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Endomycorrhizal Association in Sesame

Effects on Growth and Nutrition



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CHAPTER 1

Introduction

Sesame (*Sesamum indicum* L., Fam. Pedaliaceae) is recognized as the most ancient oil seed according to some archeological findings (Nayar, 1984; Bedigian and Harlan 1986). Its cultivation goes back to 2130 BC (Weiss, 1983). Sesame is cultivated in tropical, subtropical and southern temperate regions of the world for its seeds which is a rich source of edible oil. Sesame by virtue of its excellent quality oil is called the queen of the oil seed crops. Sesame seeds have the highest oil content (35-63%) among oilseed crops (Ashri, 1998; Baydar et al., 1999). The oil is very stable due to the presence of a number of antioxidants such as sesamin, sesamolin and sesamol (Suja et al., 2004). Therefore, it has a long shelf-life and can be blended with less stable vegetable oils to improve their stability and longevity (Chung et al., 2004; Suja et al., 2004). Recent studies have shown that the oil lowers cholesterol levels and hypertension in humans (Lemcke-Norojarvi et al., 2001; Sankar et al., 2004) and reduces the incidence of certain cancers (Hibasami et al., 2000; Miyahara et al., 2001). The observed effects have been attributed to the chemical composition of the oil characterized by a low level of saturated fatty acids and presence of antioxidants. The grains of sesame are eaten as fried, mixed with sugar or jaggery in the form sweets meats. Oil cake of sesame is a rich source of protein, carbohydrate and mineral nutrients such as calcium and phosphorus and is eaten avidly by humans as well as cattle.

India ranks both in the area and production of sesame in the world with an annual area of 2.07 million hectares and total production of 0.76 million tonnes (Anonymous, 2009a). In the state of Kerala, the crop is cultivated under an area of 533 hectares with an annual production of 171 tonnes of seeds (Anonymous, 2009b). The crop is mainly confined to the districts of Alappuzha, Palakkad and Kollam where it is cultivated in both uplands and lowlands. In the Onattukara region of Kerala, it is mainly cultivated as a summer crop in low land rice fallows.

Even though, sesame is the predominant oil seed crop of India, the per hectare productivity and the economic returns given by it are very low. It is due to the fact that the crop is very sensitive to biotic and abiotic stresses and it grows in marginal light-textured inceptisols having poor soil fertility associated with imbalance and or without fertilizer application. Under such unpredicted situations, the practice of application of both organic and inorganic fertilizer could help in bringing in profitable returns. However, due to escalating cost of production of chemical fertilizers and low subsidies for farmer, the agricultural planners are compelled to reorient their thinking towards cost effective and cheap renewable resources to supplement the chemical fertilizers.

Biofertilizers such as arbuscular mycorrhizal (AM) fungi have a potential to improve the sustainability of commercial sesame production by improving growth and yield. Such improvements of crops at given levels of inputs increases production efficiency and consequently reduce input levels to achieve the same yield. Reducing input levels can help in addressing some of the core issues of sustainability, such as eutrophication of waterways caused by excessive application of soluble P fertilizers and the depletion of non-renewable resources like rock phosphate. Improving the quality of crops by increasing their nutrient status also improves the sustainability of commercial agriculture in a less tangible, but equally important way, since the main goal of agriculture is to provide for the well being of human populations.

1. 1. Arbuscular mycorrhiza

Arbuscular mycorrhiza are mutualistic symbioses between plant roots and fungi belonging to the phylum Glomeromycota. The plant provides carbon to the fungal partner while the fungus improves the water and nutrient uptake from the soil.

Arbuscular mycorrhizal (AM) fungi have long been considered obligate symbionts with plants, since growing of the AM fungus without a host plant has not been possible. AM spores can germinate and produce hyphae, but they, die in the availability of a host. However, this view has been challenged by an experimental study showing that AM fungi can grow and form spores *in vitro*, if supplemented with

a carbon source and stimulated by particular bacterial strains (Hildebrandt et al., 2006).

Spores are asexual, multinucleate structures that are produced directly by the mycelium, either inside or outside the root. In some species sporocarps are produced, where several spores get surrounded by a periderm like structure.

The hyphae are aseptate and can grow both outside (extraradical) and inside the roots (intraradical). The intraradical mycelium produces highly ramified structures called arbuscules inside the cortical cells of roots (*Arum*-mycorrhizal type). In some other cases, hyphal coils are formed instead (*Paris*-mycorrhizal types)

Many species of Glomeromycota also produce large intraradical, globose storage cells intracellularly called vesicles. Because of this glomeromycotan fungi are sometimes also referred to as vesicular-arbuscular mycorrhizal (VAM) fungi.

1. 2. Classification and phylogenetics

Dangeard (1900) was the first to name a VAM fungus. He isolated and described a typical VA mycorrhizal from poplar and named it as *Rhizophagus populinus*. Peyronel (1924) was the first to recognize the VAM fungi as *Endogone* species. Thaxter (1922) was the first to write a monograph of the family Endogonaceae where he described seven genera in their monograph of Endogonaceae viz., *Endogone*, *Glomus*, *Gigaspora*, *Modicella*, *Graziella*, *Acaulospora* and *Sclerocystis*. From time to time, new members were added to the family Endogonaceae by different workers. Schenck and Perez (1987) reported 120 species of soil fungi forming VA mycorrhizal under the genera *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, *Entrophospora* and *Sclerocystis*. These genera were distinguished from each other on the basis of the manner of spore formation by them. The new order Glomales which included 'all soil borne fungi which form arbuscules in obligate mutualistic associations with terrestrial plants' separated AM fungi from others in the Endogonales an order in which included a group of saprophytes which may also produce ectomycorrhizae like associations. The order Glomales thus included three families, the Acaulosporaceae, Gigasporaceae and Glomaceae with six

genera: *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* (Morton and Benny, 1990).

Traditional taxonomy of AM fungi has mainly been based on spore morphology and ontogeny. The structures and characters of the mycelia, e.g. vesicles and arbuscules and coils are of great taxonomic importance. The application of molecular techniques to identify AM fungi in field studies has uncovered large cryptic diversity of new AM fungi that are not taxonomically characterized. Complete phylogenies in the Glomeromycota are based on the 18S rRNA gene (Schüßler et al., 2001). Morton and Redecker (2002) reported two ancestral clads of species discovered from deeply divergent ribosomal DNA sequences. They are classified into two new families Archaeosporaceae and Paraglomaceae. At the present time, each family consists of one genus *Archaeospora* including three species forming a typical *Acaulospora* like spores from saporiferous saccule and *Paraglomus* consists of two species forming spores indistinguishable from those of *Glomus* species.

1. 3. Ecology of AM fungi

AM fungi are of worldwide distribution. They are present in the soil in the form of chlamydospores, zygospores and azygospores and have been recovered from the soil of a variety of habitats, e.g., nutrient deficient soils (Hayman et al., 1976), forests (Mohankumar and Mahadevan, 1988), deserts (Malibari et al., 1988), sand dunes (Gemma et al., 1989), savanna (Dodd et al., 1990), saline marshy areas (Sengupta and Chauduri, 1990), eroded soils (López-Sánchez and Honrubia, 1992) and contaminated soils (Bindu and Harikumar, 2008).

AM fungal association occur widely throughout the plant kingdom (Gerdemann, 1968). They have been reported to be present in bryophytes, pteridophytes, gymnosperms and angiosperms in nearly all the geographical regions of the world (Agarwal, 2005). Most of the field crops belonging to diverse families have been shown to form mycorrhizal associations (Barea, 2000). Likewise, many of the plantation crops (Karunasinghe et al., 2009) and medicinal plants (Gupta et al., 2009) have been shown to form mycorrhizal associations.

1. 4. Benefits of AM fungi for the host plant

1. 4. 1. Host plant growth

Progress in the study of the function of the symbiosis was made in Europe in 1957, when Mosse published a report showing that arbuscular mycorrhizal infection led to improved growth of apple seedlings and clonal leaf bud cuttings. She used sporocarps of *Endogone (Glomus) mosseae* to inoculate plants growing in autoclaved soil. In 1958, Peuss showed that inoculation with mycorrhizal roots increased growth of tobacco growing in subsoil or in soil that had been fallow. In 1963 Clark, using surface-sterilized mycorrhizal roots as inoculum, reported an increase of growth of tulip poplar trees planted in fumigated soil, and Meloh (1961, 1963) showed that the growth of maize and oats could be improved by AM fungi. Various laboratory and greenhouse experiments have demonstrated that AM inoculation can improve growth and nutrition of crop plants. Khaliel and Elkhider (1987) observed that the tomato plants inoculated with *Glomus mosseae* showed greater dry weight and higher percentage of survival than uninoculated plants in low P soil. In a study of the beneficial effect of AM fungi on tuber crops, Potty (1993) and Potty and Harikumar (1995) observed an increased growth and tuber yield in sweet potato due to inoculation with the AM fungi *Glomus microcarpum*. Mohammad et al. (1998) observed that AM colonization resulted in an increased growth, dry matter production and grain yield in field grown wheat. Enhanced shoot and fruit dry weight and total dry biomass in mycorrhizal chile anchopepper has been reported (Auguilera – Gómez et al., 1999). A similar increase in growth characters due to mycorrhizal association has been reported in many crops such as subterranean clover (Grazey et al., 2004) strawberry (Stewart et al., 2005) and green gram (Idnani and Singh, 2008).

1. 4. 2. Mineral nutrition

Most research on AM effects on plant nutrition has been concerned with phosphate because it is one of the major plant nutrients. It is now well established that mycorrhizae can improve the P nutrition of host particularly in low to moderate

fertility due to the exploration of the soil by the external hyphae beyond the root hairs and P depletion zone. Absorbed P is probably converted into polyphosphate granules in the external hyphae (Callow et al., 1978) and passed to the host (White and Brown, 1979). This flow of P occurs in the presence of acid phosphatases (Gianinazzi et al., 1979) during the arbuscule life span (Cox and Tinker, 1976) or senescence (Kinden and Brown, 1975).

Experiments conducted in unsterilized soils have frequently shown that introduced fungi could stimulate more P uptake than indigenous AM fungi (Islam et al., 1980). Similarly, increased plant growth responses to AM inoculation in soils incorporated with rock phosphate and decreased plant growth in soil applied with super phosphate have been observed (Jalali and Thareja, 1985; Tang and Chen, 1986). Increased P uptake due to AM inoculation has been reported by several workers (Diop et al., 2003; Caglar and Bayram, 2006).

Higher concentrations of nitrogen in tissue have been reported in AM associated plants (Hawkins et al., 2000; Azcon et al., 2001). Habte and Aziz (1985) observed increased nitrogen uptake in mycorrhizal *Sesbania grandiflora*. Smith and Read (1997) reported that the increase in total nitrogen is due to the higher P uptake through the AM hyphae rather than increased soil uptake. Oliver et al. (1983) found that higher uptake of phosphorus in mycorrhizal plants increases the activity of NAD dependent nitrate reductase enzyme both in shoot and root, hence, the possibility of mycorrhizal fungi having a NAD dependent enzyme which might contribute to nitrate reductase activity.

Mycorrhizal effects on N nutrition have been studied under field conditions and the potential of increased uptake of N from soil, as well as P mediated effects on N fixation have been demonstrated in *Hedysarvum coronarium* (Barea et al., 1987). In mixed plantings, a twofold increase in ¹⁵N transfer from soybean to maize has been observed in mycorrhizal plots together with the relative increase in productivity of maize (Hamel et al., 1991). This suggested that mycorrhizal fungi may be involved in the redistribution of N in the plant community.

In many investigations, K was found to be at higher concentrations in the tissues of mycorrhizal plants than in those of non-mycorrhizal plants (Nielsen and Jensen, 1983; Diop et al., 2003). Elevated concentrations of K in shoots, not in the roots of mycorrhizal *Trifolium subterraneum* plants grown in P deficient soils were observed (Smith and Walker, 1981). Huang et al. (1985) reported higher K uptake in mycorrhizal *Leucaena leucocephala* and K depletion in a hyphal compartment colonized by *G. mosseae* and increased accumulation in associated mycorrhizal *Agropyron repens* has been observed (George et al., 1992).

The uptake of micronutrients such as Zn, Ca, Mg and Fe were also found to be enhanced by AM inoculation (Bagyaraj and Manjunath, 1980; Cooper and Tinker 1981; Lu and Miller 1989; An et al., 1993; Marschner and Dell, 1994; Clark et al., 1999).

1. 4. 3. Host biochemical composition

AM fungi have fundamental effects on host plant biochemistry and physiology (Smith and Gianinazzi-Pearson, 1988; Koide and Schreiner, 1992). The increased concentration of soluble carbohydrates in host root tissue attributable to mycorrhizal colonization has been observed (Steffens et al., 1963; Thomson et al., 1986; Khalafallah and Abo-Ghalia, 2008). Significant amounts of carbohydrate trehalose was found in the root of some host fungus combinations (Schubert et al., 1992) which contradicts the earlier findings of Hayman (1973) that there is no indication of fungal carbohydrate such as trehalose and mannitol. Total CHO pools increased with mycorrhizal dependency of citrus genotypes providing evidence that C allocation pattern of the host affects mycorrhizal colonization (Graham et al., 1997).

Increased P supply decreased the per cent root length converted to mycorrhizas and concentration of soluble carbohydrate (Same et al., 1983). The hypothesis that high concentration of P inhibits the formation of VA mycorrhizas by reducing concentration of soluble carbohydrate in the root was tested on leek by Amijee et al. (1990) found that even at the concentration of soil P at which the

infection was reduced, the concentration of soluble carbohydrate increased to its maximum discounting the above hypothesis.

Total amino acid levels in roots was found to be lower in mycorrhizal leek (*Allium porrum*) plants (Rolin et al., 2001) however, an optimal amino acid level, especially of glutamate necessary for growth of AM fungi within the root cells (Tawaraya et al., 1990). In a study of the efficacy of AM fungus *Glomus fasciculatum* towards amino acid levels in *Prosopis cineraria* under glass house condition, a total of twelve amino acids were quantified in mycorrhizal and non mycorrhizal plants. AM inoculation resulted in increased levels of all the amino acids as compared with control (Mathur and Vyas, 1996). Higher values of free amino acids were noted in tea leaves when grown in pots inoculated with *Glomus fasciculatum* and *Gigaspora margarita* (Lin et al., 2006). Recently, Matsubara et al. (2008) determined that inoculation of AM fungi increased total amino acid concentration and concentration of specific amino acids in strawberry plants.

The accumulation of α amino acids like proline in plant tissue is influenced by various abiological stresses. Drought stress is known to result in decline of AM colonization, but the proline level of AM plants was higher than non-mycorrhizal controls during drought period (Goicoechea et al., 1998; Valentine et al., 2006). The similar effect of AM fungi on stomatal conductance, photosynthesis and proline accumulation was observed in *Citrus jambhiri* by Levy and Krikun (2006). Further, Lioussanne et al. (2008) noted a significantly higher concentration of proline and isocyturate in the roots of tomato inoculated with *Glomus intraradices*.

Differences in qualitative and quantitative expression of protein have been shown in AM fungi (Dumas et al., 1989; Wyss et al., 1990; Arines et al., 1993; Arines et al., 1994). It seems that mycorrhiza formation increases the expression of low molecular weight protein as suggested by the results obtained in soybean (Pacovsky, 1989) tobacco (Dumas et al., 1990) and mulberry (Kumaresan, 1997).

Protein content was much higher in mycorrhizal than in non-mycorrhizal root extracts, in tobacco and onion (Dumas et al., 1989). A two to six fold higher protein content was found in mycorrhizal than in non-mycorrhizal red clover roots (Arines et

al., 1993). Other reports have not shown such a large difference in both types of roots as have been described so far, and further deep studies of this aspect are needed. Perhaps this difference is a consequence of facts such as higher metabolic activity in AM colonized root cells and the presence of internal and external fungal mycelium. It is difficult to speculate further because our actual knowledge of AM fungal proteins is very limited, and we do not know if the new proteins are of fungal (or) plant origin.

1. 5. Screening of efficient AM fungi

Use of AM fungi for the improvement of crop productivity requires selection of an efficient and appropriate fungus (Menge, 1983) since AM fungi differ in their ability to form efficient symbiosis with different crop plants. AM fungi found to be efficient for some field crops are depicted in Table 1.1.

Table 1. 1. List of efficient AM fungi for some field crops

Plant species	Efficient AM fungus/ fungi	AM fungi tested	Parameters evaluated	References
<i>Allium cepa</i>	<i>Gigaspora margarita</i>	<i>G. margarita</i> , <i>G. calospora</i> and a known AM fungus	Leaf number dry matter P uptake, bulb yield	Ramana and Babu, 1999
<i>Amorphophallus paeonifolius</i>	<i>Glomus mosseae</i> + <i>G. aggregatum</i>	<i>G. mosseae</i> , <i>G. aggregatum</i> , <i>Gigaspora albida</i> , <i>Pisolithus tinctorius</i>	Tuber yield /plant	Ganesan and Mahadevan, 1994
<i>Arachis hypogaea</i>	<i>Glomus</i> sp.	<i>G. etunicatum</i> , <i>Gigaspora margarita</i>	Shoot dry matter, nutrient uptake	Ahiabor and Hirata, 1994
	<i>Glomus fasciculatum</i>	<i>G. fasciculatum</i> and seven other AM fungi	Root shoot length dry weight P content, root colonization	Vijayakumar and Bhiravamurthy, 1999
<i>Cajanus cajan</i>	<i>Glomus clarum</i>	<i>G. fasciculatum</i> and seven other AM fungi	Plant growth	Diederichs, 1992
<i>Capsicum</i>	<i>Glomus intraradices</i>	<i>G. intraradices</i> , indigenous mixed culture, commercial inoculum (Mycorise & C)	Fruit yield	Gaur et al., 1998
<i>Colocasia esculenta</i>	<i>Glomus mosseae</i> + <i>G. aggregatum</i>	<i>G. mosseae</i> , <i>G. aggregatum</i> , <i>Gigaspora albida</i> , <i>Pisolithus tinctorius</i>	Tuber yield/plant	Ganesan and Mahadevan, 1994

<i>Cucumis sativus</i>	<i>Glomus caledonium</i>	<i>G. caledonium, Glomus sp.</i>	P uptake	Joner and Jakobsen, 1994
<i>Eleusine coracana</i>	<i>Glomus caledonium</i>	<i>G. caledonium, G. mosseae, G. fasciculatum, G. epigaeum (G. versiformae), Gigaspora calospora, G. margarita</i>	Mycorrhizal efficiency root colonization	Tiwari et al., 1993
<i>Lycopersicon esculentum</i>	<i>Glomus etunicatum</i>	<i>G. etunicatum, G. mosseae</i>	Shoot dry weight, plant height	McGraw and Schenck, 1981
<i>Manihot esculenta</i> (cassava)	<i>Glomus fasciculatum</i>	<i>Glomus fasciculatum, G. mosseae G. constrictum, G. etunicatum, Acaulospora morrowea</i>	Root colonization plant weight, shoot and root dry weight	Sivaprasad et al., 1990
	<i>G. mosseae + G. aggregatum</i>	<i>G. mosseae, G. aggregatum Gigaspora albida, Pisolithus tinctorius</i>	Tuber yield / plant	Ganesan and Mahadevan, 1994
<i>Oryza sativa</i> cv <i>Prakash</i>	<i>Glomus intraradices</i>	<i>G. intraradices, G. fasciculatum</i>	Grain yield	Secilia and Bagyaraj, 1994

<i>Oryza sativa</i> (upland rice)	<i>Acaulospora spinosa</i>	<i>Acaulospora spinosa, A. scrobiculata</i>	Plant biomass grain yield, root colonization	Ammani and Rao, 1996
<i>Phaseolus mungo</i> (mung bean) (<i>Vigna radiata</i>)	<i>Glomus intraradices</i>	<i>Glomus intraradices, Acaulospora scrobiculata</i> and four other fungi	Plant growth, crop yield	Vasuvat et al., 1987
<i>Triticum aestivum</i> wheat (var. swift)	<i>Glomus intraradices</i>	<i>Glomus intraradices, Gigaspora margarita</i>	Plant yield, number of grains/spike	Asif et al., 1995
<i>Vigna unguiculata</i>	<i>Glomus etunicatum</i>	<i>G. etunicatum, Gigaspora margarita</i>	Shoot dry matter nutrient uptake	Ahiabor and Hirata, 1994
<i>Zea mays</i> (pioneer 3905)	<i>Glomus etunicatum</i>	<i>G. etunicatum, G. mosseae, G. aggregatum, G. versiformae</i>	Leaf mass, protein concentrations	Boucher et al., 1998
<i>Vitis vinifera</i>	<i>Acaulospora laevis</i> and a mixed inocula	<i>Acaulospora laevis, A. scrobiculata, Entrophospora colombiana, Gigaspora gigantea, Glomus manihotis, Scutellospora heterogama</i> and a mixed AM inocula	Survival and growth	Harekrishna et al., 2006

1. 6. AM association in oilseed crops

Arbuscular mycorrhizal fungi are known to occur abundantly in tropical soils supporting oilseed crops (Manoharachary and Prakash, 1991). Groundnut is perhaps the most studied oil seed crops with reference to mycorrhizal symbiosis. Investigations on growth and nutrient uptake response of this crop to AM inoculation have indicated an increase in growth and uptake of nutrients such as P, Zn and Cu due to mycorrhizal inoculation in comparison to uninoculated controls (Daft and Elgiahmi, 1976; Rao and Parvathi, 1982). Concurrent changes in the biochemical constituents such as carbohydrates and proteins in groundnut have been noticed due to AM inoculation (Krishna and Bagyaraj, 1984). A preliminary survey made by Sulochana and Manoharachary (1989) has revealed the association of *Glomus constrictum*, *G. fasciculatum* and *G. mosseae* with the rhizosphere soil and root region of safflower under field conditions grown in nutrient deficient soils with less moisture.

Perennial plants that produce oil seeds such as coconut and oil palm are not an exception for AM association (Girija and Nair, 1985; Thomas and Ghai, 1987; Blal et al, 1990) and the beneficial effect of AM association on the growth and nutrition of these crops has also been reported (Harikumar and Thomas, 1991; Widiastuti and Tahardi, 1993).

1. 6. 1. AM association in sesame

Incidence of AM colonization and its beneficial effect on the crop has been examined by several workers. An overview of AM studies carried out in sesame is depicted in Table 1. 2.

Table 1. 2. An overview of AM studies carried out in sesame

Parameter	Growing situation	Results achieved	Reference
AM colonization	Field experiment	Extensive root colonization by AM fungi, which increased up to 6 weeks. Number of vesicles increased with age of the plant. Incidence of <i>Glomus</i> , <i>Gigaspora</i> and <i>Scutellospora</i> species in the rhizosphere	Vijayalakshmi and Rao, 1988
	Field experiment	Occurrence of AM fungi in the roots and rhizosphere of sesame. <i>Glomus macrocarpum</i> , <i>G. fasciculatum</i> and <i>Sclerocystis sinuosa</i> were the most prevalent species. The appressoria, arbuscules and vesicles in the roots of sesame were examined under SEM.	Selvaraj and Subramanian, 1988
	Field experiment	Fungicides carbendazim and blitox when applied to soil after 30 days of seed sowing significantly inhibited mycorrhizal colonization	Vijayalakshmi and Rao, 1993