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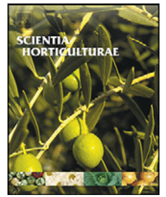

Arbuscular mycorrhizal fungi benefit mango (*Mangifera indica* L.) plant growth in the field

Sukhada Mohandas*

Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, India

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ABSTRACT

The rhizospheres of three year old-mango (*Mangifera indica* L.) rootstocks namely, Vellakulamban, Bappakai, Olour, Chandrakaran, Necker, Peach, Totapuri and Vellakulamban were studied for the spore load of arbuscular mycorrhizal (AM) fungi and root colonization at 15 cm, and 30 cm depths. Mycorrhizal spores were highest in Totapuri followed by Bappakai, Olour and Peach and Vellakulamban at 15 cm depth. Spores belonged to the genera *Glomus* and *Acaulospora* and few other genera, the predominant ones being *Glomus fasciculatum* and *Glomus mosseae* as identified by their morphology. The colonization of the root was higher in Vellakullamban and Totapuri rootstocks. Frequently occurring AM species were initially multiplied on finger millet (*Eleucine coracona* L.) in paper cups filled with soilrite and then in 12 in. pots that contained 1:1 sand soil mixture. The rootstock cultivars predominantly used for grafting mango scions in southern India were screened for their response to AM inoculation in pot culture. All the rootstock seedlings responded to mycorrhizal inoculations showed varied intensity of root colonization and improved plant height, growth and nutrient content compared to non-mycorrhizal in pot culture. Under field conditions, rootstock cv Totapuri inoculated with AM fungi and scions of mango hybrids Arka Aruna and Arka Puneeth grafted on them produced shoots earlier compared to non-mycorrhizal plants. Within two years of application of AM fungi yearly, clear difference in growth performance of mycorrhizal and nonmycorrhizal plants was observed. Plant growth studied in terms of number of branches, available soil P, leaf P, Zn and Cu improved significantly in AM colonized plants compared to uninoculated plants. This trend continued in the 8th year of sampling. The root acid and alkaline phosphatase activity was higher in six month old Arka Puneeth grafted on AM colonized Totapuri rootstock. Mycorrhizal inoculums can be easily multiplied on-farm on finger millet and applied yearly for desired results.

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1. Introduction

Mycorrhizal inoculations have been found to be beneficial in improvement of many perennial fruit crops when applied in nursery (Aguín et al., 2004; Azcón-Aguilar et al., 1992; Khade and Rodrigues, 2009; Nemec, 1983; Plenchette et al., 1983; Sukhada, 1998, 1992). Mango (*Mangifera indica* L.) the king of fruits (Bhudwar, 2002), is one of the important fruit crops of India. India is the largest producer and exporter of mangoes in the world. It occupies 22% of the total area under fruit crops comprising 2.3 million hectares forming 48% of the world output. India's production is 15 million tonnes as against world's production of 31.5 million tonnes (http://nhb.gov.in). There are a few reports of the response of mango crop to AM fungal inoculation. Xiutang (1990) tested different species of *Glomus* on *Mangifera indica* in pot culture and found that phosphorus and other mineral uptake improved due to the fungal colonization. Reddy and Bagyaraj, 1994 studied different vesicular arbuscular mycorrhizal (VAM) fungi for their symbiotic response with 'Nekkare' mango in unsterilised soil and found improvement in plant growth and shoot phosphorus content. Improvement in plant growth and nutrient content in shoots of rootstock Totapuri inoculated with *Glomus mosseae* (GM) and *Glomus fasciculatum* (GF) in pot culture was observed by Sukhada, 1998. Kamble et al. (2009) evaluated the effect of three arbuscular mycorrhizal fungi, *Glomus epigaeum*, GM and *Gigaspora calospora* and their combination on growth and root colonization of mango cv local in pot culture conditions seedlings. As there were no reports on the field response of mango crop to AM inoculations, studies were taken up to evaluate the effect of inoculation of AM fungi on establishment of rootstock and on the scions grafted on them under field conditions and study the beneficial effect on plant growth and yield.

* Tel.: +91 80 28466420; fax: +91 80 28466291.
E-mail address: sukhada.mohandas@gmail.com

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2. Materials and methods

2.1. Isolation, identification, multiplication of AM fungi and evaluation of root colonization

The rhizosphere soil from rootstocks of mango namely, Vellakulamban, Bappakai, Olour, Chandrakaran, Necker, Peach, Totapuri and Vellakulam at 15 cm, and 30 cm depths from the surface of three year old plants were screened for AM spore load. Wet sieving and decanting method of Gerdemann and Nicolson (1963) was followed to determine spore number. Two hundred and fifty grams of soil were mixed in a liter of water and allowed to settle for 2 h and sieved though series of filters of decreasing pore size 1 mm to 40 μm. The spores retained on the sieves were washed thoroughly and transferred to blotting paper placed on Petri dishes and microscopically examined. The spore load was determined per gram dry weight of the soil. The spores were identified based on spore morphology following the method of Schenck and Perez (1990). Predominant spores of AM fungi (number >50% of the total spores) in the rhizosphere were identified as *G. mosseae* (GM) or *G. fasciculatum* (GF) and were multiplied on finger millet (*Eleucine coracona* L.) initially in paper cups filled with soilrite and later in 12 in. pots filled with 1:1 sand soil mixture in polyhouse. Roots of 12 week old finger millet were studied for AM colonization and spore count (Phillips and Hayman, 1970). The initial inoculum thus multiplied was maintained in the polyhouse.

2.2. On farm incocula production

Large scale inocula production was carried out in 4 m × 4 m of land area in a corner of the field. The soil was dug up, loosened and leveled. Polythene sheet 0.15 mm thick was covered on the entire area. Moisture level of the soil was 50% of field capacity. Starter inocula of AM fungi, GM or GF was added in a row at 5 cm depth and seeds of finger millet (*Eleucine coracana* L.) were sown on them and covered. The land was irrigated regularly with a rose can. The seeds germinated within a fortnight and the roots got colonized by AM fungi. After 12 weeks the roots were checked for AM colonization. The finger millet shoots were cut at ground level and the roots were dug up to 15–20 cm below ground. The soil clumps were removed and roots were cut into small pieces and mixed in the soil. Spore count was estimated in the pooled soil. Inoculum thus produced was used in field inoculations.

2.3. Screening of rootstocks for response to AM inoculation in pot culture

Polybags, 15 cm × 20 cm, were filled with 400 g of sand, soil mixture (1:1) and 100 g of inoculum of AM either GM or GF. Uninoculated plants did not receive AM inoculums. One seedling each of popular rootstocks Bappakai, Vellakulamban and Totapuri, was planted in each polybag in ten replication. Colonization of AM was tested after 2, 4 and 6 months. Colonized plants were transferred to 12 in. pots filled with sand:soil mixture (steam sterilized by autoclaving at 120 °C and 15 pound pressure) and 250 g of AM inoculum, each gram containing 2–3 colonized root bit of finger millet and around 25–30 spores. The experiment was conducted in glass house at a minimum and max temperature of 14 and 32 °C and relative humidity of 65%. Shoot and root length, dry matter, leaf P, Zn and Cu content were studied in 10-month old plants. Phosphorus was analyzed in the tri-acid digested samples by the Vanadomolybdate phosphoric HCl procedure and assayed colorimetrically (Jackson, 1958) and Zn and Cu were analyzed by atomic absorption spectrophotometer.

2.4. Field testing

Seedlings of Totapuri rootstock were colonized with *G. mosseae* or *G. faciculatum* in polybags for three months as stated above and then were planted in pits 0.9 m × 0.9 m × 0.9 m containing 10 kg of FYM and 250 g of inoculums of either GM or GF. Plants not treated with AM fungi formed the controls. After one month the rootstock was grafted with scion of mango hybrid Arka Aruna or Arka Puneeth. The time taken for the graft to take place was recorded. Plants were fertilized with 170 g of urea, and 114 g of muriate of potash and of two doses of phosphorus, 112 g (half the level) or 224 g (full) per plant. Two uninoculated controls receiving same level of urea and potash and two doses of phosphorus, half the normal level and full level were maintained. Time taken for shoot appearance after grafting, per cent colonization of root, number of branches, available phosphorus content in the soil and leaf P, Cu and Zn and total yield were recorded at the end of 2nd year.

Plants were studied up to 8 years. After harvest each year, plants were pruned and inoculated with mycorrhizal fungi. Chemical fertilizers were applied after a fortnight, the doses increasing every year in multiples of first year dose. At the end of four years AM colonization, soil P, N, leaf P, Cu, Zn, yield and fruit TSS were recorded. At the end of 8th year soil P, leaf P, Cu, Zn and yield were recorded.

Root colonization by AM fungi in feeder roots was observed microscopically by treating them with 10% KOH, bleaching with H_2O_2 and then staining them with 1% trypan blue (Phillips and Hayman, 1970). Treating the root bits with hydrogen peroxide was necessary to see the colonization inside the roots. Twenty 1 cm pieces of the root were studied and per cent colonization of the root tissue by the fungus was calculated.

The plant growth in terms of number of branches produced and time taken for inflorescence to open were recorded at the end of 2nd year. Shoot samples were taken in 5 replication in each treatment and oven-dried at 70 °C for 72 h. The dry weight of the samples were recorded. Leaf samples along with the petiole were analyzed after 2nd, 4th and 8th year for P, Zn and Cu. Phosphorus was analyzed in the tri-acid digested samples by the Vanadomolybdate phosphoric HCl procedure and assayed colorimetrically (Jackson, 1958) and Zn and Cu were analyzed by atomic absorption spectrophotometer. The soil phosphorus and nitrogen were studied following Jackson (1958).

2.5. Acid and alkaline phosphatase content

Acid and alkaline phosphatase activity on the root surface and also in the enzyme extract of the roots of Arka Puneeth were studied. Five root tips, 1 cm in length, carefully taken from six month old Arka Puneeth grafted plants treated with 50% of phosphorus (P2) were incubated in tubes containing either a mixture of 0.5 ml of 0.25 M tris–HCL buffer (pH 9.8) for alkaline phosphatase, or 0.25 M acetate/Na^+ (buffer pH 6.0) for acid phosphatase activity, and 1.5 ml of 1 mg ml^{-1} P-nitrophenylphosphate (PNP) at 37 °C following Bartlett and Lewis (1973). The root tips were removed and 5 ml of 0.1 N NAOH was added to each tube and absorbance at 410 μm was measured. The experiment was repeated in the same way with enzyme extracted by macerating 1 g of roots in phosphate buffer at pH 6.6, and using the supernatant for assay of phosphatases instead of root pieces.

2.6. Statistical analysis

Statistical analysis was performed using two way analysis of variance with interaction between rootstocks at different depths with AM fungi (Gomez and Gomez, 1984). LSD (Least Significant Difference) was carried out to compare treatment means.

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