Vol. 16 No. 3 October 2004

## About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI, now The Energy and Resources Institute, was established in 1974. While in the initial period, focus was mainly on documentation and information dissemination activities, research activities in the fields of energy, environment, and sustainable development were initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

## The Bioresources and Biotechnology Division

Focusing on ecological, environmental, and food security issues, the division's activities include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grass-roots level, with village communities. The division functions through five areas—the Centre for Mycorrhizal Research, Microbial Biotechnology, Plant Tissue Culture and Molecular Biology, and Plant Biotechnology. It is actively engaged in mycorrhizal research. The Mycorrhiza Network has specifically been created to help scientists across the globe in carrying out research on mycorrhiza.

# The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

Established in April 1988 at TERI, New Delhi, the Mycorrhiza Network first set up the MIC (Mycorrhiza Information Centre) in the same year, and the CMCC (Centre for Mycorrhizal Culture Collection) – a national germplasm bank of mycorrhizal fungi – in 1993. The general objectives of the Mycorrhiza Network are to strengthen research, encourage participation, promote information exchange, and publish the quarterly newsletter *Mycorrhiza Netwo*s.

The MIC has been primarily responsible for establishing an information network, which facilitates sharing of information among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive databases on Asian mycorrhizologists and mycorrhizal literature (RIZA) allow information retrieval and supply documents on request.

The main objectives of the CMCC are to procure strains of both ecto and VA mycorrhizal fungi from India and abroad; multiply and maintain these fungi in pure culture; screen, isolate, identify, multiply, and maintain native mycorrhizal fungi; develop a database on cultures maintained; and provide starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction or handling.



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## Effect of temperature on development of vesicular-arbuscular mycorrhizae

## in plants

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## Introduction

Growth of plants as well as infection of plant roots by mycorrhizal fungi may be stimulated or retarded by an increase or decrease of soil temperature. The effect of other soil factors, such as soil pH, soil oxygen, or soil moisture, on the development of mycorrhiza on plant roots may also be influenced by the soil temperature, which may exert an influence on the survival of mycorrhizal fungi as spores or hyphae in soil. This write-up deals with the effect of soil temperature on the development of VAM (vesicular-arbuscular mycorrhiza) on plant roots.

# Effect of temperature on vesicular arbuscular mycorrhiza infection in plant roots

Studies conducted at the Department of Decologie et Pedologie, Faculte de Foresterie et Geodesic, Universite Laval, Quebec, Canada, on onion plants grown under three regimes of temperature and inoculated with Endogone calospora showed that the infection process followed a sigmoid curve showing a lag phase, an exponential infection period, and a plateau. All three phases were affected by the temperature conditions. The lag phase was shortest at the highest temperature (21-26 °C night/day) and longest at the lowest temperature (11–16 °C). The rate of infection indicated by the slope of the curves behaved in a comparable way; it was more rapid at 21-26 °C than at 16-26 °C and 11-16 °C. At 11–16 °C, the rate of infection remained very slow throughout the experiment. The final level of infection indicated by the plateau was highest at 21-26 °C (82%), was slightly lower (73%) at 16–21 °C, but was only 11% at 11–16 °C (Furlan and Fortin 1973).

\* Compiled from TERI database-RIZA

Pot experiment conducted at the Department of Plant Science, Leeds University, Leeds, the UK, on red clover and *Glomus caledonium*, showed that spores failed to germinate at 11 °C over a period of 40 days. The VAM infection was delayed or absent at 13.5 °C. The relative multiplication rate at entry points was lower at 16 °C or 12.5 °C than at 20 °C but there was little effect on the mean length of the infected root/entry points. Lowering the temperature reduced the relative growth rate of infected root more than that of the total root length (Sheikh and Sabders 1988).

Studies were conducted at the Department of Plant Pathology, Montana State University, Bozeman, USA, on soil incubators in the greenhouse to test the effects of three soil temperatures on growth of barley cultivars - Clark, Harmal, Steptoe, and Rihane – and on their root colonization by the VAM fungi (from agricultural soils in USA [Montana] or Syria) at different inoculum concentrations. The number of mycorrhizal plants as well as the proportion and intensity of roots colonized increased with higher soil temperatures. The VAM fungi from Montana, primarily *Glomus macrocarpum*, were cold-tolerant at 11°C while those from Syria, primarily *Glomus hoi*, were heat-tolerant at 26 °C. Inoculum potential of Montana VAM fungi was higher than Syrian VAM fungi in cool soils. Harmal, selected from the Syrian barley land races, had the highest colonization by mycorrhizal fungi of the cultivars tested (Grey 1991).

Studies conducted at the Department of Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Western Australia, Australia, on subterranean clover in the glasshouse showed that low root temperature, shading, and defoliation decreased both the percentage of root length infected by mycorrhizal fungi and concentrations of soluble carbohydrates within roots (Sam, Robson, and Abbott 1983).

In further studies at the above university, two greenhouse experiments (experiments 1 and 2) were conducted on Trifolium subterraneum with Scutellospora calospora. The differences between experiments 1 and 2 were regarding the ambient air temperature (18 °C and 31 °C, respectively), volume of substrate (400 g and 200 g of sand, respectively), and number of plants/pot (5 and 4, respectively). The percentage of root length colonized by S. calospora increased up to 49 days and 32 days in experiments 1 and 2, respectively, after which it declined. At this stage, there was no further increase in mycorrhizal root length but there was a sharp increase in non-mycorrhizal root length. In the first experiment, the concentration of soluble carbohydrates in the roots decreased during the first 49 days, followed by a sudden increase, coinciding with the decline in the percentage of the root length colonized. One week before this, the inflow of P (phosphorus) to the shoot also decreased. In both experiments, sporulation by S. calospora occurred after a decline in colonization. During sporulation, there was no further spread in colonization within the root and this may have been a cause of increase in the concentration of soluble carbohydrates within the roots (Pearson and Schweiger 1993).

Studies at the Department of Plant Pathology, University of Georgia, Athens, Georgia, USA, on cotton cultivars showed that maximum mycorrhizal development by Gigaspora margarita and the subsequent cotton growth stimulation occurred at 30 °C and 24 °C, and was slight or absent at 19 °C or 14 °C. Growth stimulation of five cotton cultivars varied considerably; Coker 310 and Stoneville 213 responded best. Acala 1517-70 and Deltapine 16 were marginally influenced and Paymaster 909 was relatively unaffected. In the P-deficient soil, inoculum levels of either 200 or 400 azygospores of G. margarita per plant significantly stimulated cotton growth, whereas rates of 10, 50, and 100 spores per plant had little or no effect (Pugh, Roncadori, and Hussey 1981).

In further studies at the above university, cotton (Gossypium hirsutum) cv. Stoneville 213 inoculated with Glomus intraradices, Glomus ambisporum, or Gigaspora margarita and grown in soil temperature tanks in the glasshouse at  $18 \,^{\circ}$ C,  $24 \,^{\circ}$ C,  $30 \,^{\circ}$ C, and  $36 \,^{\circ}$ C showed that the per cent root colonization by the VAM fungi was less than 10% at  $18 \,^{\circ}$ C and was 57%–80% at temperatures of  $24 \,^{\circ}$ C and higher (Smith and Roncadori 1986).

Studies at the Soil Science and Plant Pathology Departments, University of Florida, Gainesville, Florida, USA, on *Gigaspora albida* (INVAM isolate GABD 185), *Glomus etunicatum* (LETC236), and *G. intraradix* (LINR208), grown in association with *Allium cepa* in pasteurized Arredondo loamy sand, showed that inocula of *G. etunicatum* and *G. albida*  placed at 1 cm depth produced greater lengths of germination hyphae and at a greater rate than at 13-cm inoculum-placement depth. G. intraradices showed no difference in germination hyphae between the two depths. Soil temperature at 1 cm depth was generally 2 °C warmer than at 13 cm depth. For G. etunicatum, there was an eight-day delay in penetration when the inoculum was placed 13 cm below the seed compared to placement at 1 cm. The colonized root length and sporulation were always greater for inoculated plants, with the inoculum placed at 1 cm depth. G. intraradices penetrated the root after 13 days when placed 1-cm below the seed, four days sooner than 13-cm-placed inoculum. The colonized root length and sporulation were greater for inocluated plants, with the inoculum placed at 13 cm depth by six weeks. The total root length generally decreased with depth below 9 cm. Sporulation was also affected by the inoculumplacement depth in soil. Delay in penetration and subsequent development by eight days reduced the colonization and sporulation of G. etunicatum. The delay of four days for G. intraradices had little effect on colonization and sporulation as the development proceeded more aggressively when inoculated at 1 cm depth. Colonization by the VAM fungi placed 13 cm below the seed was dependent on root growth to the inoculum while colonization by the VAM fungi placed at 1 cm depth was dependent on the inherent penetration rate of the fungus (Jarstfer and Schenck 1990).

In studies at the Departments of Plant Pathology and Agronomy, Kansas State University, Manhattan, USA, a warm-season grass, Andropogon gerardii, or a cool-season grass, Bromus inermis, were grown at two temperatures on one side of a pot divided by a 48 m (micron) nylon root barrier. The mycorrhizal function was assessed by measuring the amount of 32 P translocated from one side of the pot to plants on the other side. As a control, mycorrhizal hyphae crossing the barrier were severed manually. Approximately, 100-times more 32 P was observed in *B. inermis* when grown at 18 °C than at 29 °C. In contrast, A. gerardii accumulated four-times more 32 P at 29 °C than at 18 °C. Thus, it appears that mycorrhizal hyphae are most active in both the highly dependent and weakly dependent plant species at temperatures most conducive to the growth of each species. It remains enigmatic why the mycorrhizal hyphae are so active in the cool-season host when no biomass response occurs in that host (Wilson, Hetrick, and Schwab 1993).

Studies conducted at the Department of Soil Science, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, Australia, on barley cv. Galleon, grown on sterilized soil inoculated with *G. intraradices* and maintained at soil temperatures of 10 °C, 15 °C, or 20 °C showed that the vesicular-arbuscular mycorrhiza formation was significantly reduced as soil temperature decreased (Baon, Smith, and Alston 1994).

Studies at the faculty of Agriculture, Hokkaido University, Sapporo, Japan, showed that in-vitro spore germination rate was greater at 25 °C in G. etunicatum and at 25 °C or 30 °C in G. margarita. Hyphal growth was best at 20 °C, 25 °C, or 30 °C in G. etunicatum and at 25 °C, 30 °C, or 35 °C in G. margarita. Inoculation of Asparagus officinalis cv. MW 500W seedlings with both the fungi showed that the extent of mycorrhizal infection (percentage of infected portions in the root system) was greatest at 25 °C in G. etunicatum-inoculated plants and at 25 °C or 30 °C in G. margaritainoculated plants. When the bed soil temperature was made to fluctuate diurnally in the range of 15–25 °C or 18–30 °C, little difference in the extent of mycorrhizal infection was found between the two temperature regimes in G. etunicatum-inoculated plants, but in G. margarita-inoculated plants, the percentage was higher under the 18–30 °C regime than under the 15-25 °C regime (Matsubara and Harada 1996).

In a field experiment conducted at the Department of Natural Resources and the Environment, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan, winter wheat (*Triticum aestivum* L.) was grown in a dry land with or without *G. intraradices* inoculation at a rate of 5000 spores per 30 cm in each row and with or without P fertilizer. The roots were sampled at five growth stages to quantify the VAM. No VAM infection following seeding was evident during the fall months, which were characterized by a low soil temperature. During spring, the VAM increased gradually with the rising soil temperatures (Mohammad, Pan, and Kennedy 1998).

### Effect of temperature on vesiculararbuscular mycorrhiza efficiency in plants

Studies at the Plant Pathology Department, University of Georgia, Athens, USA, on cotton (Gossypium hirsutum L.) cv. Stoneville 213, grown in soil temperature tanks in the glasshouse and inoculated with G. intraradices, G. ambisporum, or G. margarita showed that growth response in fresh and dry weights of shoots and plant height were linear for non-inoculated plants but non-linear for mycorrhizal plants at soil temperatures of 18 °C, 24 °C, 30 °C, and 36 °C. Total length of the root and length of mycorrhizal root were positively correlated and increased as the soil temperatures increased. However, the total root length was not significantly changed by the soil temperature in non-mycorrhizal plants. At 18 °C, shoot and root growth were not improved by the mycorrhizae and the total root length was actually suppressed by the endophytes. At 24 °C, 30 °C, and 36 °C, mycorrhizae stimulated plant growth (Smith and Roncadori 1986).

Studies at the Department Decologie et Pedologie, Faculte de Foresterie et Geodesic, Universite Laval, Quebec, Canada, on onion plants grown under three regimes of temperature and inoculated with *Endogone calospora* showed that the growth of plants was strongly stimulated after infection with mycorrhizal fungi at 21-26 °C and 16-21 °C but a parasitic decrease (P < 0.05) of growth could be observed on the eighth week at 11-16 °C. Spore production curves followed very closely the growth-enhancement curves of inoculated plants and the final spore production per plant was 2600, 1800, and 50 for the 21-26 °C, 16-21 °C, and 11-16 °C regimes, respectively (Furlan and Fortin 1973).

Studies conducted at the Departmento de Microbiologia, Estacion Experimental del Zaidin, Consejo Superior de Investigaciones Científicas, Granada, Spain, on wheat cultivars showed that different plant-growth temperatures, and the sport given by culture media and inoculation with different spores affected the VAM colonization levels. After the VAM reinoculation, plant dry weight of Castan and 7-Cerros cultivars increased, but not that of Negrillo and Champlein cultivars. VAM increased the shoot dry weight of 7-Cerros only, but not of Champlein, when grown at 35–24 °C, and had no effect on the dry weight of either cultivar grown at 18-12 °C and 42-24 °C . Inoculation with Glomus mosseae increased the dry weight of the cultivars more than inoculation with G. fasciculatum or G. aggregatum. Effect on the plant dry weight was greater in plants grown in soil than in sand/ vermiculite pots. Inoculation with sterilized and unsterilized spores of G. mosseae, either in soil pots or in sand/vermiculite tubes, did not increase the plant dry weight (Vierheilig and Ocampo 1991).

Glasshouse studies at the Department of Soil Science, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, Australia, on barely cv. Galleon grown in pots in sterilized soil inoculated with G. intraradices and maintained at soil temperatures of 10 °C, 15 °C, or 20 °C showed that a reduction in plant growth due to a low temperature was more pronounced in mycorrhizal plants than in non-mycorrhizal plants. The lower the soil temperature, the higher was the root-shoot ratio. The ratio was also higher in non-mycorrhizal plants than in mycorrhizal plants. A significant interaction between mycorrhiza and the soil temperature was observed for root dry matter and specific P uptake (P uptake/unit weight of root). Compared with the non-mycorrhizal plants, the specific P uptake in mycorrhizal plants was higher (Baon, Smith, and Alston 1994).

In studies conducted at the Department of Biology, University of Ottawa, Canada, seven-weekold seedlings of spring and winter wheat (*Triticum aestivum*) cultivars (Glenlea and A C Ron) were subjected to a one-week cold treatment when inoculated with *Glomus mosseae*. The combined effect of the VAM and low temperature on the two cultivars showed that the dry biomass was higher in Glenlea than AC Ron at week five and did not significantly change at week eight in both cultivars with either the mycorrhizal or cold treatment. Chlorophyll content was higher in mycorrhizal than in nonmycorrhizal plants in cv. Glenlea at 5 °C but was unaltered in AC Ron or in either cultivar at 25 °C. The reducing and total sugar contents were higher in AC Ron than in Glenlea cultivar. Protein content was higher in Glenlea than AC Ron at 25 °C but remained constant regardless of the mycorrhizal or cold treatment (Paradis, Dalpe, and Charest 1995).

In further studies at the above university, seeds of two hybrids of maize (*Zea mays*) (Pioneer 3902 and Pride 5) were grown in soil inoculated with *Glomus mosseae*. Germination tests at 10 °C and 25 °C showed that Pride 5 was more resistant to chilling than Pioneer 3902. Plants grown at 25 °C for six weeks were given a one-week chilling treatment at 10 °C. Mycorrhizal plants at 10 °C had greater biomass, carbohydrates, and protein contents than the non-mycorrhizal plants (Charest, Dalpe, and Brown 1993).

Studies at the Institut für Tropischen und Subtropischen Pflanzenbau, Grisebachstr., Göttingen, Germany, showed that mycorrhizal plants of *Eupatorium odoratum* supplied with insoluble phosphate  $Ca_{5}(PO_{4})_{3}OH$  responded to soil temperature with a steep rise in growth from 20 °C to 30 °C and a fall from 30 °C to 35 °C, whereas non-mycorrhizal plants showed no response. Soil temperature seems to affect the physiological activity of the mycorrhiza more than its development as the plants being kept at optimum temperature  $(30 \,^{\circ}\text{C})$ for 20 days before being moved to lower or higher temperatures showed the same inhibition of growth as plants kept permanently at the unfavourable temperatures. Diursnally changing soil temperatures with 35 °C during the day only were not harmful to the functioning of mycorrhiza if followed by lower night temperatures. Contrary to E. odoratum, nonmycorrhizal Guizotia abyssinica and Sorghum bicolor did respond to temperature in presence of insoluble phosphate although less so than mycorrhizal plants. Shading had no effect on non-mycorrhizal plants but decreased the efficiency of the mycorrhiza (Moawad, Nyabyenda, Graw, et. al 1979).

In studies at the Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada, barley was grown at soil temperatures of 12 °C, 16 °C, and 20 °C (air temperature 22–18 °C; day/night) in pasteurized (control) and mycorrhizal soil to determine the effect of mycorrhiza on root tip growth. Examination of the segmented root system showed that in pasteurized soil, 16 °C was the apparent optimum temperature for growth of root and shoot. The mycorrhizal infection appeared to shift the optimum downwards and growth was less at higher temperatures. Mycorrhizal plants had a higher shoot/root ratio (dry weight) and greater root proliferation than non-mycorrhizal plants. The fungus was associated with finer roots (Volkmar and Woodbury 1981).

In a field experiment conducted at the Department of Natural Resources and the Environment, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan, in a dryland winter wheat (Triticum aestivum L.), winter wheat was seeded within a day after inoculation with G. intraradices (5000 spores per 30 cm in each row) and with or without P fertilization. Increases in wheat grain yields by the enhanced VAM were of similar magnitude to the response obtained from P fertilization. However, responses differed at intermediate growth stages. At the tillering stage, the P uptake was mainly increased by P fertilization but not by fungal inoculation. At harvest, the enhanced VAM increased the P uptake regardless of whether or not the fertilizer P was added. Thus the VAM symbiosis increased with the rising soil temperatures in spring, in time to enhance the late-season P accumulation and grain production. (Mohammad, Pan, and Kennedy 1998).

#### Effect of temperature on relative efficiency of VAM fungi

Two greenhouse experiments at the Centro Internacional de Agricultura Tropical, Cali, Colombia, to determine the effect of soil temperatures (20 °C and 30 °C ) on the efficiency of 10 isolates (9 species) of VAM fungi (7 from cold regions and 3 from warm climates) on cassava clone MCol113 showed that all isolates were effective at higher soil temperatures, excepting Acaulospora *morrowae* and *Scutellospora pellucida*, which are generally ineffective mycorrhizal fungi. Only three isolates were effective at the lower temperature, Glomus manihotis (isolated from a warm climate), A. laevis (from a cold climate), and one isolate of *Entrophosphora colombiana*, which originated from a cold climate. Another isolate of E. colombiana from a warm climate was not effective at 20 °C (Sieverding 1988).

Glasshouse studies at the Plant Pathology Department, University of Georgia, Athens, USA, on the effects of four soil temperatures (18 °C, 24 °C, 30 °C, and 36 °C) on growth and root colonization by three VAM fungi of Stoneville 213 cultivar of cotton (Gossypium hirsutum) showed that mycorrhizae stimulated plant growth at 24 °C, 30 °C, and 36 °C. At 18 °C, shoot and root growth were not improved by mycorrhizae and the total root length was suppressed by the endophytes. Shoot dry weights were maximum at 30 °C on plant mycorrhiza with G. margarita and G. intraradices, and at 36 °C, plants inoculated with G. ambisporum had maximum shoot dry weights. All mycorrhizal plants had increased leaf tissue concentrations of P, Cu (copper), Zn (zinc), and Mn (manganese), with concentrations of Cu, Zn, and Mn greatest in plant mycorrhiza with G. ambisporum. The three mycorrhizal fungi generally stimulated plant growth equally well at 24 °C and 34 °C and G. ambisporum was slightly more effective as a symbiont at 36 °C

than either G. intraradices or G. margarita (Smith and Roncadori 1986).

Studies at the Department of Agronomy, University of Nebraska, Lincoln, USA, on sorghum plants grown in growth chambers at 20 °C, 25 °C, or 30°C in a low P Typic Arguidoll (3.65 microg (micrograms) P/g soil, pH 8.3) and inoculated with Glomus fasciculatum, G. intraradices, or G. macrocarpum showed that sorghum root colonization and plant responses to the VAM fungi were temperature-dependent. G. macrocarpum-colonized sorghum roots had best and enhanced plant growth and mineral uptake, considerably more than the other VAM fungi species, especially at 30 °C. G. macrocarpum and G. fasciculatum enhanced the shoot growth at 20 °C and 25 °C and mineral uptake only at 20 °C. G. intraradices depressed the shoot growth and mineral uptake at 30 °C. G. macrocarpum enhanced the shoot P, K, and Zn above that, which could be accounted for by an increased biomass content at all temperatures, and iron content at 25 °C and 30 °C (Raju, Clark, Ellis, et. al 1990).

In a greenhouse experiment conducted at the Plant Pathology Department, University of Florida, Gainesville, USA, six VAM fungi were compared for their response on soybean (*Glycine max*) at four soil temperatures (18 °C, 24 °C, 30 °C, and 36 °C) with greenhouse air temperatures being at 26/33 °C night/ day temperatures. The VAM inoculation was conducted by placing 50 spores 2.5-cm below seed in each plastic pot with 2 kg of Arredondo fine sand (pH 5.5, P 38 parts per million). The mean values for all fungus and plant parameters in three tests were generally greatest at 30 °C and lowest at 18 °C soil temperatures. Soybean flower numbers, pod set, and seed yield varied considerably in the three tests but plant height was little affected by soil temperature or fungal species. Non-mycorrhizal control plants were lowest in shoot and root weight at 36 °C but they were usually superior to plants colonized by Acaulospora laevis, Gigaspora pellucida, and Glomus clarus at 18 °C, 24 °C, and 30 °C. Root and shoot weights of plants colonized by G. mosseae, G. claroideus, and Gigaspora gregaria were usually superior to the non-mycorrhizal plants at all soil temperatures. Spore size was generally less at soil temperatures of 30 °C than at 24 °C but this varied with fungal species and test. Four species produced the greatest number of spores per gram of colonized root at 36 °C while G. pellucida and A. laevis produced the maximum number at 30 °C. Maximum root colonization was attained by G. gregaria at 36 °C, G. mosseae at 18 °C, G. claroideus and A. laevis at 30 °C (Schenck and Smith 1981).

Studies at the Faculty of Agriculture, Hokkaido University, Sapporo, Japan, on A. officinalis cv. MW500W seedlings inoculated with G. margarita or G. etunicatum showed that there were no growth enhancements eight weeks after inoculation at 20 °C with both the mycorrhizal species. At 25 °C or 30 °C, both G. etunicatum- and G. margaritainoculated plants were taller than non-inoculated plants. At 30 °C, the G. margarita-inoculated plants were taller than G. etunicatum-inoculated plants. At 25 °C, the G. etunicatum- or G. margarita-inoculated plants had more shoots and storage roots than those at other temperatures. Root colonization for G. etunicatum was greatest at 25 °C but for G. etunicatum, root colonization was greatest at 25 °C or 30 °C. When the bed-soil temperature was made to fluctuate diurnally at 15-25 °C or 18-30 °C, G. etunicatum-inoculated plants were taller, with more shoots and storage roots, eight weeks after inoculation than G. etunicatuminoculated plants under the 15–25 °C regime while the reverse was observed under the 18-30 °C regime. Per cent root infection was higher under the 18-30 °C regime than under the 15-25 °C regime in G. margarita-inoculated plants but there was little difference between the two temperature regimes in G. etunicatum-inoculated plants (Matsubara and Harada 1996).

### Effect of temperature on vesiculararbuscular mycorrhiza efficiency at various pH levels

Studies at the Institute Pflanzenbau und Tierhygience in den Tropen und Subtropen, University of Göttingen, Germany, on Burley tobacco, grown in a greenhouse at pH 5,6, or 7; soil temperatures of 20 °C, 25 °C, 30 °C, or 35 °C; and fertilized with the MCP (monocalcium phosphate) or HA (hydroxylapatite) showed that the HA plants inoculated with G. mosseae and non-inoculated MCP plants responded similarly to changes in the pH or temperature. Response of the host plant to environmental factors dominated the reaction; inoculation modified it only slightly. The temperature optimum tended to shift from 30 °C at pH 5 to 25 °C at a higher pH. Inoculation increased the P uptake and growth considerably even in plants supplied with the MCP. Development of fungus in roots was fair to strong in all treatments. The best development of mycelium was at 30 °C, of arbuscles at 25-30 °C, and of vesicles also at 25-30 °C (Khanaqa 1987).

Pot culture experiments were conducted at the above institute on *Triticum aestivum* and *S. bicolor* to study the effect of the VAM fungi, *G. macrocarpum* and *G. manihotis*, under four soil pH levels (pH 4.5, 5.5, 6.5, and 7.5); four soil temperatures (20 °C, 25 °C, 30 °C, and 35 °C); and under fertilization with different rock phosphates. Controls were kept with soluble phosphate (MCP) and with no P fertilizer (0P). Increase in shoot dry weight up to 143% could be found with mycorrhizal *T. aestivum* using Kodjari rock phosphate as a P source. In presence of the VAM, *S. bicolor* and *T. aestivum* fertilized with any of the rock phosphates, except Kola, produced yields comparable with those of plants fertilized with the MCP at soil pH 5.5 to

7.5 and at 25 °C soil temperature. As the soil pH increased from 5.5 to 7.5, dry weight declined. Likewise, yields decreased with increasing soil temperature and at 35 °C, VAM showed no effect in all treatments (Fabig, Moawad, and Achtnich 1989).

#### Effect of temperature on vesiculararbuscular mycorrhiza efficiency at different soil moisture levels

Studies were conducted at the Texas A & M University and Texas Agricultural Experiment Station, College Station, USA, on water relations of glasshouse-grown container plants of Berberis thunbergii cv. Atropurpurea, Buxus microphylla var. japonica, and Pittosporum tobira cv. Wheeler, inoculated or not with G. etunicatum and G. fasciculatum under high-temperature root stress conditions. Pre-dawn xylem water potential in stems (psi stem) increased initially (more positive) in response to high root-zone temperatures (40–50 °C) and then decreased over a five-day period. Stomatal conductance (Gs) and ET (evapotranspiration) were reduced incrementally over time in response to high root zone temperatures. Root damage occurred as indicated by reductions in root quality and Gs at 35 °C and 40 °C for B. thunbergii and P. tobira, and 40 °C and 45 °C for the more high-temperatureresistant B. microphylla. Colonization increased Gs and ET of B. microphylla at ambient (25 °C) and high (45 °C) temperatures and increased the ET of B. thunbergii at 25 °C. Colonized plants had lower (more negative) psi shoot with initial exposure to increased root-zone temperatures. However, throughout the remainder of the study, there was little reduction in plant stress with the mycorrhizal isolates used. Root hydraulic conductivity (Lp) increased markedly in B. thunbergii compared with B. microphylla at 40 °C and 45 °C, indicating less high-temperature resistance in *B. thunbergii* roots (Newman and Davies 1988).

Studies at the Institute of Tropischen, Subtropischen, Pflanzenbau, Grisebachstrasse 6, Göttingen, Germany, showed that shoot dry matter of the weed, Eupatorium odoratum, fertilized with soluble or insoluble phosphate and inoculated with G. macrocarpus was higher than that of nonmycorrhizal plants fertilized with soluble phosphate, at all temperatures except at 20 °C. At 20 °C, plants did not respond to the treatments. Non-mycorrhizal plants reacted with more sensitivity to water shortage than mycorrhizal plants with both P sources, particularly at the high soil temperatures of 30 °C and 35 °C. In the driest water regime, growth of mycorrhizal plants fertilized with insoluble P and grown at the sub- and supra-optimal temperatures of 25 °C and 35 °C was similar to that of nonmycorrhizal plants fertilized with soluble phosphate and grown under optimum conditions (30 °C and full water supply). An improved growth in mycorrhizal plants was caused mainly by an

increased uptake of P. Mycorrhizal plants fertilized with soluble phosphate absorbed excessive amounts of P. Besides P, mycorrhizal plants took up higher amounts of K (potassium) and Mg (magnesium) than non-mycorrhizal plants. The uptake of Ca (calcium) by mycorrhizal plants fertilized with hydroxylapatite was low; these plants had a very high K: Ca ratio in their shoots, which may have contributed to stabilization of water relations. Influence of different soil temperatures and water regimes on the development of mycorrhiza was small. High efficiency of the VAM at 25 °C, 30 °C, and 35 °C must be ascribed to the activation of physiological processes. At these temperatures, water utilization of the mycorrhizal plants was considerably improved (Sieverding 1983).

In studies conducted at the Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, USA, large intact soil cores from nearly pure stands of *Pascopyrum smithii* (Elymus smithii) and *Bouteloua gracilis*, extracted from the Central Plains Experimental Range in North-east Colorado, USA, were transferred to controlled environment chambers and exposed to a variety of water, temperature, and CO<sub>2</sub> regimes for four annual growth cycles. Root samples were harvested after second and fourth annual growth cycles (winter time) for mycorrhiza examination. After two growth cycles in the growth chambers, 54% of the root length was colonized in P. smithii, compared to 35% in B. gracilis. Field control plants had significantly lower colonization. Increasing the CO<sub>2</sub> increased mycorrhizal colonization in *B. gracilis* by 46% but had no effect in P. smithii. Temperatures 4 °C higher than normal decreased colonization in *P. smithii* by 15%. Increased annual precipitation decreased colonization in both species. Simulated climatic change conditions (increased CO<sub>2</sub>, increased temperature, and reduced precipitation) decreased colonization in P. smithii but had less effect on B. gracilis. After four growth cycles in P. smithii, trends of treatments remained similar but the overall colonization rate decreased (Monz, Hunt, Reeves, et al. 1994).

In glasshouse experiments at the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, University of Western Australia, Nedlands, Australia, an early break, a late break, and a false break (followed by a late break) were applied to a field soil collected dry in summer and filled in pots. In each break, pots were watered to field capacity and planted with the subterranean clover (*T. subterraneum*) or capeweed (*Arctotheca calendula*) with soil temperature maintained at 30 °C for the early and false breaks, and 18 °C for the late break. In both, early and late breaks, pots were watered to field capacity when plant and mycorrhizal variables were assessed. In a false break, pots were watered to field capacity for seven days, after which soil was allowed to dry and newly emerged plants were allowed to die. These pots were then re-watered and

replanted at the same time as pots receiving a late break. The following year, soil temperature was maintained at 31 °C or 18 °C in both early and late breaks and pots were planted with clover and watered to field capacity. The VAM colonization of both clover and capeweed was initially low in an early break (only *Glomus* spp.) compared with the levels observed in a late break (Glomus spp., Acaulospora spp., and *Scutellospora* spp.). Colonization was decreased by a false break because of a decrease in the formation of mycorrhizae of *Glomus* spp. In the second year, mycorrhizae of Glomus spp. predominated in warm soil in both early and late breaks and mycorrhizae of Acaulospora spp., *Scutellospora* spp., and fine endophytes occurred in greater abundance in cool soil in early and late breaks. These results indicated that soil temperature at the time of the break had a large impact on both, the overall levels of VAM colonization of pasture plants and colonization by different fungi. Fungi that remained quiescent in warm soil avoided damage in a false break (Braunberger, Abbott, and Robson 1997).

Studies at the United States Department of Agriculture, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Athens, Georgia, on rooted cuttings of poinsettia (*Euphorbia pulcherrima*) infected with *G. margarita* showed that the mycorrhizal cuttings withstood the transplant shock under high temperature and low moisture conditions better than non-mycorrhizal cuttings. Thus, endomycorrhizal plants grew better than nonsymbiotic plants under conditions of low fertility and moisture stress (Barrows and Rocadori 1977).

### Effect of temperature on vesiculararbuscular mycorrhiza efficiency at different oxygen levels

In pot experiments conducted under growth room conditions at the Institute fur Tropischen und Subtropischen, Pflanzenbou der Universitat, Göttingen, Germany, E. odoratum was inoculated with G. macrocarpus in soil fertilized with hardly soluble  $Ca_3(PO_4)_3OH$  and supplied continuously with oxygen at the rate of one litre per hour. Dry matter increased markedly with rising oxygen up to 16% and with soil temperature up to 30 °C. In the 35 °C treatment, dry matter increased with up to 21% oxygen. The inoculation effect at 20 °C and 25 °C decreased with increasing oxygen up to 21% but at 30 °C and 35 °C, it increased continuously up to 21%. The effect in the 35 °C treatment at 16% and 21% oxygen was greater than at corresponding oxygen concentrations in the 20 °C treatment. Uptake of all elements increased with rising oxygen up to 16% and with soil temperature up to 30 °C. In 35 °C treatment, mineral uptake increased up to 21% oxygen, except N and Mg. The P uptake was more strongly improved by inoculation than the uptake of other elements. The mineral concentration at 20 °C, 25 °C, and 30 °C was generally little

affected by soil oxygen, but at 35 °C it increased up to 21% oxygen. The P concentration increased but the concentration of N decreased with increasing soil temperature up to 30 °C and the concentration of Ca and Mg were little affected by the soil temperature. Inoculation increased the concentrations of P, K, and Mg but decreased N and Ca concentrations. The incidence of mycorrhiza was lower at 2% than at higher oxygen concentrations at all soil temperatures. The percentage of root length infected and the number of vesicles increased with rising soil temperature up to 30 °C, with greatest values at 16% and 21% oxygen. The incidence of mycorrhiza at 35 °C was appreciably higher than at 20 °C except at 2% oxygen where the infection rate was very low and no arbuscules were observed. Generally, the percentage of root segments with arbuscules increased from 20 °C to 25 °C and then decreased at 30 °C and 35 °C. The arbuscule development was strongest in roots at 25 °C with 8% and 16% oxygen in the soil atmosphere and in unaerated control roots (Saif 1981; 1983).

### Effect of fire on survival of vesiculararbuscular mycorrhiza fungi in soil

In studies at the United States Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experimental Station, Tempe, Arizona, USA, all material, including living, downed, and dead matter, was burnt in the fall of 1989 in one hectare of mature pinyon-juniper wood land using drip torches. Soil cores were taken from interspaces and beneath the canopies of pinyon and juniper in the spring of 1989, and immediately prior to and 96 hours following the burn (spring samples were taken to assess seasonal variability of spores). During spring, there were no differences in species richness under pinyon, juniper canopies, or interspaces. G. fasciculatum and G. aggregatum were the two most frequently observed species. In preburn samples, species richness was slightly lower than in spring in each of the three cover types. In post-burn samples, G. fasciculatum, G. deserticola, and G. macrocarpum were the only remaining species. Spore density varied under each of the three cover types in spring and pre-burn samples but in postburn samples, spore numbers were significantly reduced (in all but one pine sample), particularly under tree canopies (up to 91% loss) as compared with the interspaces (47% loss). In the bioassay, the per cent colonization of pearl millet was significantly greater when grown in juniper soils than in the other two cover types. All post-burn bioassays were significantly reduced and in several canopy soils, there was no mycorrhizal colonization. Overall, losses under canopies were higher than in interspaces. Loss of mycorrhizae was correlated with soil temperature and heating duration, which varied with the amount of litter (and duff under tree canopies) burnt. Highest temperatures were reached

in soils under canopies. The large fuel load, in addition to the total combustion of organic material, contributed to a more intense burn. Smouldering of duff and tree stumps maintained these high temperatures for several days. Overall, burning temperatures in interspaces were low as interspaces had low fuel load, little aboveground vegetation and litter, and no duff (Klopatek, Friesec, Allen, *et al.*1990).

Studies at the Kansas State University, Division of Biology, AcKert Hall, Manhattan, Kansas, USA, on tallgrass prairie sites subjected to 10 years of annual burning, mowing, N or P fertilization, or untreated showed that spring burning of native prairie field plots significantly reduced the VAM fungal species diversity while increasing spore abundance. This increase in the total spore number was due to a general increase in most of the 17 species present. In general, the management treatments had larger effects on the richness component of the diversity than on the evenness of the VAM species abundance. Burning and mowing had no significant effect on the VAM fungal colonization of roots or extraradical mycorrhizal hyphal development. N fertilization significantly increased the root colonization and extraradical mycorrhizal hyphae while P amendment decreased the extraradical hyphae. Fertilization had no significant effect on the VAM spore abundance, fungal species diversity or richness, but decreased the fungal species evenness (Eom, Hartnett, Wilson, et al. 1999).

## Effect of temperature on survival of vesicular-arbuscular mycorrhiza in storage

Studies at the United States Department of Agriculture, Horticulture Research Laboratory, Orlando, Florida, USA, on survival of G. intraradices during storage at five temperatures showed that survival was poorest at the two highest temperatures (16 °C and 21 °C in test 1 and 4 °C and 21 °C in test 2). G. mosseae stored at 12% soil moisture level for 56 days stimulated a growth response in sour orange in all treatments, but not after treatment for 196 days. G. intraradices, stored at the same soil-moisture level for 196 days survived all treatments. Freezing inocula of both species for one, two, and three 14-hour periods, with 10-hour thawing periods between freezes and a 62-hour continuous freeze before inoculation, did not affect the development of G. intraradices in the root but the root weight was decreased in the three-longest freeze treatments. The effect of freezing on G. mosseae suggested that spore dormancy factors were reduced by freezing because plant growth and fungus development in roots increased. Five heat treatments, 43-66 °C, reduced the activity of G. mosseae, G. intraradices, and G. deserticola inocula even at the lowest temperature. Viability of G. mosseae and G. intraradices did not occur beyond 60 °C and for G. deserticola not beyond 54 °C (Nemec 1987).

In studies at the Department of Land Resource Science, and Department of Botany, University of Guelph, Ontario, Canada, maize (Z. mays) was grown in pots containing pasteurized soil inoculated with VAM fungus 'Elora 500' isolate. A pair of  $43 \,\mu\text{m}$  nylon mesh pouches (270 cm<sup>3</sup>) containing pasteurized soil were placed in each pot and establishment of the VAM symbiosis was ensured for six weeks. After six weeks, the paired mesh pouches containing the VAM mycelium were removed from the pots and buried in the field soil at Elora Research Station near Guelph, Ontario, Canada. Half of the pairs of pouches were sealed in polyethylene prior to transplanting to prevent entry of the fungal hyphae from the surrounding soil. Transplanting occurred in November 1991 when soil temperature at 10 cm depth under a nearby sod plot was 6.5 °C. The soil froze in mid-December in 1991; it reached a minimum temperature under sod of 3.3 °C at 5 cm depth and 1.7 °C at 10 cm depth in March 1992. Pouches were sampled at the time of transplanting in March 1992 when the soil was still frozen and in April 1992 when soil temperature under sod at 10 cm depth was 6.3 °C. At each sampling, soil from one pouch of each pair was used to obtain the measurement of (1) extra-radical hyphal density using a membrane filtration method, (2) hyphal viability using the vital stain fluorescein diacetate, and (3) the VAM spore density. The remaining soil from the sampled pouch was disturbed and passed through a 2 mm sieve. Soil in the other pouch of each pair was left undisturbed. A growth room bioassay was conducted at each sampling time using disturbed and undisturbed soil with Sudan grass (Sorghum sudanens) as the bioassay plant. The length of viable hyphae decreased from 7.0 m per gram soil in November to 2.3 m per gram in March, but increased to 10.0 m per gram in April. The proportion of hyphal length that was viable decreased from 0.4 m per gram of soil in November to 0.2 m per gram of soil in March but increased to 0.7 m per gram in April. The results were similar in pouches sealed in polyethylene. Extensive fine branching of the viable hyphae was observed in the April samples. Colonization was observed in all bioassay plants, thus the extra-radical mycelium was an effective inoculum following the thawing of soil. Results of the experiment show that the extra-radical mycelium of at least some VAM fungi remains viable over winter and begins to grow in absence of either a living or dead host plant (Addy, Schaffer, Miller, et al. 1992).

In studies at the Department of Agricultural Biochemistry, Waite Agricultural Research Institute, Glen Osmond, South Australia, potting mix with or without inoculum of *G. intraradices* mixed throughout was placed in pots, moistened to field capacity, and then placed in controlled root temperature tanks at 22 °C, 30 °C, and 38 °C. Germinated seeds of mung bean (*Vigna radiata*) were planted into the potting mix at zero, three, and six weeks after the mix was moistened and then evaluated after two-week plant growth. In non-fallowed potting mix, infection after two weeks was greatest at 30 °C under all fallow treatments. When potting mix was followed for three or six weeks prior to the two-week plant growth, the level of infection at 38 °C approached the level of infection, which occurred in the 30 °C soil, but this infection did not give greater shoot dry weight accumulation than the non-inoculated treatments. In absence of the inoculum, shoot dry matter accumulation was greatest at 38 °C. At 22 °C, shoot dry matter accumulation and the level of infection were consistently low. This form of G. intraradices was able to survive and retain infectivity in fallow moist soil for up to six weeks at 38 °C although 30 °C appeared optimal for infection (Haugen and Smith 1990).

In studies at the Department of Biological Sciences, University of Albrta, Edmonton, Canada, extra-radical mycelia of two Glomus species were produced in fine mesh pouches, which excluded roots but not the hyphae. The mycelia in these pouches were exposed to freezing conditions either in the field or in a controlled temperature chamber. Bioassay plants were grown directly in the pouches and mycorrhizal colonization was assessed after one month. The mycelia remained infective in frozen soil over winter. This survival was not dependent on either the presence of the root pieces or on the connection of mycelia to roots. Spores were not an effective inoculum in these bioassays. Over the period, winter survival of mycelia would enable plants to become incorporated into functional mycorrhizal associations early in spring (Addy, Miller, and Peterson 1997).

In a similar experiment conducted at the Department of Horticulture, Pennsylvania State University, USA, fine nylon pouches containing extra-matrical mycelium of the VAM grown in soil were exposed to freezing, both in field and under controlled conditions. Following freezing, soil in half of the pouches was disturbed by sieving. In an additional experiment, pouches contained only isolated spores. The relative infectivity of the mycelia and spores following freezing was determined by the growing bioassay plants directly in pouches. Spores were not an effective inoculum in any of the bioassays, regardless of the freezing. Soil disturbance after freezing reduced the subsequent colonization of bioassay plants, indicating that the mycelia were responsible for mycorrhizal formation following freezing (Heather, Miller, and Peterson 1997).

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## **Research findings**

# Effect of vesicular-arbuscular mycorrhiza fungi and vermicompost on drought tolerance in papaya

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## Introduction

Papaya being one of the most economically important fruit crops of the tropical and subtropical areas, has a unique place among the cultivated horticultural crops in Karnataka because of its high production (87 tonnes/hectare) per unit area. Also, its cultivation is gaining importance at a faster rate in the arid and semi-arid areas of the state because of high returns. However, in these areas, cultivation is often affected by drought conditions. Therefore, the present investigation was carried out to know the effect of the VAM (vesicular-arbuscular mycorrhiza) and vermicompost on drought tolerance of papaya.

## Material and methods

An investigation was carried out at the Department of Pomology, Kittur Rani Channamma College of Horticulture at Arabhavi and University of Agricultural Sciences at Dharwad to know the effect of two VAM fungi – viz., *Glomus fasciculatum* and *Sclerocystis dussii* – and vermicompost on drought. VAM inoculation was done by placing the inoculum uniformly at the rate of five grams per bag, at fivecentimetres depth, and seeds were sown in the bags. Fifty-day-old, uniform, and healthy seedlings were transplanted in pots. Application of vermicompost at the rate of 650 grams per plant as per the treatment was done while planting. Observation on relative water content was estimated according to the standard method developed by Barrs and Weatherly (1962). Soil moisture was recorded by taking a known quantity of soil and keeping it in a hot-air oven at 60 °C to attain a constant weight and calculated in percentage. Days taken for leaf weathering and complete wilting of the plant were taken by counting the number of days after imposition of drought (Table 1).

## **Results and discussions**

Highest per cent relative water content was observed in S. dussii + vermicompost (67.87%) applied plants, which was statistically at par with G. fasciculatum + vermicompost (64.65%) and significantly least in control (Table 2). A significantly higher soil moisture was recorded in G. fasciculatum + vermicompost treated pots (9.62%), followed by S. dussii + vermicompost (8.15%) whereas soil in control pots recorded least per cent of water (3.78%). After 70 days of drought, the imposition of G. fasciculatum + vermicompost (0.42%) and S. dussii + vermicompost (0.42%) was found to

 Table 1
 Effect of vesicular-arbuscular mycorrhizal fungi and vermicompost on days taken for leaf abscission and complete wilting of plants after imposition of drought

Treatment	Days taken for leaf abscission	Days taken for complete wilting
T,	32.50	41.10
T <sub>2</sub>	37.50	47.00
T_	39.25	61.00
T,	41.50	62.03
T <sub>5</sub>	36.75	55.50
T <sub>e</sub>	39.23	60.20
S. Em ±	1.45	2.36
C.D. at 5%	4.33	7.01

 $T_1 = Control$ 

 $T_2 =$  Vermicompost at the rate of 650 g/plant

T<sub>3</sub> = Glomus fasciculatum

 $T_A = Glomus fasciculatum + vermicompost$ 

T<sub>5</sub> = Sclerocystis dussii

T<sub>6</sub> = Sclerocystis dussii + vermicompost

C.D. - critical difference

S. Em – standard error of mean

 
 Table 2
 Effect of vesicular-arbuscular mycorrhizal fungi and vermicompost on relative water content of leaf- and soil-moisture content

		Moisture content (%)			
Treatment	Relative water content (%)	Before drought	After drought		
T,	45.45	3.78	0.31		
T,	54.77	6.57	0.33		
Τ <sub></sub>	59.53	7.47	0.39		
T	64.65	9.62	0.42		
T_	60.97	5.75	0.38		
T	67.87	8.15	0.42		
S. Em ±	1.92	0.41	0.01		
C.D. at 5%	5.70	1.24	0.04		

 $T_1 = Control$ 

 $T_2$  = Vermicompost at the rate of 650 g/plant

T<sub>3</sub> = Glomus fasciculatum

T<sub>4</sub> = Glomus fasciculatum + vermicompost

T<sub>5</sub> = Sclerocystis dussii

 $T_6 = Sclerocystis dussii + vermicompost$ 

C.D. - critical difference

S. Em - standard error of mean

contain statistically at par per cent soil moisture with least soil moisture in control (0.31%). *G. fasciculatum* + vermicompost treated plants took maximum days for leaf abscission (41.50), which was at par with *S. dussii* + vermicompost (39.23), while the least number of days taken for leaf abscission in control plants was 32.50. However, plants treated with *G. fasciculatum* and grown in media containing vermicompost took maximum days for complete wilting, while days taken for wilting of plants treated with *G. fasciculatum*, *S. dussii* + vermicompost were statistically at par with *G. fasciculatum* + vermicompost. Significantly, the least number of days for complete wilting of plants were recorded in control plants.

The treatments G. fasciculatum + vermicompost and S. dussii + vermicompost further recorded significantly higher per cent relative water content and led to a retention of maximum per cent of soil moisture of 0.92 and 0.92, respectively.

The VAM symbiosis appears to affect drought, mostly through the mechanism of drought avoidance, often associated with improved 'P' nutrition. From the literature (Augè 2001 and Adivappar 2001), 80% of the mycorrhizal studies reporting plant growth during drought revealed the VAM plant to be larger than non-mycorrhizal plants.

The anatomical characteristics of mycorrhizal roots (Augè 2001) suggest several means by which the fungus could lower the resistance of leaf tissue to water transport. The VAM may act as a lowresistance pathway through the root cortex or it could increase the surface-area availability for water absorption through increased root mass and hyphal growth. Alternatively, fungus may increase the surface area by stimulating root growth, which shows an increased number of roots and root length influenced by G. fasciculatum + vermicompost and S. dussii + vermicompost. Thus, being the largest in size, plants inoculated with G. fasciculatum + vermicompost and S. dussii + vermicompost used most of the water, leading to a difference in control and treated plants in water-use efficiency, with advancing water scarcity in the VAM-treated plants.

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# Arbuscular mycorrhizal association of weeds found with different plantation crops and nursery plants

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## Introduction

Mycorrhizal association with plants is a universal occurrence. Plant taxa belonging to such widely separated phyletic groups, that is, angiosperms, gymnosperms, pteridophytes, and bryophytes, are potential hosts of the mycorrhizal fungi (Doddridge 1986; Shannon and Kendrick 1982). Recently, various surveys have been conducted for the AM (arbuscular mycorrhizal) plants in different regions of India (Senthilkumar, Venugadeswar, and Krishnamurthy 2000; Tholkappian, Sivasaravaanan, and Sundaram 2000; and Prasad, Manunath, and Reddy 2000). Occurrence of the AM infection and root colonization in several weed species was reported by Gupta and Ali (1996) and Manoharachary, Ramarao, and Sulochana (1988). Most experiments with mycorrhiza were conducted under controlled greenhouses or environmental growth chambers. However, there are very few reports on the AM-weed association in open-field environments (Bagyaraj and Varma 1995). In the present report, our observations on the distribution and colonization pattern of the AM fungi in different species of weeds found with the field and nursery crops, and open fallow lands are given.

### Materials and methods

The study was conducted at the botanical gardens of the Regional Plant Resource Centre, Bhubaneswar. Weeds occurring in flower beds of marigold, chrysanthemum, and rose; in plantations of mango and jamun; in the nursery of ornamental plants; and in a natural stand of forest species were studied for mycorrhizal infections.

Different species of weeds with the root system were carefully collected in polybags and brought to the laboratory for study. Plants were identified and voucher specimens were preserved in the herbarium of Regional Plant Resource Centre. All study material was thoroughly washed to clear soil and dirt adhering to the roots. The AM colonization in roots was determined according to the root clearing and staining methods of Phillips and Hayman (1970). Slides were prepared and microscopically examined for the AM associations with the root of weed species, and observations were made on the infection of intracellular fungal hyphae along with arbuscles and vesicles or spores related to the plant–AM symbiosis.

### Results

A total of 98 different species of weeds belonging to 32 families viz. Acanthaceae, Amaranthaceae, Asclepiadaceae, Asteraceae, Boraginaceae, Caesalpiniaceae, Capparidaceae, Chenopodiaceae, Commenlinaceae, Compositae, Convolvulaceae, Cucurbitaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Graminae, Malvaceae, Molluginaceae, Onagraceae, Oxalidaceae, Papilinonaceae, Passiflorae, Poaceae, Portulacaceae, Rubiaceae, Sapindaceae, Scrophulariaceae, Solanaceae, Sterculiaceae, Tiliaceae, Urticaceae, and Verbenaceae were studied, out of which 51 species showed incidence of arbuscular infection and colonization (Figures 1–8).

The percentage of colonization varied from 2% to 100% in Euphorbia hirta and Ichonocarpus frutescens, respectively. Highest colonization was recorded in I. frutescens (100%) growing in association with mango, followed by Ageratum conyzoides (90%) and Scoparia dulcis (90% and 50%) growing in the fallow and mixed-forest vegetation as well as in the rose beds. Cassia tora collected from the marigold bed showed 90% colonization and Croton sparsiflorus from a perennial plant had 80% colonization in the chrysanthemum bed (Table 1).

Most predominant weeds found in more than four experimental sites were Amaranthus spinosus, A. conyzoides, Heliotropium indicum, and Cleome rutidospermum. The three weed plants, A. spinosus, A. conyzoides, and S. dulcis, were commonly found in all fields. But a variation in their colonization percentage was evident. Similarly, A. conyzoides was

 Table 1
 Maximum colonization scores of host weed plants by sites

Experimental sites	Host plants	Colonization (%)
Fallow land Nursery of perennial	Scoparia dulcis Euphorbia hirta,	90
ornamentals	Passiflora foetida	2
Marigold (seasonal herbaceous)	Cassia tora	90
Chrysanthemum (perennial herbaceous)	Croton sparciflorus	80
Rose (woody perennial)	Scoparia dulcis	50
Dry deciduous forest	Scoparia dulcis	90
Jamun plantation crops	Vernonia cineria	50
Mango scion banks	lchonocarpus frutescens	100



# Figure 1 Mycorrhization of weed host plants growing in barren field

Figure 1 Weeds in baren field

Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Aegaratum conyzoides	Aega cony	40	 Erigeron astiroides	Erige asti	0	Panicum bravifolium	Pani brav	0
Amaranthus viridus	Amar viri	0	Eclipta prostata	Ecli pros	0	Passiflora foetida	Pass foet	20
Atylosia scarbioides	Atyl scar	10	Elusine indica	Elusindi	0	Phyllanthus freturnus	Phyl fret	0
Aristida setacea	Aris seta	0	Euphorbia prostata	Euph pros	30	Phyllanthus niruri	Phylniru	0
Blumea lacera	Blum lace	0	Ficus conosa	Ficu cono	0	Phyllathus symplex	Phylsymp	0
Blumea hara	Blum hara	0	Fimbristylis acuminata	Fimb acum	0	Physelis minima	Phys mini	0
Cassia tora	Cass tora	15	Gnaphellium indicum	Gnap indi	55	Rungia pectinata	Rungpect	30
Cleome rutidospermum	Cleo ruti	60	Hemidesmus indicus	Hemi indi	15	Scoparia dulcis	Scop dulc	75
Coldenia procumbens	Cold proc	10	Mucuna pruriens	Mucu prur	40	Sida acuta	Sida acut	0
Crotolaria pallida	Crot pall	15	Mimosa pudica	Mimo pudi	15	Solanum nigrum	Sola nigr	75
Cynedon dactylon	Cyne dact	15	Melochia chorcorifolia	Melo chor	30	Spermacoe articularis	Sper arti	20
Cyperus rotandus	Cype rota	0	Mollugo pentaphylla	Moll pent	0	Trema orientalis	Trem orie	15
Cyperus haspen	Cype hasp	20	Morinda tinctoria	Moritinc	0	Tridax procumbens	Trid proc	20
Cucumis trigonus	Cucu trigo	25	Panicum repens	Pani repe	0	Vernonia cineria	Vern cine	15
Desmodium gangeticum	Desm gang	0		·				



**Figure 2** Mycorrhization of weed host plants growing in horticulture nursery

#### Figure 2 Weeds in mixed horticulture

Species	Abbreviation	n Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Ageratum conyzoides	Agercony	0	Echinochloa colona	Echi colo	0	Oxalis corniculata	Oxal corn	0
Amaranthus viridus	Amarviri	0	Euphorbia hirta	Euph hirt	2	Passiflora foetida	Pass foet	2
Centella asiatica	Cent asia	20	Gnaphellium indicum	Gnap indi	0	Portulacca spp.	Port sp.	0
Coldenia procumbens	Cold proc	0	Lindenia crustacea	Lind crust	0	Scoparia dulcis	Scop dulc	0
Cynedon dactylon	Cyne dact	0	Ludwigia parrenis	Ludw parr	0	Spermacoe articularis	Sper arti	0
Dactvoctenium aegypticum	Dact aegy	0	Maius megoses	Maiu mego	0	Trema orientalis	Trem orie	0
Digitaria ciliaris	Digit cili	0	, 5	, ,				



Figure 3 Mycorrhization of weed host plants growing in marigold field

#### Figure 3 Weeds in marigold garden

Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Ageratum conyzoides	Agercony	20	Croton bonplandenianum	Crotbonp	70	Melilotus alba	Mali alba	30
Amaranthus spinosus	Amarspin	0	Digitaria cilliaris	Digi cill	40	Melochia chorcorifolia	Melo chor	0
Cassia occidentale	Cass occi	60	Euphorbia hirta	Euph hirt	0	Mulugo pentaphylla	Mulu pent	0
Cassia tora	Cass tora	90	Euphorbia pulcherrima	Euph pulc	50	Peniselum pedicellatum	Peni pedi	20
Chenopodium album	Chen albu	0	Gnaphellium indicum	Gnap indi	0	Physelis minima	Phys mini	0
Cleome rutidospermum	Cleo ruti	0	Heliotropium indicum	Heli indi	0	Portulaca oleracea	Portoler	0
Coldenia procumbens	Cold proc	15	Ipomoea pest-tigridis	lpom pest	50	Richardia scabra	Rich scab	50



#### Figure 4 Mycorrhization of weed host plants growing in chrysanthemum field

#### Figure 4 Weeds in chrysanthemum garden

Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Amaranthus spinosus	Amarspin	0	Phylanthus fraternus	Phyl frat	20	Eragnostsis aspera	Eragaspe	0
Cassia oxidentale	Cass oxid	20	Sesbenia sesban	Sesb sesb	20	Melolotus indica	Melo indi	0
Cleome rutidosperma	Cleo ruti	0	Echinocloa colona	Echi colo	0	Portulacca grandiflora	Port gran	0
Cleome viscosa	Cleo visc	30	Elusine indica	Elus ind	40	Petunia hybrida	Petu hybr	50
Cyperus cupressus	Cype cupr	0	Digitaria longifolia	Digi long	35	Scoparia dulcis	Scop dulc	30
Cyperus iria	Cype iria	0	Digitaria ciliaris	Digi cili	0	Melochia chorcorifolia	Melo chor	0
Croton sperciflorus	Crot sper	80		-				



#### Figure 5 Weeds in rose garden

Species	Abbreviation Colonization (%)		Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Aegaratum conyzoides	Aega cony	9	Euphorbia hirta	Euph hirt	0	Mollugo pentaphylla	Moll pent	0
Amaranthus spinosus	Amarspin	0	Gnephalium polycaulon	Gnep poly	1	Scoparia dulcis	Scop dulc	50
Digitaria ciliaris	Digi cili	1	Heliotropium indicum	Heli indi	0	Urena lobata	Uren loba	0
Elusine indica	Elus indi	3	Lagera alata	Lage alat	3	Vernonia cineria	Vern cine	1



## **Figure 6** Mycorrhization of weed host plants growing in mixed forest

#### Figure 6 Weeds in mixed forest plantation

Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Ageratum conyzoides	Ager cony	50	Dactyoctenium aegypticum	Dact aegy	0	Perotis sp.	Pero sp.	0
Amaranthus spinosus	Amar spin	0	Digitaria longifolia	Digi long	30	Phyllanthus niruri	Phyl niru	50
Brassica campestris	Brass camp	0	Emelia sonchifolia	Emel sonc	0	Phyllathus symplex	Phyl symp	0
Cardiospermum sp.	Card sp.	70	Euphorbia hirta	Euph hirt	40	Richardia scabra	Rich scab	70
Cassia occidentale	Cass occi	70	Gnaphellium sp.	Gnap sp.	0	Scoparia dulcis	Scop dulc	90
Celosia argentia	Celo arge	0	Heliotropium indicum	Heli indi	40	Sida cordata	Sida cord	0
Cleome rutidospermum	Cleo ruti	0	Hemidesmus indicus	Hemi indi	0	Spermacoe sp.	Spersp.	0
Commelia benghalensis	Comm beng	0	Lucas aspera	Luca aspe	0	Sporobolus fertilis	Spor fert	0
Crotolaria pallida	Crot pall	0	Oldenlandia corvmbosa	Olde corv	0	Triumfetta neglecta	Triu negl	0
Croton bonplandenianum	Crot bonp	80	5	,		5	0	



#### Figure 7 Weeds in Jamun plantation

Plant Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Ageratum conyzoides	Ager cony	30	Hemidesmus indicus	Hemi indi	20	Scoparia dulcis	Scop dulc	25
Amaranthus spinosus	Amarspin	10	Lageera alata	Lage alat	0	Triumfetta neglecta	Triu negl	0
Cyperus rotandus	Cype rota	0	Panicum bravifolium	Pani brav	0	Urena lobata	Uren loba	0
Dactyoctenium aegipticum	Dact aegi	0	Paniselum pedicellatum	Pani pedi	0	Vernonia cineria	Vern cine	50



#### Figure 8 Weeds in mango plantation

Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)	Species	Abbreviation	Colonization (%)
Ageratum conyzoides	Agercony	90	Eclipta prostrata	Ecli pros	0	Phyllanthus fratescens	Phyl frat	0
Amaranthus spinosus	Amarspin	0	Gnaphellium polycolon	Gnap poly	60	Physelis minima	Phys mini	0
Cleome rutidospermum	Cleo ruti	0	Heliotropium indicum	Heli indi	0	Ricardia scabra	Rica scab	10
Cleome viscosa	Cleo visc	0	Ichnocarpus frutescens	lchn frut	100	Scoparia dulcis	Scop dulc	20
Croton bonplandenianum	Crot bonp	0	Lantana camara	Lant cama	20	Sida acuta	Sida acut	35
Cvnedon dactvlon	Cvne dact	30	Panicum repans	Pani repa	15	Spilanthus acmella	Spil acme	45
Cyperus haspan	Cype hasp	0	Peniselum pedicellatum	Peni pedi	2	Vernonia cineria	Vern cine	0
Dactyoctanium aegypticum	Dact aegy	0						

#### Table 2 Most abundant host weed plants found in different vegetation sites

Host plants	Barren field	Horticultural nursery	Marigold field	Chrysanthemum field	Rose field	Mixed forest	Jamun field	Mango field
Ageratum conyzoides	+	+	+	-	+	+	+	+
Amaranthus spinosus	+	_	+	+	+	+	_	_
Cleome rutidospermum		+	+	+	-	+	_	+
Euphorbia hirta	_	+	+	-	+	+	_	_
Dactyoctenium aegiapticum	+	_	_	-	+	+	+	_
Digitaria cilliaris	+	+	+	+	-	-	_	_
Heliotropium indicum	+	_	_	-	+	+	_	+
Scorparia dulcis	+	+	+	+	+	+	+	_

found to be with a wide range of mycorrhization (10%-90%) in all the studied sites except in the horticultural field (Table 2).

The maximum colonized families were Asteraceae, Euphorbiaceae, and Rubiaceae. Weeds growing in horticultural nursery showed very poor colonization. Weeds of the rose field showed poor performance whereas weeds of marigold and chrysanthemum showed high mycorrhization. Similarly, weeds in the jamun field got poor colonization whereas weeds in the mango field received a promising colonization percentage.

#### Discussion

Prevalence of the AM fungi in different vegetation sites of the Regional Plant Resource Centre became evident from the result of this survey. An analysis of the weed plants growing wildly in fields has shown 51 species as mycorrhizal. None of the members of Tiliaceae, Portulacaceae, Oxalidaceae, Onagraceae, Molluginaceae, Chenopodiaceae, and Commelinacee had the AM infection. According to Jagpal and Mukherji (1988); Manoharchary, Ramarao, and Sulochana (1988); and Kehri, Chandra, and Maheshwari (1988), Commelina benghalensis was reported as mycorrhizal but in this study, it was found non-mycorrhizal. The occurrence of mycorrhization in Cyperus haspan is of special interest as it was reported as non-mycorrhizal earlier. If not weeded out wholly, the surviving plants might be able to transfer the AM infection from one plant to another and ultimately to the soil through roots to increase the AM infection of the other hosts. Successional appearance of weed plants may be useful for the perennation and multiplication of incidental AM fungi. The occurrence and mycorrhization of weeds like A. spinosus, S. dulcis, and A. conyzoides in most of the experimental sites confirms the unspecific infectivity of the AM fungi irrespective of the rhizosphere soil. It may be accepted that they have a positive role in the AM propagule perennation. However, there is a lack of reporting on exclusive colonizations between a mycorrhizal fungus and a host plant. Factors that determine host-symbiont affinities have not yet been thoroughly investigated and no doubt, are of

considerable importance, especially in the field environmental conditions.

## Acknowledgements

We thank Dr P C Panda, Senior Scientist at the Regional Plant Resource Centre, Bhubaneswar, and his associates for identification of weeds and other field assistance, and for preserving the weed specimens in the herbaria of Regional Plant Resource Centre.

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# Genotypical response of tobacco to inoculation with arbuscular mycorrhizal fungus, *Glomus aggregatum*

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The AM (arbuscular mycorrhizal) fungi form symbiotic associations with most economically important crop plants. These fungi can improve plant growth under low fertility conditions, confer tolerance to plants against some pathogens, improve the water balance of plants, and contribute to formation of soil structure (Jeffries 1987).

Phenotypic and genotypic variations in response to the AM colonization between the plant cultivars or lines/genotypes of a single species with respect to nutrient acquisition and growth have been reported (Krishna, Shetty, Dart, et al. 1985; Boyetchko and Tewari 1995; Rosewarne, Barker, and Smith 1997; Singh, Singh, and Johri 2002). Wani and Konde (1996) reported the existence of genotype-dependent variation in nutrient uptake, particularly P (phosphorus). The genetic make-up and physiological need for P can decide the extent of colonization as the mycorrhizae play an important role in the P uptake (Krishna, Shetty, Dart, et al. 1985). However, differences in the relative mycorrhizal dependency between crop species or even cultivars are related to inherent factors such as root structure, metabolism, and plant growth rates that could affect nutrient demand (Koide 1991). The genetic basis of variation among genotypes and among species for the plant mycorrhizal interaction is not well understood, and little information is available for tobacco (Nicotiana tabacum L.) on this aspect.

Tobacco is an important commercial narcotic cash crop in Tamil Nadu. The application of P to tobacco has a significant influence on its yield. Significant differences have been observed in the growth rates of cultivars of several host species due to mycorrhizal inoculation (Indi, Konde, Wani, *et al.* 1992; Patil, Konde, Wani, *et al.* 1992; Wani and Konde 1996). However, little information is available on the genotype-dependent variation in the AM fungal symbiosis in tobacco. The present study was undertaken to evaluate the response of ten genotypes of tobacco to inoculation with *Glomus aggregatum* in a greenhouse.

### Materials and methods

The soil for this experiment was sandy loam with a pH of 6.2; with organic carbon 0.62%; and 0.08%, 0.012%, and 0.15% available nitrogen, P, and potassium, respectively. Soil was autoclaved before potting, with no obvious alteration in its structure. The earthen pots (24 cm in diameter and 16 cm in height) were surface disinfected with copper sulphate solution (5%), and were filled with sterile soil at the rate of 5 kg/pot. The experiment was laid out in a completely randomized block design with three replications. There were two treatments for each variety viz., inoculation with *G. aggregatum* and non-inoculated control.

Ten genetically diverse tobacco genotypes were analysed (Table 1). The seedlings of tobacco varieties were raised separately on seed beds (30 x 15 x 10 cm) using sterile sand: soil (1:1) mix. There were 20 beds, 2 beds for each variety. The AM fungus *G. aggregatum*, used as inoculum, was maintained on onion (*Allium cepa* L.) grown in sterile soil for a period of 90 days. For each bed, *G. aggregatum* inoculum containing spores, infected root bits, and mycelial bits was added 
 Table 1
 Per cent root colonization, spore number, shoot and root phosphorus content due to Glomus aggregatum inoculation on tobacco genotypes

			Phosphorus uptake (mg/plant)			
	Developter	Number of energy (	Shoot		Root	
Type/cultivars	mycorrhiza root colonization	100 g rhizosphere soil	Non- mycorrhizal	mycorrhizal	Non- mycorrhizal	Mycorrhizal
Cigar						
Vellai Vazhai (VV2)	54.5	396	30.2	52.6	11.4	42.4
Karu Vazhai (KV1)	28.4	205	29.4	48.2	10.5	38.8
Country Cheroot						
Oosikappal (1737)						
(narrow leaf)	32.5	215	29.6	53.2	11.8	42.9
Ossikappal (OK1)						
(Broad leaf)	45.5	342	28.6	62.5	12.4	48.2
Chewing						
Monnai (164)	53.4	362	32.6	68.4	10.8	50.6
Vazhaikappal (l 115)	75.4	470	30.2	69.6	11.3	44.2
Vadamugam (VD1)	68.2	412	30.1	64.3	11.8	30.8
Vattakkappal (VTK 1)	48.5	360	30.1	68.0	12.1	32.4
Vedaranyam (VR2)	82.5	620	31.8	72.5	12.4	52.2
Periya Vedaranyam (PV7)	43.0	320	30.4	62.4	11.6	45.2
SEM ±	2.4	4.8	3.2	6.2	1.6	3.2
CD at 5% level	8.2	10.4	6.8	12.4	3.8	9.6

CD - critical difference; SEM - standard error of mean

at the rate of 250 g inoculum. One seed bed of each variety and 250 g of inoculum were layered 2-cm below soil. The seeds were sown in the nursery bed; they germinated in about 10 days. Twenty-five-day-old seedlings were transplanted to pots containing 5 kg of soil. One seedling was transplanted to each pot.

Plants were harvested after 90 days of transplanting. The plant height, number of leaves, leaf length and breadth, shoot and root dry weight, and mycorrhizal dependency were determined (Plenchette, Fortin, and Furlan 1983). The per cent root colonization of tobacco genotypes was determined by staining with 0.5% trypan blue in lactophenol (Phillips and Hayman 1970) and counting AM colonization percentage by root intersect method (Giovanetti and Mosse 1980). Rhizosphere soil samples were analysed for the AM fungal spore number by employing the wet sieving and decanting technique (Gerdemann and Nicolson 1963). Shoot and root P content were determined by vanadomolybdate yellow colour method (Jackson 1973). The data obtained were subject to analysis of variance by randomized complete block design.

#### **Results and discussion**

There was a considerable variability among the tobacco genotypes with respect to mycorrhizal colonization and spore number in the rhizosphere soil. Among the genotypes, VR2 (Vedaranyam) local recorded the maximum per cent root colonization and highest spore number than rest of the genotypes. Significant variations within different genotypes were also observed (Table 1). Root colonization by the mycorrhizal fungi varied considerably depending on the genotype, which might also be related to specific interactions between the mycorrhizal fungi and host species. Similar results have also been reported by Indi, Konde, Wani, *et al.* (1992) in genotypes of brinjal; Patil, Konde, Wani, *et al.* (1992) in chilli; Wani and Konde (1996) in garlic, and Singh, Singh, and Johri (2002) in maize lines.

Genotypical variation in the AM root colonization could be due to an interaction between host genotype and the AM strain preference. The number of infection sites available on the root might also be one of the factors leading to variations in the AM root colonization of different genotypes. Higher root colonization allows more host fungal contact and exchange of nutrients, and helps in better plant growth (Abbott and Robson 1982). Menge (1983) believed that rapid and high levels of colonization may be the prime determinant of efficiency of the symbiosis. Therefore, genotypes that prevent or allow lower levels of colonization are destined to derive meagre benefits from the AM symbiosis. From the present investigation it is observed that the genotypes viz., VR2 local and I 115, seem to be highly mycorrhizal compared to rest of the genotypes.

The results of plant height, dry weight, leaf length and breadth, and the number of leaves differed significantly between the genotypes (Table 2). Genotype VR2 local recorded significantly highest plant height, plant dry weight, and leaf yield than the

			Plant bic	omass (g/ p	lant)		Amin	رو د د	1 004101	0.04+b		4+000	
	Plant h	neight (cm)	Shoot		Root		leaves	er ur s/plant	toppe.	d) (cm)	topper u	d (cm)	
Type/cultivars	MN	Σ	MN	Þ	MN	Σ	MN	Σ	WN	Þ	MN	Þ	wycorrnizai dependency (%)
Cigar Vellai Vazhai (VV2)	50.2	58.2	510.6	580.5	130.5	136.4	20	52	75.2	82.0	32.8	34.2	111.8
Karu Vazhai (KV1)	36.5	40.3	380.2	424.2	127.5	132.5	19	20	65.4	70.2	35.2	36.4	109.7
<b>Country Cheroot</b> Oosikappal (1737)													
(narrow leaf)	48.0	52.0	430.2	520.5	120.6	130.4	19	20	66.2	72.4	12.4	13.2	118.2
(broad leaf)	49.2	59.5	485.3	610.4	128.4	133.6	19	21	62.4	65.2	25.8	26.6	121.2
Chewing													
Monnai (1 64)	32.0	39.8	410.4	440.5	127.5	135.8	20	20	65.1	69.4	45.2	46.2	107.1
Vazhaikappal (l 115)	48.6	58.0	435.6	576.8	132.5	133.8	21	24	62.2	74.2	36.0	39.2	125.1
Vadamugam (VD1)	35.0	44.0	385.2	465.2	128.8	135.4	19	20	68.0	72.4	35.2	38.4	116.8
va uahkappai (VTK 1)	45.2	51.0	415.2	512.4	130.6	131.4	20	22	64.2	69.2	44.2	45.2	118.0
Vedaranyam (VR2)	48.0	62.0	432.4	640.5	132.5	138.8	21	24	68.2	76.4	43.8	44.2	138.0
Periya Vedaranyam (PV7)	38.0	44.0	395.6	460.0	127.4	130.5	20	21	66.2	68.0	49.2	48.4	112.9
SEM ±	1.8	1.4	15.4	18.2	6.2	4.4	1.2	1.2	3.2	3.8	1.4	2.4	I
CD at 5% level	3.6	2.8	45.8	48.4	18.4	12.8	2.4	2.4	6.4	9.2	2.8	4.8	I

CD – critical difference; M – mycorrhizal; NM – non-mycorrhizal; SEM – standard error of mean Mycorrhizal dependency of the plants has been calculated by the authors with the following formula.

Biomass of M plants Biomass of NM plants × 1000

Table 2 Effect of Glomus aggregatum inoculation on plant growth response and mycorrhizal dependency of tobacco genotypes

rest of the genotypes. Genotypes KV1 and I 64 recorded the least plant growth parameters.

Observations on the plant growth parameters indicated a variation in tobacco genotypes due to inoculation with *G. aggregatum*. Similar results were recorded by Indi, Konde, Wani, *et al.* (1992) in chilli genotypes. An increase in the plant height and biomass (Table 2) could be attributed to an increase in the P uptake as influenced by mycorrhizal inoculation (Table 1).

From the results it was clear that the tobacco genotypes also displayed varying degrees of the AM dependency. The mycorrhizal dependency percentage was 107.1%–138.0% (Table 2). Maximum dependency was recorded in the genotype of VR2 followed by I 115 and OK1, while the least was by the genotypes KV1 and I 64. The inoculation of G. aggregatum recorded significantly higher uptake of P than the uninoculated control (Table 1). Among the genotypes, VR2 local was significantly superior overall than other genotypes, while KV1 had the least P uptake. Wani and Konde (1996) observed variations in enhanced P uptake in different genotypes of garlic as influenced by G. mosseae. An increase in the P uptake by onion cultivars due to the AM inoculation was observed by Powell, Clark, and Verberne (1982). A variation in the P uptake by the tobacco genotype could be recorded since the AM fungi are considered to play a major role in increasing the P uptake, and a varied per cent root colonization by G. aggregatum in tobacco genotypes was recorded. The genotype VR2 local of tobacco was most responsive to G. aggregatum, while it may be used in future plantbreeding programmes to conserve this trait for better plant growth in the P-deficient sandy loam soils.

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## New approaches

## Use of fatty acids for identification of arbuscular mycorrhizal fungi

FAME (fatty acid methyl ester) analysis performed by Madan R, Pankhurst C, Hawke B, Smith S. 2002 (Soil Biology Biochemistry 34[1]: 125–128) on the spores of four AM (arbuscular mycorrhizal) fungi (Glomus coronatum, G. mosseae, Gigaspora margarita, and Scutellospora calospora) showed 16:1 omega 5C to be the dominant fatty acid present. In addition, spores of G. margarita contained large quantities of 18:1 omega 9C and three 20-C fatty acids (20:1 omega 9C, 20:2 omega 6C, and 22:1 omega 9C) that were not present in spores of the other two species. Addition of a known number of spores of each AM species to the soil demonstrated that the spore fatty acids could be readily detected and quantified against the background of soil fatty acids. An addition of different combinations and quantities of spores to soil gave the expected ratios of the marker fatty acids in the soil FAME profiles. The results confirm the use of 16:1 omega 5C as a marker fatty acid for the AM fungi in controlled environments and suggest that 18:1 omega 9C, 20:1 omega 9C, 20:2 omega 6C, and 22:1 omega 9C could be used as possible markers for the detection of *G. margarita*.



## **Centre for Mycorrhizal Culture Collection**

# Comparative study of different phosphorus fertilizer doses on population and diversity of arbuscular mycorrhizal fungi

#### Reena Singh and Alok Adholeya

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Agricultural ecosystems subject to continuous high intensification invariably exhibit degradation trends over a period of time (although to different degrees depending upon the climatic zones). A more holistic, long-term approach to food production in sustainable agro ecosystems is therefore urgently needed. There are many beneficial soil microbes, which play an important role in the soil fertility, biological nitrogen fixation, biological control of disease, etc., which should be exploited for an enhanced crop productivity. One such microorganism is mycorrhizal fungi, which form a symbiotic association with roots of higher plants. The mycorrhizal fungi form an intricate network of extraradical mycelium, where they absorb mineral nutrients, in particular P (phosphorus), and transfer them to roots of host plants. It has been documented in a vast literature that mycorrhizal plants show an improved growth, health, and resistance to abiotic and biotic stresses as compared to nonmycorrhizal controls.

In most cases, beneficial effects of mycorrhizae are associated with an increased P uptake (Abbott and Robson 1984). Increased concentration of P in arbuscular mycorrhizal plants may be due to (1) increased physical exploration of soil, (2) increased movement of P into the AMF (arbuscular mycorrhizal fungus) hyphae, (3) modifications of root, (4) increased storage of absorbed P, (5) efficient transfer of P to plant roots, and (6) efficient utilization of P within the plant. Within the context of sustainable agriculture, interaction between the AMF and P fertilization is complex. While on one hand, high levels of soil P, although transient (Bolan 1991), are deleterious to the AMF (Abbott and Robson 1984), on the other, AMF decrease the need for fertilizers by contributing to the satisfaction of a crop plants' P demand at noninhibitive levels of P supply (Koide 1991). Thus, the potential for AMF utilization in the P nutrition is clearly present, but requires testing and evaluation of the supply and demand relationships of the symbiosis. The effect of phosphatic fertilizer dose on mycorrhiza was investigated in wheat-rice agricultural systems. Three fields (LL1 [low-input low yielding]; LL2 [lowinput high yielding]; and HH [high-input high yielding]) of the rice-wheat production system were selected from Budaun (mid-western plain zone of Uttar Pradesh, India). In the LL1 field, the phosphatic fertilizers had not been applied for the past 4–5 years and yield was very low. In LL2 field, there was no application of the phosphatic fertilizers and yet the field showed a yield comparable to the HH field where the recommended dosages of fertilizers were applied. Wheat was in cultivation for more than 20–25 years in all selected fields.

From each collection site, four replicates were taken from the rhizosphere of the host plants at a depth of 0–30 cm, each replicate a composite of five samples. Samples were air-dried in shade to a point where there was no free moisture and were filled in plastic bags, sealed, and stored at 4 °C in a cold room until processed. Analysis of the soil was done for its chemical parameters. A soil suspension of 1: 2.5 (soilto-water mixture) was made. The pH of the soil suspension was measured by digital pH meter and electrical conductivity was measured by digital electrical conductivity meter. A protocol by Datta, Khera, and Saini (1962) was followed for measuring the per cent organic carbon. The total N was calculated using Kjendahl's method of Bremner (1960). The available P was determined using Olsen's method of determination of the available P (Olsen, Cole, Watanabe, et al. 1954). Estimation of the available K was done with the help of flame photometer. The soil was analysed for its mycorrhizal parameters, which include species diversity, population density, infectivity potential, and intraradical colonization of the AMF. The AMF spores were isolated following the modified wet sieving and decanting technique (Gerdemann and Nicolson 1963). Intraradical colonization by the AMF was quantified following the clearing and staining





LL2 - low input, high yield; HH - high

input, high yield; LSD - least square

deviation

Gaur, Bhatia, et al. (1996). Results showed that the field with low input of chemical fertilizers harbours more mycorrhiza in the Budaun region (Figure 1). The per cent colonization appeared unaffected by any of the fertilizer doses. Species diversity of the AMF also showed no difference amongst different fertility doses. Five AM fungal species were observed in all the fields: Glomus albidum Walker & Rhodes, G. fasciculatum (Thaxter) Gerdemann & Trappe emend. Walker & Koide, G. intraradices Schenck & Smith, G. mosseae Nicolson & Gerdemann, and Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders.

technique of Phillips

and Hayman (1970)

potential by protocol

and the infectivity

given by Sharma,

This is in contrast to observations by earlier workers that phosphate fertilizers reduce diversity and colonization of host plant roots by the AMF. Contradictory findings of our study are not surprising, considering the complexity of mycorrhizal systems and the myriad undefined variables and interactions inherent in the field research. Some variables likely to be important in predicting mycorrhizal responses to fertilization include the original fertility of soil, organic matter content of fertilizer and soil, balance of nutrients within the fertilizer, and mycorrhizal dependency of crop species or cultivar. The difference in our results may be because of the fact that it is the P status of the plant rather than that of the soil, which regulates the development of AM.

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## **Recent references**

The latest additions to the network's database on mycorrhiza are published here for the members' information. The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge. This list consists of papers from the following journals.

- Applied and Environmental Microbiology
- Applied Soil Ecology
- Botanical Bulletin of Academia Sinica
- Current Science
- Ecology Letters
- European Journal of Histochemistry
- Fems Microbiology Ecology
- Functional Ecology
- Geomicrobiology Journal

- Journal of Plant Physiology
- Journal of Tropical Ecology
- Mycorrhiza
- Northwest Science
- OIKOS
- Plant and Soil
- Plant Ecology
- Symbiosis

Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos [*address of the corresponding author]
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## **Forthcoming events**

# Conferences, congresses, seminars, symposiums, and workshops

Lima, <b>Peru</b> 4–7 April 2005	Ninth Plant Virus Epidemiology Symposium International Potato Center, P O Box 1558, Lima 12, Peru
	Fax +51 (1) 317 5326• E-mail plant-virus-epidemiology-symp@cgiar.orgTel. +51 (1) 349 6017Website www.cipotato.org/training/PlantVirusEpidemSymp05/index.htm
Florida, <b>USA</b> 10–14 April 2005	Second Joint Conference of the International Working Groups on Legume (IWGLV) and Vegetable Viruses (IWGVV) Gail C Wisler, University of Florida/IFAS, Department of Plant Pathology, 1453 Fifield Hall, P O Box 110 680, Gainesville, Florida 32 611, USA
	Fax +1 (352) 392 6532• E-mail gcwisler@ifas.ufl.eduTel. +1 (352) 392 3631• Website http://conference.ifas.ufl.edu/vegleg/
Durban, KwaZulu-Natal, <b>South Africa</b> 17–21 April 2005	International Edible Legume Conference/Fourth World Cowpea Congress IELC Secretariat, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa
	Fax +27 12 420 4588• E-mail legume@up.ac.zaTel. + 27 12 420 2799• Website www.up.ac.za/conferences/ielc
Gent, <b>Belgium</b> 3 May 2005	Fifty-sixth International Symposium on Crop Protection K De Jonghe, Department of Crop Protection, University of Gent, Coupure Links 653, B-9000 Gent, Belgium
	<i>Fax</i> +32 (9) 264 6 238 • <i>E-mail</i> Kris.DeJonghe@rug.ac.be <i>Tel.</i> +32 (9) 264 6 022

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