Growth Characteristics of Tropical and Temperate Arbuscular Mycorrhizal Fungi in Onion (*Allium cepa* L.) Roots

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Abstract

Onion (*Allium cepa.* L) plants were inoculated with isolates of three tropical and one temperate arbuscular mycorrhizal fungi isolated from field soils, with the objective of selecting infective and rapidly spreading mycobionts for the production of fungal inoculum for field applications. Onion plants grown in quartz sand and calcined atapulgite (clay mineral) medium in a 5:1 ratio were inoculated with propagules of arbuscular mycorrhizal fungi under greenhouse conditions. Sequential harvests of the plants were done at 10-day intervals. Roots were cleared of cytoplasmic contents and stained with trypan blue in acidic glycerol to assess root colonisation. Fungal entry points into root epidermal cells and colonisation or spread of fungal hyphae, vesicles and arbuscules were estimated microscopically. The number of entry points per cm root length varied considerably among the different isolates over time. Mean entry points per cm of root length ranged from 0.29 to 2.69, with the *Scutellospora* sp. isolate having the highest value 31 days after inoculation. Percent root colonised, an indication of hyphal spread within the root cortex, ranged from 9.9% to 60%. At the final harvest, 71 days after inoculation, the highest colonisation was associated with the temperate *Glomus mosseae* isolate followed by the G*igaspora gigantea*, the tropical *Glomus mosseae* and the *Scutelospora* sp. isolate in that order. The aggressiveness of the fungal isolates during the early colonisation phase was not correlated with the internal spread of the fungi in the root cortex. The tropical isolates *Gigaspora gigantea, Glomus mosseae* and the temperate isolate, *Glomus mosseae,* were, therefore, desirable for selection compared to *Scutelospora* sp. although the latter fungus was more aggressive in the initial soil phase of the colonisation process.

Introduction

Arbuscular mycorrhizal fungi are obligate biotrophic eukaryotic organisms which form symbiotic associations with the roots of about 80% of terrestrial plants. The fungi, which have coenocytic to sparsely septate mycelia have been placed in the new division Glomeromycota (Schubler *et al*., 2001). The mutualistic association, known as mycorrhiza, confers numerous benefits to plants, such as increased inflow of phosphorus, disease resistance, improved water relations and enhanced growth (Sorensen *et al.*, 2005; Wright, 2005; Auge, 2004). Indigenous populations of arbuscular mycorrhizal (AM) fungi in field soils are seldom monospecific and may consist of several species belonging to different genera. In agricultural production systems, increased benefits from the mutualism between plants and AM fungi can be optimized by the manipulation of indigenous or introduced species through appropriate crop and soil management practices (Miller *et al*., 1995).

The successful introduction of mycorrhizal fungi depends on the effectiveness or their ability to benefit plant growth. In recent years, more efficient technologies have become available for inoculum production, and the lower cost of inoculum

and ease of production have made such technologies applicable in highly mechanized agricultural systems in developed countries as well as in some less developed agricultural systems (Jarstfer & Sylvia, 1993). The selection of fungi for application in field soils as inoculum is dependant on the infectivity or spread of the endophytes within roots as well as the efficiency of the symbiosis once it is established. In ecosystems with low soil fertility, fungi with high colonization capacities could, therefore, be desirable since extra and intra-radical fungal spread is important for the onset of nutrient uptake process and overall growth response (Martin *et al*., 1993).

The introduction of beneficial AM fungi in peasant agricultural systems of developing countries, where the benefits of mycorrhizas are yet to be realized, ought to be preceded by the selection of appropriate mycobionts with desirable characteristics. Presently in Ghana, very limited information is available on the diversity and ecology, and the potential benefits of indigenous arbuscular mycorrhizal fungi in soils of the various agro-ecologcal zones. To harness the benefits of the mutualistic association in crop plants, grown in low-input agricultural and horticultural production systems, would require the selection of suitable symbionts with the potential to infect and colonise crop plants characteristics. The study was, therefore, conducted to quantitatively determine differential patterns of infection and fungal spread within onion roots by three indigenous tropical isolates and one temperate isolate of arbuscular mycorrhizal fungi, with a view to selecting aggressive or rapidly spreading isolates for use in inoculum production and subsequent field application.

Materials and methods

Fungal isolates

Tropical isolates of *arbuscular-mycorrhizal* fungi used in previous studies (Asmah, 1995) were selected for ability to enhance nutrient uptake in tropical soils. The fungal isolates were *Glomus mosseae* (Isolate UCGLM 1), *Gigaspora gigantea* (UCGG 1), and *Scutelospora* sp. (UCS 1). Using standard protocol (Brundrett *et al*., 1994), single spore cultures were established in maize (*Zea mays*) roots and stored at a room temperature of 24 ^{o}C for 2 months before being used in the study. A temperate isolate of *Glomus mosseae* was also obtained from a mixed population of AM fungi in an arable field at Sonning, Reading, United Kingdom. Spore cultures of the isolate were established on maize plants grown in media consisting of sand and calcined atapulgite (Terra-Green^{Tm}). Mineral nutrition for the plants was provided by periodic watering with a modified Steiners Universal Nutrient Solution (Steiner, 1984). The temperate isolate was included after preliminary investigations had revealed the quantitative dominance of spores of the fungal species and extensive root colonization of plants growing at the site.

Growth medium

A mixture of sterilized quartz sand and calcined atapulgite in the ratio of 5:1, respectively, was used as the growth medium. Mineral nutrition was provided by a nutrient solution in which the P level had been adjusted to limit plant growth and to promote fungal growth (Snowball & Robson, 1984). Polypropylene pots were sterilized by immersion in 15% H_2O_2 solution for 3 min and rinsed with sterile distilled water. Growth medium (800 dm^3) were placed in the pots

and covered with three layers of globular polyethylene granules to prevent aerial contamination.

Treatments and growth conditions

Treatments consisted of four fungal isolates and seven harvesting times after inoculation. Onion seeds were surface-sterilized with 15% H_2O_2 and pregerminated on sterile filter paper in petri dishes. When radicles were 1 cm long, inoculum consisting of 50 spores of the AM fungi, pre-germinated on Millipore™ mixed-ester membrane filters, were placed with the onion root in a 2-cm depression in the growth medium. Treatments were replicated three times and pots were arranged in the glasshouse to conform to a randomised complete block design. The plants were irrigated with 50 dm^3 of nutrient solution at 3-day intervals.

Environmental conditions in the glass house were as follows: temperature- 25/15 ^{0}C (max/min), daily illumination for 12 h was provided by four 400 Watt discharge lamps and photosynthetic active radiation (PAR), measured with a quantum sensor, was 95 µM m^{-2} s^{-1}. Plants were harvested at 10-day intervals for seven times from the eleventh day after inoculation.

Estimation of AM fungal infection and colonization

At each harvest, fresh roots were separated from shoots and weighed. Roots were separated from growth medium by immersing the experimental pots in a 3-litre bucket filled with water and gently agitated to prevent loss of fine roots. All roots from each plant were heated in 10% potassium hydroxide (KOH**)** solution in a water bath at 85 ^{o}C for 1 h to clear the roots of cytoplasmic contents.

Cleared roots were stained using trypan blue in acidic glycerol (Koske & Gemma, 1989) and viewed with a compound microscope to determine the presence of mycorrhizal fungal structures (vesicles, arbuscules and hyphae). Root colonization was estimated on 2 cm long root pieces by the method of Biermann & Lindermann (1981), and entry points were counted directly on the root pieces laid out during estimation of percentage colonization. Data collected was transformed into angular form (arcsin) and analysed. Treatments were compared using Tukey's honestly significantly difference method (Sokal & Rohlf, 1995).

Results

The mean number of hyphal entry points per cm length of roots in the onion plants ranged from 0.29 to 2.7. The minimum significant difference for entry points using Tukey's honestly significant difference test ($P < 0.01$) for interactions between fungal isolate and days after inoculation was 1.42 entry points per cm root length. Entry points in roots increased from 11 days to 31 days after inoculation for all the four fungal isolates with no significant differences between the isolates to 21 days (Fig. 1). Entry points associated with *Scutelospora* sp. at 31 days was 2.69 and significantly greater than that for the tropical *G. mosseae* isolate and *G. gigantea* which recorded 0.94 and 0.59, respectively. Mean entry points associated with the temperate *G. mosseae* was 1.3 per cm root length which was not significantly different from the *Scutellospora* sp. isolate.

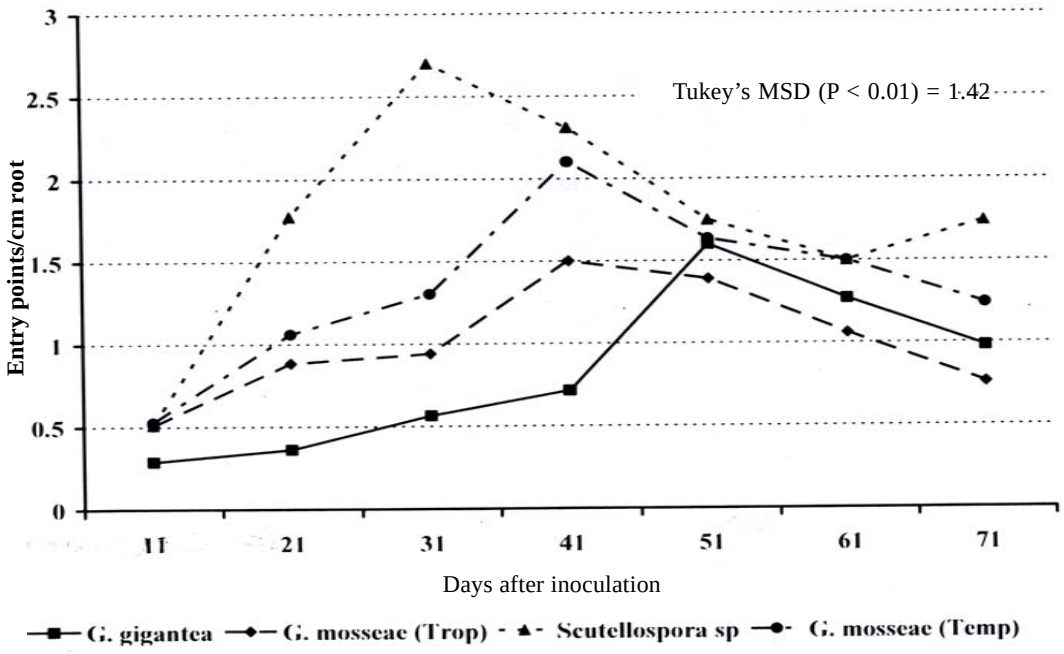

Fig. 1. Fungal entry points in onion roots after inoculation with AM fungi

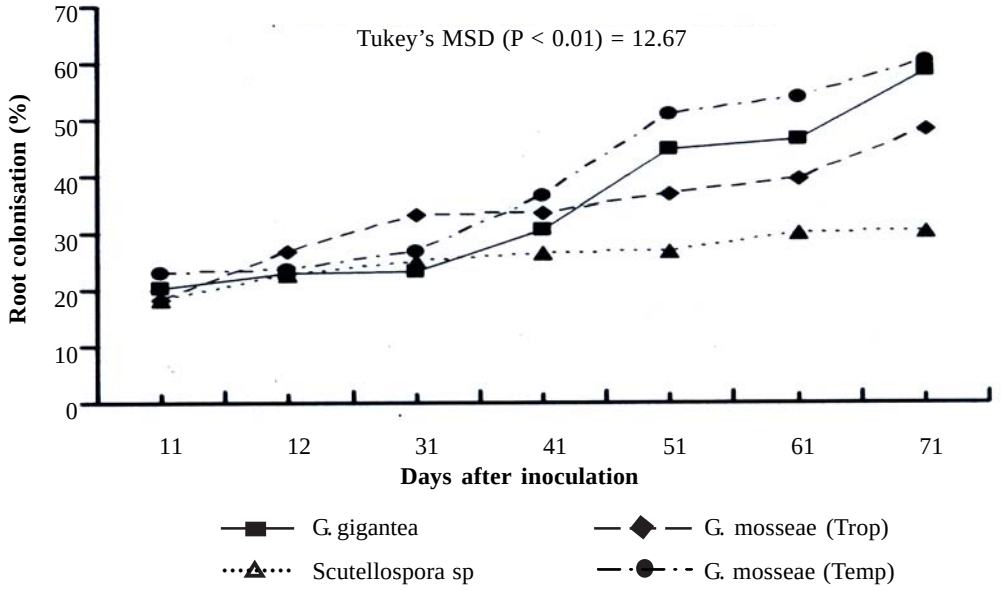

Fig. 2. Root colonisation by four AM fungi in onion

Forty-one days after inoculation, entry points associated with *Scutellospora* sp. was 2.31 and was significantly greater ($P < 0.01$) than that of *G. gigantea* which had mean entry point of 0.71 per cm root length. Differences between *Scutellospora* sp. and the two *G. mosseae* isolates with entry points 2.10 and 1.50, respectively, were not significantly ($P < 0.01$) different. Entry points for *G. gigantea* continued to increase sharply at 51 days after inoculation and then decreased finally whilst that for the two *G. mosseae* isolates decreased from 41 days to 71 days. Entry points for *Scutellospora* sp. decreased from 31 days to 61 days and then increased at 71 days. At 41 days and thereafter, no significant differences ($P < 0.01$) were found in entry points associated with all the fungal isolates.

Colonisation of roots by AM fungi ranged from 18.3% by the tropical *G. moseae* sp. isolate to 60.3% by the temperate *G. mosseae* isolate. The minimum significant difference for percent root colonisations using Tukey's honestly significant difference test ($P < 0.01$) for interactions between fungal isolate and days after inoculation was 12.67 per cent. Per centum root length of onion plants colonised by all fungal isolates increased from the 11 days to 71 days after inoculation. Up to 41 days a after inoculation, no significant differences were found in root length colonised by the four isolates.

Fifty one days after inoculation, colonisation by *G. gigantea* was 44.7% and was significantly ($P < 0.01$) greater than that for *Scutellospora* sp. which colonised 26.8% of roots. Colonisation by the temperate *G. mosseae* isolate was 50.9% and significantly greater ($P < 0.01$) than that for the *Scutellospora* sp. and tropical *G. mosseae* which colonised 26.8% and 36.7% of roots, respectively.

Root colonisation levels increase to 61 days after inoculation, colonisation in roots associated with *G. gigantea* was 46.4% which was significantly greater ($P < 0.01$) than that for *Scutellospora* sp. associated roots with 30.6% root length colonisation. Roots associated with the temperate *G. mosseae* at 61 days had 53.9% colonisation which was significantly greater ($P < 0.01$) than that for the roots colonised by the tropical *G. mosseae* and *Scutellospora* sp. isolates. Levels of colonisation for the isolates were 39.4% and 30.11%, respectively.

At the final harvest, 71 days after inoculation, root colonisation by *G. gigantea* and the two *G. mosseae* isolates were significantly ($P < 0.01$) greater than that for the *Scutellospora* sp. isolate. Levels of colonisation were 58.6%, 48.2%, 60.3% and 30.4% with respect to roots associated with *G. mosseae*, the two *G. mosseae* and *Scutellospora* sp.

Discussion

Under a given set of environmental conditions, processes involved in determining the overall infection of roots include: (i) the frequency of infection of roots by propagules in soil, and (ii) the rate of growth of fungus once inside the root cortex. Entry points in roots colonised by AM fungi are points along the root surface where fungi enter intact living root cells. They occur from single hypha and multiple branches produced by a single hyphae or multiple hyphae from different propagules. Single hypha from germinating spores have been observed to produce branches and then to, subsequently,

initiate infection through multiple entry points. The differences in entry points per unit length as observed in the investigation was a reflection of the relative aggressiveness of the different isolates. Entry points observed in this study could have been initiated either from single hyphae arising from single propagules or multiple branches of single hyphae.

The *Scutelospora* isolate was more aggressive as the rate of increase in entry point formation as well as the number of entry points per cm root length, observed 31 days after inoculation, was the highest compared to the other fungi. The reason for the decrease in entry points per unit length of root for each of the isolates after reaching a peak could not be easily determined but it is probable that the rate of initiation of new entry points from extra-matrical hyphae had decreased compared to extension of root length. Counts of entry points have been used by some investigators as a measure of completion of soil phase of colonisation (Smith & Smith, 1981a; Smith & Walker, 1981). In earlier studies, wide variations in entry points, ranging from 0.2 per millimetre to 26 points per millimetre of root length, have been reported by some workers (Jasper *et al.*, 1979; Cox & Sanders, 1974). The range found in this study was 0.6–2.7 which occurred 31 days after inoculation before reduction in number relative to root lengths were observed.

The spread of infection inside the root cortex as determined by percentage root length colonised showed that the aggressive initiation of infection with multiple entry points 31 days after inoculation, when the highest number was observed, did not correspond to colonisation inside roots. The highest colonisation was associated with the tropical *G. mosseae* isolate and not the *Scutelospora* isolate which had the highest number of entry points. The *G. gigantea* isolate was relatively less aggressive as both entry points and colonisation percentage were the lowest. The rapid changes that occurred in rates of spread in root cortex after 31 days might be attributed to the interactions between host plant and the mycobionts.

The colonisation of roots by the two tropical isolates *G. gigantea* and *G. mosseae* and the temperate *G. mosseae* isolate continued to increase with the rates following a sigmoid curve while that for the *Scutelospora* isolate had levelled off. Among the tropical isolates, *G. gigantea* and *G. mosseae* with higher colonisation percentages may, therefore, be more desirable for selection since it is recognised that the spread of infection is important for the onset of growth response in plants. Considering the low host-specificity/wide host range of AM fungi and their adaptability to diverse ecosystems, the temperate *G. mosseae* isolate, with the highest root colonisation relative to the other isolates, would also be desirable for selection to produce inoculum.

Since an important requirement of AM fungal inocula is that it must be infective in the agricultural system in which it is targeted (Jarstfer & Sylvia, 1993), the two tropical isolates, *G. gigantean* and *G. mosseae,* and the temperate *G. mosseae* isolate could be considered for the production of inoculum as mono-specific pot cultures. Subsequent usage of the inocula will depend on the effectiveness of the symbiosis in producing desired growth responses or any of the benefits that the mutualistic association is

known to confer on plants under field conditions.

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