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Full Length Article

Response of soilless grown *Basella alba* L. inoculated With AM Fungi- A Strategy for Mass Multiplication

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi are obligate symbioants, generally multiply in association of other living host plants. Therefore, its multiplication status in roots is extremely varied and different with different plants. A low cost, low maintenance system for soilless production of AM fungal spores and inoculum was developed and adapted for production of *Basella alba*L. Plants. Preliminary experiments were undertaken on seven plants. An ideal soil less mixture could hold sufficient water for plant grown and simultaneously permit good aeration. Earthen pots had a porous texture with numerous air spaces into which the mycorrhiza lpropagules fit well. A greenhouse study was conducted under nursery conditions to study the responses of soilless grown *Basella alba* L. plant. The seedlings raised in presence of AM fungi (*Glomus faciculatum*) with vermiculite and perlite significantly showed an increased in dry weight of shoot and root and phosphours uptake in shoots over the control (non-inoculated plants).

Key Words: Arbuscular mycorrhizal (AM) fungi, vermicompost, perlite, seedlings and per cent root colonization, *Basella alba* L.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi occur in all soils, and commonly colonize roots of many plant species. These fungi can increase plant growth and reproduction by enhancing uptake of nutrients, especially those immobile in soil like phosphours (Lakshman, 2009). AM fungi, can also benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses and increasing resistance to pest. The primary effect of AM fungi on their host plant is an increase in plant growth and nutrient uptake (Bagyaraj, 2006) mycorrhizal inoculation reduces the quantity of fertilizer application, making it less than normally required for non inoculated plant conditions (Charron *et al.,* 2001). The AM fungi are absent in soilless substrates) Babu *et al*., 2001; Mosse, 1990). Uses of AM fungi on soil less grown pepper plants in open substrate systems have been previously reported. The results suggested that mycorrhiza could promote plant growth and increase fruit yield. Moreover, in recycling soil less systems, mycorrhizal response was not investigated. In hydroponic production all necessary nutrients are required in root medium with optimal ranges and beneficial forms by the plants. However, in closed systems, nutrient composition of the re-circulating solution is different with time from that of the solution initially supplied to the crop.

The aim of this study is to ascertain whether the presence of mycorrhizal fungus alleviates disadvantages of closed soil less systems. Considering the water, nutrients, and environmental concern with open system, an alternative approach of closed system with better root condition control via mycorrhiza has been discussed in the paper.

MATERIALS AND METHODS

One week old seedlings of Niger, cowpea, green gram, Red gram, black gram, Maize and Tomato were raised in (20 × 20 cm) pots containing 4 kg of soil i.e. sterilized garden loam and pure sand in 1:1 ration. The pots were inoculated with/mixed inoculum10g AM fungi of *Rhizophagus fasciculatus* by placing 4 cm below the surface in the experimental pot soils seeds were surface sterilized in (2% sodium hypochlorite) and they were grown. The used soil for the experiments was sandy loam with pH; 6.8 Organic Carbon; 0.84%, Nitrogen; 1.41 mg/kg, potassium; 2.41 mg/kg, phosphours; 0.18 mg/kg, Zinc; 2.02 mg/kg, Copper; 1.04 mg/kg, Magnesium; 1.42 mg/kg, E.C; 10.17 m.mho/cm. the soil was steam sterilized for one hour on two consecutive days. The physico-chemical properties of the soil were determined as per Jackson (1973). Percent of organic matter was determined according to Piper (1950). Electric conductivity was measured using Bridge meter and pH 1:1 (w/v) soil to water ration. All the seven seeds were procured from University of Agricultural Science Department of Horticulture, Dharwad. Germinated in small glass cups containing sterilized soil. Data on plant height, root length, shoot dry weight. Root weight, percent of root colorization and spore number was recorded at two intervals.

Green house experiments were conducted in earthen pots filled with vermiculation and perlite without soil.Five treatments were set in triplicate of *Basellaalba*L. Plants at nursery condition as follows.

1. Control (CN)
2. Mycorrhiza *(Rhizophagus fasciculatum)* inoculated
3. Mycorrhiza *(Rhizophagus fasciculatus)* inoculated + Vermiculite
4. Mycorrhiza *(Rhizophagus fasciculatus)* inoculated +Perlite
5. Mycorrhiza *(Rhizophagus fasciculatus)* inoculated + Vermiculite + Perlite

Experiment pots were kept free of weeds and pests. Stem cutting were irrigated every alternate day in order to maintain moisture. The plants were harvested after 30, 60 and 90 days interval after AMF inoculation. Observations such as dry weight of plants shoot and root was oven dried at 70^0C until a constant weight was obtained to determine the dry weight.

AM inoculum production

AM fungal spores were isolated from rhizosphere soil where *Allium sativum*is being cultivated in U.A.S Dharwad. AM fungal spores were isolated following the wet sieving and decanting method (Gerdmann and Nicolson, 1963). The spores were identified by using Schenck and Perez manual (1990). Important AM spores of *Glomus, Sclerocystis, Acaulospora, Scutellospora* and *Enterophospora* were recovered from 100g of rhizosphere soil samples. *Rhizophagus fasciculatus* was the most dominant in the soil. Therefore it was selected and used in this experiment. The selected spores were multiplied using Jowar (*Sorghum vulgare* L.) as host plant for three months in culture pots measuring 15cm height and 30 cm diameter. The pots were filled with autoclaved soil) (121^0C for one hour at 15 psi). After 60 days jowar was cut at ground level, the roots were chopped into 1 cm pieces and mixed with the soil from rhizosphere of host plants. This soil based inoculum was used for inoculation. Fifteen grams of air dried AM fungal inoculum was placed to each pot as thin layer at a depth of 2 cm below the soil surface. The inoculum consist 3 g root bits plus 12g rhizospheric soil of host plants with hyphae and sporocarps (105 clamydospores per 50g soil approximately). Hogland nutrient solution without phosphorus was given to the seedlings at the interval of 15 days.

Root Colonization

The per cent root colonization was evaluated microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described by Phillips and Hayman (1970). The following formula was used to calculate the root colonization according to Giovannetti and Mosse (1980).

$$\text{Root Colonization (\%)} = \frac{\text{Number of Colonized segments}}{\text{Total Number of segments examined}} \text{ X } 100$$

Mycorrhizosphere spores determination

Spores were separated from the soil by wet sieving and decanting technique (Gerdemann and Nicolson, 1963), where 50 g of soil were collected and mixed with water. The mixture was poured through different sieve size (250, 106, 45 um). After several time of sieve washing the supernatant was collected in petridish and spores were counted under binocular-microscope.The phosphorus content in the shoots was determined by the vanado – molybdate phosphoric acid yellow color method outlined by Jackson (1973).

RESULTS AND DISCUSSION

Preliminary experiments on seven selected plants viz : Niger, cowpea, green gram, red gram, black gram, maize and tomato showed the that there was a significant plant and root growth, with increased shoot and root weight in Niger, Cowpea, green gram, red gram, black gram, Maize and Tomato plants. There plants were inoculated with indigenous AM fungus *Rhizophagus fasciculatus*. All the seven plants have shown steadily increase from 30 to 60 days. However, the percent root colonization and spore number varied from 30 to 60 days (Table1). The percentage of AM Fungal colonization was increased in all the seven plants. The increased spore population was noted down among the plants of Maize, Niger, Cowpea, black gram and tomato from 30 to 60 days. Acontrast to this data the decreased spore population was recorded in green and red gram rhizosphere soils.

Significant increase in growth was observed in plant treated AM fungal inoculation along with soilless materials on *Basella alba* L. with the inoculation of (*Rhizophagus fasciculatus +* vermiculite *+ Perlite*) shown in (Table 2). After 60 to 90 days plants showed increased dry weight of shoot and root. These results were positive with the inoculation of *Rhizophagus fasciculatus* + Vermiculite *Rhizophagus fasciculatus* + perlite, *Rhizophagus fasciculatus* alone and control (non-inoculated) plants respectively.

The response in soilless growth *Basella alba* L. which received AM fungi (*Rhizophagus fasciculatus*) inoculums showed the Per cent mycorrhizal root colonization in experimental plants was low at 30 days. But there was a steadily increased growth after 60 days shown in (Table -2). However, it was observed that after 90 days of inoculation there was increased per cent root colonization and spore number *Basella alba* L. stem cuttings (Table 1). The hyphae, arbuscules and vesicles were predominant sign of infection in *Basella alba* L. similar results were documented by Cortas *et al*., 2004; Lakshman, 2009)

Basella alba L. plant treated with soilless material *Rhizophagus fasciculatus* + vermiculite + perlite performed significantly and showed increased dry weight of shoot and root and P-uptake values are found dominant over other treatments *Rhizophagus fasciculatus* + vermiculite, *Rhizophagus fasciculatus* + perlite, *Rhizophagus fasciculatus* alone or control. Plants inoculated with AM fungi (*Rhizophagus fasciculatus*) alone inoculated plants showed increased per cent root colonization and spore number/50g of soil over control. This is mainly due to AM fungal inoculation with vermiculite and perlite treatment. The present investigation is in consistent with early workers of (Babu *et al*., 2001; Cavender *et al.,* 2003 and Gutierrez miceili *et al.,* 2008.)

Mycorrhizal colonization increased when vermiculite were added (Gutierrez Micelie*tal.,* 2008). Few studies have examined the effect of vermiculite on AM fungi. Cavender *et al.,* (2003) have reported an increase in AM fungal colonization of roots of *Sorghum bicolor* after vermiculite application. Mycorrhizal root colonization was increased after 60 to 90 days at a rate of 72%, 81% 93% respectively. Mycorhizal colonization with AM fungal (*Rhizophagus fasciculatus*) inoculation showed significantly improved phosphorus in shots root length due to the enhancement of the total root surface by hyphal growth. Perlite lacks beneficial symbiotic association with AM fungi, the intermediate of roots lead to symbiotic association among crops in sustainable systems (Lakshman, 1997 and 2009). In contrast to this there was negative impact of perlite on AM fungi (Mauritz Vestbery *et al.,* 2008, Lakshman and Kadam, 2011). Perlite has been found to inhibit AM fungal colonization. In the present study it can be concluded that *Rhizophagus fasciculatus* with inoculation of soilless material in pots could be more suitable that it can promote the growth of *Basella alba* L. stem cuttings at nursery.

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Table :1 Mass multiplication of AM fungi in seven host preferencewith the inoculation of AM fungus *Rhizophagus fasciculatus*

	Shoot Length (cm)	Root Length (cm)	Short weight (g)	Root weight (g)	AMF colonization (%)	Spore count/ 50g/soil
Niger						
30 days	56.2	72.2	7.9	16.8	76.2	103.2
60 days	74.1	51.3	15.3	17.4	68.2	110.6
Cowpea						
30 days	37.9	13.5	6.8	0.8	62.3	107.0
60 days	86.2	11.7	9.6	1.4	71.5	119.2
Green gram						
30 days	38.6	10.1	2.3	0.6	58.7	97.1
60 days	35.9	24.5	3.2	3.8	51.4	61.4
Red gram						
30 days	38.2	11.6	1.4	0.6	48.2	81.0
60 days	41.5	3.8	3.3	1.2	56.4	54.1
Black gram						
30 days	31.1	12.6	3.2	0.4	89.6	178.2
60 days	12.2	15.3	6.5	5.1	47.4	99.1
Maize						
30 days	66.1	73.1	8.5	17.5	77.4	103.1
60 days	94.5	46.3	16.7	17.8	69.3	108.4
Tomato						
30 days	62.1	14.3	18.9	5.6	48.8	162.5
60 days	81.4	11.8	44.1	14.8	52.3	93.2

Table 2: Showing the effect soil inoculation with AM fungi (*Rhizophagus fasciculatus*) and soilless materials on dry weight of shoot (DWS) and root (DWR), per cent colonization (PC), spore number (SN) and phosphorus (P) uptake in shoot *Basella alba* L. plants. CN-Control; GF-*Rhizophagus fasciculatus.*

Tretaments (%)	Dry weight of shoot (g)	Dry weight of root (g)	Percent colonization (%)	Spore number	P-uptake
30 days intervals					
CN	2.1	1.6	0.0	0.0	005
GF	4.0	2.2	72	80	0.10
GF+Vermicompost	5.9	3.4	76	83	0.13
GF + Perlite	5.1	3.6	77	85	0.15
GF + Vermicompost + Perlite	7.6	4.5	80	78	0.16
60 days intervals					
CN	3.7	2.3	00	00	0.05
GF	5.2	3.1	81	80	0.11
GF+ Vermicompost	6.1	3.5	78	77	0.14
GF+ Perlite	6.2	3.8	81	78	0.15
GF + Vermicompost + Perlite	8.2	4.7	82	79	0.17
90 Days intervals					
CN	4.1	2.5	00	00	0.06
GF	5.6	3.4	93	81	0.11
GF + Vermicompost	6.5	3.6	81	78	0.14
GF + Perlite	6.6	3.8	80	79	0.16
GF + Vermicompost + Perlite	8.9	5.0	83	81	0.17

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