47.0	6.0	33.3
		P3	43.0	44.0	44.0	43.7
G	N0	P0	47.0	47.0	47.0	47.0
		P1	48.0	47.0	45.0	46.7
		P2	46.0	40.0	48.0	44.7
		P3	49.0	48.0	45.0	47.3
	N1	P0	56.0	45.0	45.0	48.7
		P1	44.0	43.0	44.0	43.7
		P2	49.0	46.0	45.0	46.7
		P3	45.0	45.0	43.0	44.3
	N2	P0	45.0	46.0	42.0	44.3
		P1	44.0	46.0	48.0	46.0
		P2	46.0	46.0	43.0	45.0
		P3	42.0	44.0	45.0	43.7
	N3	P0	47.0	46.0	45.0	46.0
		P1	45.0	46.0	46.0	45.7
		P2	46.0	46.0	45.0	45.7
		P3	45.0	45.0	47.0	45.7

Appendix Table 2 Shoot dry matter (g pot^{-1}) of maize as affected by AM fungal treatments, N rates and P rates (Experiment 2).

AM fungi	N rate	P rate	Rep. 1	Rep. 2	Rep. 3	Average
CK	N0	P0	33.6	18.2	14.6	22.1
		P1	63.7	47.1	38.0	49.6
		P2	75.8	59.2	58.5	64.5
		P3	92.5	65.5	97.8	85.3
	N1	P0	23.3	2.3	12.9	12.8
		P1	38.7	49.2	31.8	39.9
		P2	85.2	77.8	77.7	80.2
		P3	93.7	123.9	109.9	109.2
	N2	P0	13.8	12.1	10.9	12.3
		P1	44.5	19.2	36.1	33.3
		P2	79.9	36.0	61.8	59.2
		P3	95.0	86.2	79.3	86.8
	N3	P0	10.3	8.1	8.4	8.9
		P1	35.9	43.8	63.3	47.7
		P2	47.1	50.8	54.1	50.7
		P3	105.6	66.5	112.1	94.7
A	N0	P0	55.6	79.1	63.2	66.0
		P1	92.4	79.9	63.8	78.7
		P2	75.2	93.8	84.4	84.5
		P3	104.4	97.4	108.8	103.5
	N1	P0	87.9	55.8	63.2	69.0
		P1	92.4	103.0	83.4	92.9
		P2	118.8	95.7	88.0	100.8
		P3	131.5	103.7	99.1	111.4
	N2	P0	66.4	66.9	72.9	68.7
		P1	98.5	83.8	87.6	90.0
		P2	109.6	95.1	96.6	100.4
		P3	106.3	104.7	87.4	99.5
	N3	P0	62.5	56.9	76.5	65.3
		P1	75.7	83.6	63.2	74.2
		P2	114.5	115.1	93.2	107.6
		P3	116.1	116.5	108.8	113.8
G	N0	P0	108.5	113.6	102.4	108.2
		P1	123.8	89.7	118.5	110.7
		P2	109.5	100.2	99.7	103.1
		P3	129.8	97.6	121.2	116.2
	N1	P0	88.1	108.3	104.6	100.3
		P1	128.2	91.9	81.6	100.6
		P2	107.9	93.6	105.3	102.3
		P3	125.5	101.6	124.9	117.3
	N2	P0	97.6	78.6	84.3	86.8
		P1	105.1	111.2	118.1	111.5
		P2	107.4	104.3	111.7	107.8
		P3	124.8	149.7	137.2	137.2
	N3	P0	105.1	65.8	69.3	80.1
		P1	110.0	107.1	99.9	105.7
		P2	131.0	92.8	131.7	118.5
		P3	134.1	138.8	105.7	126.2

Appendix Table 3 Root colonization (%) of *A. spinosa* as affected by different rates of N and P fertilizers (Experiment 2).

N rate	P rate	Rep. 1	Rep. 2	Rep. 3	Average
N0	P0	70.0	62.8	87.3	73.4
	P1	84.3	78.3	75.3	79.3
	P2	52.1	45.8	49.0	49.0
	P3	57.1	43.1	67.9	56.0
N1	P0	50.1	60.3	50.6	53.7
	P1	42.4	34.4	65.3	47.3
	P2	49.4	64.5	50.1	54.7
	P3	57.8	80.5	66.6	68.3
N2	P0	83.0	53.8	39.8	58.8
	P1	67.5	40.1	61.8	56.5
	P2	66.3	54.3	64.9	61.8
	P3	70.8	51.6	17.0	46.4
N3	P0	81.1	57.3	39.1	59.2
	P1	56.6	57.4	67.3	60.4
	P2	34.1	71.3	61.3	55.6
	P3	75.8	49.6	23.4	49.6

Appendix Table 4 Root colonization (%) of *G. aggregatum* as affected by different rates of N and P fertilizers (Experiment 2).

N rate	P rate	Rep. 1	Rep. 2	Rep. 3	Average
N0	P0	41.2	45.0	51.8	46.0
	P1	56.2	28.3	62.4	49.0
	P2	78.7	38.9	54.5	57.4
	P3	18.6	19.5	21.3	19.8
N1	P0	60.5	58.2	76.3	65.0
	P1	32.3	43.7	25.4	33.8
	P2	21.1	7.3	15.2	14.5
	P3	27.4	25.7	22.1	25.1
N2	P0	45.9	85.7	84.0	71.9
	P1	61.3	62.8	44.0	56.0
	P2	25.5	27.4	43.7	32.2
	P3	4.9	31.1	9.5	15.2
N3	P0	30.1	25.7	32.6	29.5
	P1	33.2	28.2	52.1	37.8
	P2	36.4	43.7	61.3	47.1
	P3	43.3	8.2	25.2	25.6

Appendix Table 5 Spore intensity (sp g^{-1} soil) of *A. spinosa* as affected by different rates of N and P fertilizers (Experiment 2).

N rate	P rate	Rep. 1	Rep. 2	Rep. 3	Average
N0	P0	2.75	4.00	5.75	4.17
	P1	2.25	1.25	1.50	1.67
	P2	1.50	2.50	4.00	2.67
	P3	5.00	1.00	5.00	3.67
N1	P0	7.25	3.25	6.50	5.67
	P1	9.75	2.50	2.00	4.75
	P2	3.50	3.25	12.00	6.25
	P3	3.75	2.75	3.50	3.33
N2	P0	9.50	1.50	7.00	6.00
	P1	3.00	2.25	2.75	2.67
	P2	6.75	5.25	3.50	5.17
	P3	1.25	1.25	2.00	1.50
N3	P0	3.00	10.25	11.25	8.17
	P1	2.25	6.25	1.75	3.42
	P2	2.00	3.25	2.00	2.42
	P3	0.75	3.50	0.50	1.58

Appendix Table 6 Spore intensity (sp g^{-1} soil) of *G. aggregatum* as affected by different rates of N and P fertilizers (Experiment 2).

N rate	P rate	Rep. 1	Rep. 2	Rep. 3	Average
N0	P0	169.3	180.0	225.5	191.6
	P1	158.5	160.0	238.8	185.8
	P2	88.5	175.3	246.5	170.1
	P3	86.5	91.3	134.5	104.1
N1	P0	274.3	240.8	315.3	276.8
	P1	152.5	219.0	146.8	172.8
	P2	156.0	59.3	124.5	113.3
	P3	125.0	109.5	73.0	102.5
N2	P0	167.8	291.0	176.8	211.9
	P1	83.0	274.0	144.3	167.1
	P2	116.8	113.8	162.3	131.0
	P3	45.0	137.8	75.5	86.1
N3	P0	75.8	83.0	113.3	90.7
	P1	112.5	125.0	176.8	138.1
	P2	132.3	208.0	270.8	203.7
	P3	225.0	75.3	136.8	145.7

Appendix Table 7 Height (cm) of maize at 30 DAP as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	18.5	15.4	16.4	16.8
	S	26.3	23.6	25.2	25.0
	G	29.8	25.0	25.7	26.8
SW1C11	NI	21.0	17.7	19.0	19.2
	S	27.5	26.4	28.6	27.5
	G	27.8	27.8	28.8	28.1
SW2301	NI	14.2	13.3	13.2	13.6
	S	22.8	19.6	22.4	21.6
	G	23.9	24.2	22.1	23.4
SW3851	NI	19.3	18.5	18.4	18.7
	S	31.6	26.7	26.4	28.2
	G	33.5	29.9	28.3	30.6

Appendix Table 8 Height (cm) of maize at 45 DAP as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	48.0	34.5	35.3	39.3
	S	62.5	61.0	68.4	64.0
	G	70.7	62.8	54.9	62.8
SW1C11	NI	43.4	35.2	52.2	43.6
	S	58.2	57.3	57.3	57.6
	G	64.2	55.2	66.4	61.9
SW2301	NI	26.3	29.3	29.6	28.4
	S	49.6	43.2	49.0	47.3
	G	49.8	49.5	50.6	50.0
SW3851	NI	50.0	45.8	45.8	47.2
	S	70.1	63.0	66.7	66.6
	G	73.6	65.6	65.0	68.1

Appendix Table 9 Height (cm) of maize at 62 DAP as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cutivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	139.3	116.3	105.7	120.4
	S	145.3	134.0	144.0	141.1
	G	147.0	143.0	119.0	136.3
SW1C11	NI	151.0	102.0	147.3	133.4
	S	160.3	147.3	156.0	154.5
	G	164.7	143.0	157.0	154.9
SW2301	NI	95.0	100.7	95.7	97.1
	S	111.0	109.0	114.3	111.4
	G	107.7	107.0	112.7	109.1
SW3851	NI	141.0	138.3	115.3	131.5
	S	139.7	140.7	139.7	140.0
	G	154.0	136.7	138.7	143.1

Appendix Table 10 Tasseling age (days) of maize as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	61.3	64.3	66.3	64.0
	S	55.0	56.7	56.3	56.0
	G	55.7	55.0	54.7	55.1
SW1C11	NI	61.7	67.3	62.0	63.7
	S	58.0	60.0	58.7	58.9
	G	57.0	58.3	57.0	57.4
SW2301	NI	62.7	61.0	62.3	62.0
	S	55.3	56.3	55.0	55.5
	G	54.7	54.7	54.7	54.7
SW3851	NI	61.3	61.3	62.3	61.6
	S	57.0	58.0	58.3	57.8
	G	57.0	57.3	58.0	57.4

Appendix Table 11 Silking age (days) of maize as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	67.5	72.5	70.0	70.0
	S	61.0	64.5	62.5	62.7
	G	62.5	62.5	61.5	62.2
SW1C11	NI	65.0	73.5	68.0	68.8
	S	60.5	63.0	62.5	62.0
	G	64.5	63.0	59.5	62.3
SW2301	NI	66.5	69.5	65.5	67.2
	S	56.0	59.5	60.0	58.5
	G	58.5	56.0	58.5	57.7
SW3851	NI	75.0	69.0	69.0	71.0
	S	59.0	62.0	63.0	61.3
	G	61.0	63.0	65.0	63.0

Appendix Table 12 Shoot dry weight (g pot^{-1}) of maize as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	88.3	66.5	60.3	71.7
	S	119.6	120.2	117.7	119.2
	G	127.8	130.2	124.2	127.4
SW1C11	NI	78.1	69.9	100.0	82.7
	S	149.1	139.7	145.2	144.7
	G	142.4	143.3	142.8	142.8
SW2301	NI	53.9	65.4	54.9	58.1
	S	126.0	113.0	115.9	118.3
	G	116.1	135.9	111.4	121.1
SW3851	NI	91.3	80.2	74.1	81.9
	S	138.1	134.4	128.7	133.7
	G	144.1	133.6	125.7	134.5

Appendix Table 13 N uptake (mg N pot^{-1}) in shoot of maize as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	465	446	372	428
	S	487	491	475	484
	G	504	498	470	490
SW1C11	NI	477	434	511	474
	S	576	523	547	549
	G	554	506	544	534
SW2301	NI	368	367	401	379
	S	563	452	422	479
	G	479	588	459	508
SW3851	NI	493	444	424	454
	S	536	493	483	504
	G	555	492	466	505

Appendix Table 14 P uptake (mg P pot^{-1}) in shoot of maize as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	86.0	72.0	71.0	76.3
	S	86.0	93.0	77.0	85.3
	G	116.0	79.0	99.0	98.0
SW1C11	NI	94.0	80.0	80.0	84.7
	S	89.0	104.0	86.0	93.0
	G	135.0	85.0	98.0	106.0
SW2301	NI	59.0	65.0	61.0	61.7
	S	117.0	100.0	72.0	96.3
	G	106.0	124.0	84.0	104.7
SW3851	NI	88.0	74.0	71.0	77.7
	S	87.0	78.0	66.0	77.0
	G	129.0	97.0	75.0	100.3

Appendix Table 15 K uptake (mg K pot^{-1}) in shoot of maize as affected by maize cultivar and AM fungal treatments (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	3944	2936	3343	3408
	S	3972	3862	3856	3897
	G	4103	3530	3709	3780
SW1C11	NI	3753	3377	3539	3556
	S	4432	4214	4375	4340
	G	4196	3731	3736	3888
SW2301	NI	2389	2804	2764	2652
	S	3928	3783	3723	3811
	G	3664	3738	3415	3606
SW3851	NI	3527	3544	3328	3466
	S	4062	4003	3947	4004
	G	4142	4094	3471	3903

Appendix Table 16 Spore intensity (spores 100 g^{-1} soil) of the inoculated AM fungal treatments in soil after harvest (Experiment 3).

Cultivar	AM fungi	Rep. 1	Rep. 2	Rep. 3	Average
SW1C0	NI	0.0	0.0	0.0	0.0
	S	2.0	100.0	66.0	56.0
	G	46.0	64.0	82.0	64.0
SW1C11	NI	0.0	0.0	0.0	0.0
	S	2.0	140.0	80.0	74.0
	G	30.0	52.0	42.0	41.3
SW2301	NI	0.0	0.0	0.0	0.0
	S	1.0	110.0	52.0	54.3
	G	64.0	100.0	46.0	70.0
SW3851	NI	0.0	0.0	0.0	0.0
	S	132.0	24.0	64.0	73.3
	G	44.0	82.0	36.0	54.0

Appendix Table 17 Shoot dry weight (kg ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E and 01L. 96-01 = year 1996-2001, D = dry season; E = early rainy season; L = late rainy season; C = non-inoculated with AM fungi; T6 = inoculated with T6-AM fungal species from Germany; A = inoculated with *A. spinosa*; S+G = inoculated with *S. fulgida* and *G. aggregatum* (Experiment 4).

Cropping season	AM fungal inoculation	Rep.				Average
		1	2	3	4	
96L	C	8507	7901	7229	6231	7467
	T6	6030	8361	8130	6555	7269
	A	6939	8639	6557	6931	7267
	S+G	7815	8477	8957	6734	7996
97E	C	8540	9249	7677	8590	8514
	T6	9098	9200	8628	7636	8641
	A	8113	9554	8605	8858	8783
	S+G	8786	9020	9110	8275	8798
98D	C	8710	10314	9240	7961	9056
	T6	8017	10083	10090	9211	9350
	A	9513	10124	10245	8982	9716
	S+G	10356	9894	10869	8317	9859
98L	C	10051	7651	6698	6187	7647
	T6	6287	8089	7921	5376	6918
	A	7339	8469	6831	6877	7379
	S+G	8242	7966	7875	5702	7446
99D	C	9283	10285	9104	9139	9453
	T6	8486	10611	9539	8473	9277
	A	9674	9677	9259	8643	9313
	S+G	11254	10717	10932	9151	10514
99L	C	5768	5652	5104	4987	5378
	T6	4727	5941	5704	5237	5402
	A	6284	6942	5298	5031	5889
	S+G	6032	6324	7257	4208	5955
00E	C	5870	5027	4580	4083	4890
	T6	6643	4883	4134	5552	5303
	A	5437	5508	5541	4904	5348
	S+G	6853	4975	4619	4927	5344
00L	C	3375	4548	3495	4107	3881
	T6	3666	4472	5601	4735	4619
	A	3973	4555	4690	4602	4455
	S+G	4338	3280	5253	4822	4423
01E	C	8483	9695	8041	7873	8523
	T6	7422	10237	8043	9176	8720
	A	8433	9077	8648	8022	8545
	S+G	10043	8040	8455	8480	8755
01L	C	5593	6896	6109	5578	6044
	T6	5318	7327	6346	6139	6283
	A	6315	7338	6747	6162	6641
	S+G	6711	7498	6792	6094	6774

Appendix Table 18 Grain yield (kg ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 96L, 97E, 98D, 98L, 99D, 99L, 00E, 00L, 01E and 01L (Experiment 4).

Cropping season	AM fungal inoculation	Rep.				Average
		1	2	3	4	
96L	C	4266	3970	3363	2555	3539
	T6	2615	4503	4711	2570	3600
	A	3370	4429	3296	3370	3616
	S+G	3844	4422	4926	3252	4111
97E	C	4557	5077	4169	4788	4648
	T6	5221	5820	5005	4121	5042
	A	4368	5593	4633	5401	4999
	S+G	5348	5592	5707	5029	5419
98D	C	4104	5507	4361	3421	4348
	T6	4302	4843	4953	4680	4695
	A	4486	5527	4289	4379	4670
	S+G	4811	5617	5054	4388	4968
98L	C	3994	3617	2986	2686	3321
	T6	2973	4490	4110	2560	3533
	A	3425	4072	3212	3930	3660
	S+G	3829	4327	4433	2850	3860
99D	C	5493	5921	4768	4835	5254
	T6	4798	6375	4890	4784	5212
	A	5212	5178	5114	5054	5140
	S+G	5633	5966	5995	5081	5669
99L	C	3100	3159	2985	2455	2925
	T6	2306	3263	3064	2553	2797
	A	3784	4088	2821	3095	3447
	S+G	2900	3596	4105	2324	3231
00E	C	2456	2408	1765	1830	2115
	T6	2788	2106	1217	1912	2006
	A	2647	2684	2386	2326	2511
	S+G	2070	1578	2022	2189	1965
00L	C	1754	2572	1753	2143	2056
	T6	1923	2505	3262	2269	2490
	A	2117	2553	2204	2670	2386
	S+G	2341	1781	2636	2881	2410
01E	C	3209	4008	2621	3270	3277
	T6	2752	4577	3200	3630	3540
	A	3465	3714	3429	3571	3545
	S+G	4374	2977	3735	3752	3710
01L	C	2156	2146	1992	1608	1976
	T6	3047	2446	1941	1616	2263
	A	2008	2638	2209	2088	2236
	S+G	2226	2698	2274	1770	2242

Appendix Table 19 N uptake (kg N ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 98D, 98L, 99D, 99L, 00L, 01E and 01L (Experiment 4).

Cropping season	AM fungal inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
98D	C	77.67	94.17	79.15	67.01	79.5
	T6	69.94	88.86	88.25	78.36	81.4
	A	79.61	90.79	79.78	74.60	81.2
	S+G	98.96	94.16	97.90	74.77	91.4
98L	C	90.77	66.63	56.99	53.58	67.0
	T6	51.00	82.09	71.58	46.50	62.8
	A	58.91	78.71	57.46	63.24	64.6
	S+G	74.92	69.55	77.74	46.43	67.2
99D	C	88.59	95.46	84.43	76.89	86.3
	T6	74.83	103.74	85.19	77.25	85.3
	A	86.86	84.44	87.75	77.49	84.1
	S+G	108.26	105.62	101.22	83.50	99.7
99L	C	58.06	54.87	49.97	43.92	51.7
	T6	46.05	58.73	55.30	50.36	52.6
	A	63.42	68.84	48.84	50.01	57.8
	S+G	53.23	62.77	72.19	41.24	57.4
00L	C	29.89	39.61	28.39	33.23	32.8
	T6	30.91	37.42	44.03	35.99	37.1
	A	33.61	36.61	36.07	37.12	35.9
	S+G	36.68	28.27	41.77	40.98	36.9
01E	C	61.26	64.97	55.64	57.91	59.9
	T6	53.29	74.93	60.93	62.24	62.8
	A	61.81	66.22	62.83	70.80	65.4
	S+G	72.14	59.89	67.98	75.45	68.9
01L	C	67.14	58.70	62.13	57.18	61.3
	T6	68.48	69.57	58.43	57.69	63.5
	A	67.41	71.28	70.05	70.94	69.9
	S+G	67.39	62.76	64.57	57.49	63.1

Appendix Table 20 P uptake (kg P ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 98D, 98L, 99D, 99L, 00L, 01E and 01L (Experiment 4).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
98D	C	20.55	22.32	17.91	17.77	19.6
	T6	17.37	18.97	19.93	18.51	18.7
	A	19.90	19.99	18.14	14.16	18.0
	S+G	22.04	21.05	20.32	15.94	19.8
98L	C	19.85	15.28	13.69	15.15	16.0
	T6	14.94	19.86	15.42	10.28	15.1
	A	14.37	16.73	15.32	12.15	14.6
	S+G	20.87	13.83	15.38	11.42	15.4
99D	C	26.06	23.12	21.17	19.48	22.5
	T6	20.70	21.56	18.40	18.00	19.7
	A	21.95	19.32	22.05	16.65	20.0
	S+G	29.49	23.05	19.44	19.11	22.8
99L	C	13.99	14.23	15.70	11.46	13.8
	T6	11.81	11.82	14.70	13.96	13.1
	A	15.87	16.91	14.81	13.97	15.4
	S+G	12.31	14.44	16.24	10.55	13.4
00L	C	8.43	10.94	8.38	9.77	9.4
	T6	8.98	11.41	15.41	11.45	11.8
	A	10.31	11.02	10.37	11.74	10.9
	S+G	11.22	8.18	11.54	11.77	10.7
01E	C	15.48	16.66	13.49	15.28	15.2
	T6	13.60	18.49	14.69	16.56	15.8
	A	15.62	16.59	14.83	16.71	15.9
	S+G	17.19	13.41	15.88	15.64	15.5
01L	C	12.10	10.64	10.16	8.78	10.4
	T6	12.28	12.09	9.69	8.75	10.7
	A	9.95	11.28	10.20	10.14	10.4
	S+G	10.64	10.91	11.03	9.29	10.5

Appendix Table 21 K uptake (kg K ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 98D, 98L, 99D, 99L, 00L, 01E and 01L (Experiment 4).

Cropping season	AM fungal inoculation	Rep.				Average
		1	2	3	4	
98D	C	82.3	99.5	68.5	73.8	81.0
	T6	66.0	90.0	88.6	85.1	82.4
	A	84.6	86.0	61.5	52.4	71.1
	S+G	88.5	81.6	90.6	66.2	81.7
98L	C	84.1	65.2	51.4	56.7	64.3
	T6	59.1	54.7	50.1	43.8	51.9
	A	66.3	71.3	39.9	42.8	55.1
	S+G	73.4	54.1	59.3	48.5	58.8
99D	C	77.5	92.3	99.0	91.5	90.1
	T6	67.7	84.7	98.0	79.3	82.4
	A	75.6	92.4	85.8	75.8	82.4
	S+G	97.8	92.4	109.0	81.9	95.3
99L	C	44.5	44.2	41.1	35.4	41.3
	T6	35.3	49.3	48.8	41.9	43.8
	A	44.8	48.5	43.2	33.9	42.6
	S+G	48.3	46.3	60.8	34.2	47.4
00L	C	34.4	50.5	33.8	44.2	40.7
	T6	35.5	38.6	46.1	46.3	41.6
	A	43.0	45.4	46.7	41.3	44.1
	S+G	39.5	31.2	46.9	37.5	38.8
01E	C	97.1	91.8	93.8	78.2	90.2
	T6	80.7	79.9	74.4	98.5	83.4
	A	78.0	74.0	90.6	71.6	78.6
	S+G	99.5	74.7	76.6	75.7	81.6
01L	C	94.1	104.2	81.6	76.5	89.1
	T6	54.2	105.4	105.1	79.2	86.0
	A	80.4	103.0	98.4	80.2	90.5
	S+G	103.7	104.2	93.7	80.5	95.5

Appendix Table 22 Abundance (%) of *G. aggregatum* spore in soil collected from the field of each treatment of each cropping in 99D, 99L, 00L, 01E and 01L (Experiment 4).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
99D	C	-	0.00	0.00	0.00	0.00
	T6	0.00	0.00	0.00	0.00	0.00
	A	0.00	0.00	0.00	0.00	0.00
	S+G	6.45	4.26	2.27	0.00	3.25
99L	C	-	21.52	0.00	0.00	7.17
	T6	0.00	51.35	51.09	0.00	25.61
	A	0.00	36.36	0.00	20.99	14.34
	S+G	52.38	38.46	30.93	17.91	34.92
00L	C	-	9.09	4.55	1.32	4.98
	T6	2.30	2.13	10.20	3.26	4.47
	A	5.68	7.34	7.48	9.47	7.49
	S+G	4.84	9.85	10.79	9.89	8.84
01E	C	-	1.00	1.58	0.00	0.86
	T6	0.64	0.54	0.85	1.18	0.80
	A	0.99	0.31	0.00	1.24	0.64
	S+G	0.70	0.79	1.10	1.62	1.05
01L	C	-	2.03	4.00	1.20	2.41
	T6	2.67	1.55	2.05	2.92	2.30
	A	4.40	4.05	7.52	3.66	4.90
	S+G	7.26	5.07	7.69	5.47	6.37

Appendix Table 23 Abundance (%) of *A. spinosa* spore in soil collected from the field of each treatment of cropping in 99D (Experiment 4).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
99D	C	0.00	0.00	0.00	0.00	0.00
	T6	0.00	2.15	0.00	0.00	0.54
	A	7.37	9.09	2.03	4.00	5.62
	S+G	0.00	0.00	0.00	0.00	0.00

Appendix Table 24 Shoot dry weight (kg ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 99D, 99L, 00E, 00L and 01E. (C = non-inoculated with AM fungi; S = inoculated with *S. fulgida*; G = inoculated with *G. aggregatum*; S+G = inoculated with both AM fungi) (Experiment 5).

Cropping season	AM fungal inoculation	Rep.				Average
		1	2	3	4	
99D	C	11057	12287	12244	11584	11793
	S	12513	11912	10477	9737	11160
	G	11697	13121	12159	11891	12217
	S+G	12064	10868	10967	10441	11085
99L	C	4413	4971	5879	5568	5208
	S	5292	4965	4229	4124	4653
	G	5640	4481	5746	6740	5652
	S+G	5231	5096	4052	4738	4779
00E	C	4294	4613	3977	3413	4074
	S	3938	4513	4952	4498	4475
	G	4494	4748	4861	4740	4711
	S+G	4149	3982	5469	4773	4593
00L	C	4954	4980	5312	2927	4543
	S	3958	3638	4266	4005	3967
	G	4562	4870	4090	5541	4766
	S+G	5162	5229	3961	4885	4809
01E	C	7235	7245	7647	7480	7402
	S	7305	8809	8169	7256	7885
	G	8016	7401	8113	8306	7959
	S+G	8204	6459	8052	8302	7754

Appendix Table 25 Grain yield (kg ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 99D, 99L, 00E, 00L and 01E (Experiment 5).

Cropping season	AM fungal inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
99D	C	7314	7304	6259	6885	6941
	S	7261	7410	6261	6395	6832
	G	7250	7298	7624	6926	7275
	S+G	7362	7192	6877	6652	7021
99L	C	2369	2869	2760	2434	2608
	S	2226	2115	2152	2225	2180
	G	2282	2134	2643	3067	2532
	S+G	2429	2701	1905	2707	2436
00E	C	1845	871	1346	1129	1298
	S	1472	1294	1998	1425	1547
	G	1923	1821	2401	1090	1809
	S+G	1611	1273	2403	2525	1953
00L	C	3394	2603	2570	1185	2438
	S	2522	2008	2295	2342	2292
	G	3014	2418	2222	3216	2718
	S+G	3301	3075	2118	2610	2776
01E	C	2256	2168	2598	2586	2402
	S	2872	2520	3211	2739	2836
	G	2830	2225	3076	3171	2826
	S+G	2832	2347	2828	3442	2862

Appendix Table 26 N uptake (kg N ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 99D, 99L, 00L and 01E (Experiment 5).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
99D	C	114.1	136.6	115.4	118.7	121.2
	S	129.4	134.7	111.6	102.7	119.6
	G	128.7	137.0	133.8	125.2	131.2
	S+G	125.4	120.8	108.1	110.3	116.2
99L	C	41.1	48.3	50.8	48.9	47.3
	S	49.8	43.1	43.0	41.7	44.4
	G	55.5	42.6	51.4	65.4	53.7
	S+G	47.2	48.6	39.1	47.5	45.6
00L	C	49.7	47.1	43.8	22.9	40.9
	S	38.6	31.9	36.3	34.6	35.3
	G	43.4	39.0	36.9	47.4	41.7
	S+G	47.1	45.8	32.1	40.7	41.4
01E	C	51.6	51.0	54.2	54.1	52.7
	S	54.9	56.0	57.4	55.5	55.9
	G	61.3	53.5	69.4	73.5	64.4
	S+G	58.1	49.9	55.0	65.2	57.0

Appendix Table 27 P uptake (kg P ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 99D, 99L, 00L and 01E (Experiment 5).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
99D	C	28.4	31.5	25.1	21.9	26.7
	S	28.8	32.0	25.8	21.0	26.9
	G	29.1	35.8	30.7	31.1	31.7
	S+G	30.9	33.4	23.1	23.3	27.7
99L	C	15.8	14.3	16.6	13.3	15.0
	S	17.7	16.6	15.4	11.9	15.4
	G	18.9	18.8	16.0	18.3	18.0
	S+G	16.3	18.6	13.3	15.9	16.0
00L	C	13.8	12.3	13.2	5.7	11.2
	S	10.5	9.6	11.3	10.4	10.4
	G	14.4	11.8	10.6	14.8	12.9
	S+G	14.8	14.4	10.1	10.7	12.5
01E	C	10.7	11.0	12.1	13.1	11.7
	S	11.7	12.2	12.9	12.5	12.3
	G	12.9	11.2	13.5	14.3	13.0
	S+G	12.6	11.3	12.6	14.3	12.7

Appendix Table 28 K uptake (kg K ha^{-1}) of maize as affected by AM fungal inoculation in the field of each cropping in 99D, 99L, 00L and 01E (Experiment 5).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
99D	C	92.9	126.8	90.2	80.6	97.6
	S	118.1	111.3	76.4	77.7	95.9
	G	116.5	115.4	100.4	115.9	112.0
	S+G	124.2	90.7	79.5	81.1	93.9
99L	C	47.9	47.3	49.7	50.5	48.8
	S	58.2	57.8	37.0	37.4	47.6
	G	62.4	49.9	50.0	65.8	57.0
	S+G	56.0	51.3	38.2	42.1	46.9
00L	C	47.0	48.6	52.0	26.5	43.5
	S	40.4	37.1	33.9	37.1	37.1
	G	43.4	43.7	35.6	55.3	44.5
	S+G	50.3	47.2	33.5	43.9	43.7
01E	C	81.2	103.6	76.3	66.1	81.8
	S	85.8	121.1	74.7	81.3	90.7
	G	98.5	96.4	97.0	96.3	97.0
	S+G	94.1	69.5	80.7	80.0	81.1

Appendix Table 29 Abundance (%) of *G. aggregatum* spore in soil collected from the field of each treatment of each cropping in 99D, 99L, 00L and 01E (Experiment 5).

Cropping season	AM fungal inoculation	Rep. 1	2	3	4	Average
99D	C	0.0	0.0	1.2	0.0	0.3
	S	0.0	0.0	0.0	1.1	0.3
	G	2.4	4.1	4.1	3.0	3.4
	S+G	13.2	3.1	4.0	0.0	5.1
99L	C	0.0	20.3	23.0	13.5	14.2
	S	0.0	17.5	20.0	27.2	16.2
	G	0.0	42.6	0.0	23.6	16.6
	S+G	9.1	35.3	33.3	19.7	24.4
00L	C	8.3	5.1	2.4	7.1	5.7
	S	0.0	3.2	8.5	18.6	7.6
	G	9.1	10.0	26.4	32.6	19.5
	S+G	5.3	20.0	11.6	22.4	14.8
01E	C	0.0	0.0	nd	1.1	0.4
	S	0.0	0.0	3.3	4.7	2.0
	G	1.8	3.1	4.6	4.4	3.5
	S+G	2.8	2.2	2.5	4.0	2.9

Appendix Table 30 Shoot dry weight (g plant^{-1}) of maize as affected by AM fungal inoculation in the pot of cropping in 99D (Experiment 5).

AM fungal	Rep.					Average
inoculation	1	2	3	4	5	
C	63.3	70.0	58.6	72.8	nd	67.4
S	74.8	75.7	43.5	74.2	78.6	69.3
G	78.7	93.5	80.8	74.3	95.3	84.5
S+G	90.5	97.2	71.1	70.7	75.4	81.0

Appendix Table 31 N uptake (mg N plant^{-1}) of maize as affected by AM fungal inoculation in the pot of cropping in 99D (Experiment 5).

AM fungal	Rep.					Average
inoculation	1	2	3	4	5	
C	655.8	368.7	486.1	361.1	nd	465.0
S	557.7	542.9	302.1	544.1	502.3	489.8
G	645.2	584.3	511.6	514.2	530.8	557.2
S+G	779.2	573.6	566.8	478.9	568.7	593.4

Appendix Table 32 P uptake (mg P plant^{-1}) of maize as affected by AM fungal inoculation in the pot of cropping in 99D (Experiment 5).

AM fungal	Rep.					Average
inoculation	1	2	3	4	5	
C	142	111	117	111	nd	120
S	116	116	85	126	109	110
G	171	160	142	153	137	153
S+G	187	153	135	128	126	146

Appendix Table 33 K uptake (mg K plant^{-1}) of maize as affected by AM fungal inoculation in the pot of cropping in 99D (Experiment 5).

AM fungal	Rep.					Average
inoculation	1	2	3	4	5	
C	426	919	608	916	nd	688
S	514	691	514	815	577	622
G	853	831	767	929	686	813
S+G	782	883	799	634	544	728

[1/] nd: not determined

Appendix Table 34 Abundance of *G. aggregatum* spore (%) in soils collected from the pot after harvest of the specified cropping seasons as affected by inoculation with different AM fungi (Experiment 5).

AM fungal	Rep.					Average
inoculation	1	2	3	4	5	
C	0.0	2.7	0.0	0.0	nd	0.6
S	0.0	0.0	0.0	0.0	0.0	0.0
G	13.9	25.4	8.0	17.6	4.6	13.9
S+G	17.1	4.6	12.6	8.3	7.6	10.0

[1/] nd: not determined

Appendix Table 35 Shoot dry weight (kg ha^{-1}) of maize as affected by AM fungi in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	5501	6594	7077	6679	6463
S	5375	5697	6504	6322	5975
G	5658	5843	7157	6199	6214
S+G	4690	5972	5535	5820	5504

Appendix Table 36 Grain yield (kg ha^{-1}) of maize as affect by inoculated AM fungi in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	1983	2285	2320	2373	2240
S	1603	2206	2300	1993	2026
G	1951	1641	2703	2314	2152
S+G	840	1918	1801	1877	1609

Appendix Table 37 N uptake (kg N ha^{-1}) of maize as affected by AM fungi in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	51.8	58.7	63.0	61.4	58.7
S	47.0	54.1	60.2	56.9	54.6
G	52.8	52.8	66.9	57.6	57.5
S+G	39.4	51.3	53.0	55.3	49.7

Appendix Table 38 P uptake (kg P ha^{-1}) of maize as affected by AM fungi in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	8.3	9.4	9.9	9.9	9.4
S	8.0	9.4	9.9	8.9	9.0
G	9.1	8.7	11.4	10.2	9.9
S+G	5.7	9.0	8.6	9.3	8.2

Appendix Table 39 K uptake (kg K ha^{-1}) of maize as affected by AM fungi in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	82.1	81.5	99.2	93.4	89.1
S	87.7	64.5	92.4	88.4	83.3
G	76.4	72.4	92.3	91.6	83.2
S+G	73.0	71.4	84.3	75.7	76.1

Appendix Table 40 Total spores (spores 100 g^{-1} soil) of each treatment in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	185.3	196.0	256.3	184.9	205.6
S	194.3	222.3	237.9	186.7	210.3
G	234.3	243.7	214.0	207.3	224.8
S+G	172.7	212.3	246.3	182.3	203.4

Appendix Table 41 Number of *G. aggregatum* spore (spores 100 g^{-1} soil) of each treatment in the first repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	0.00	0.00	7.30	6.74	3.51
S	1.70	0.00	5.79	9.30	4.20
G	10.70	7.00	13.70	12.00	10.85
S+G	5.30	6.30	4.70	10.00	6.58

Appendix Table 42 Grain yield (kg ha^{-1}) of maize as affect by inoculated AM fungi in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	3529	4281	4361	5187	4340
S	4758	4340	4676	4527	4575
G	3888	4640	4894	6067	4872
S+G	3351	4254	4333	5488	4357

Appendix Table 43 Shoot dry weight (kg ha^{-1}) of maize as affected by AM fungi in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	6818	7825	7767	9305	7929
S	9415	10377	10652	10568	10253
G	9043	9549	10047	10981	9905
S+G	7819	9370	8019	10974	9046

Appendix Table 44 N uptake (kg N ha^{-1}) of maize as affected by AM fungi in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	63.3	60.7	67.8	85.3	69.3
S	73.1	70.6	84.1	95.7	80.9
G	67.4	72.2	74.9	100.5	78.7
S+G	61.2	73.9	72.3	97.0	76.1

Appendix Table 45 P uptake (kg P ha^{-1}) of maize as affected by AM fungi in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	20.6	16.2	19.5	20.8	19.3
S	19.3	18.6	26.9	21.9	21.7
G	22.1	25.6	21.9	24.2	23.4
S+G	21.1	23.1	21.3	31.4	24.2

Appendix Table 46 K uptake (kg K ha^{-1}) of maize as affected by AM fungi in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	47.1	54.3	51.3	47.6	50.0
S	63.3	63.7	92.6	69.8	72.4
G	69.6	90.3	74.4	77.7	78.0
S+G	67.1	73.0	58.7	87.5	71.6

Appendix Table 47 Total spores (spores 100 g^{-1} soil) of each treatment in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	758.0	580.0	456.0	252.0	511.5
S	1046.0	480.0	456.0	224.0	551.5
G	734.0	548.0	470.0	216.0	492.0
S+G	570.0	520.0	376.0	238.0	426.0

Appendix Table 48 Number of *S. fulgida* spore (spores 100 g^{-1} soil) of each treatment in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	6.0	8.0	0.0	2.0	4.0
S	12.0	50.0	12.0	12.0	21.5
G	2.0	36.0	8.0	4.0	12.5
S+G	2.0	8.0	6.0	8.0	6.0

Appendix Table 49 Number of *G. aggregatum* spore (spores 100 g^{-1} soil) of each treatment in the second repetitive inoculation cropping (Experiment 6).

Inoculation	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average
C	0.0	0.0	16.0	10.0	6.5
S	34.0	0.0	32.0	22.0	22.0
G	52.0	24.0	70.0	34.0	45.0
S+G	40.0	14.0	12.0	12.0	19.5