Influence of host plant and rice straw as substrate on mass multiplication of arbuscular mycorrhizal fungi for large-scale agricultural application

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Abstract
Purpose Rice straw is a waste product from the harvesting of Oryza sativa L. It is recurrently burned resulting in producing overheating, soil degradation, and air and water pollution.
Methods A pot experiment was designed under polyhouse conditions to evaluate the effects of rice straw as a substrate with Sorghum bicolor (L.) Moench, Hordeum vulgare L. and Triticum aestivum L. as different host on mass multiplication of Glomus mosseae.
Results AM fungi multiplication was greatly influenced by the presence, concentration (0, 25, 50 or 100 g/pot) and type of substrates. Soil amendment with compost rice straw proved more suitable for AM fungal multiplication over dry substrate. Sorghum bicolor acts as a suitable host and produced the most inoculum followed by Triticum aestivum.
Conclusion Assessment of the effects of substrates and hosts on the multiplication of pure culture of AM Fungi is an important prerequisite condition for large-scale agricultural application and exploration as biofertilizers.

Keywords Glomus mosseae · Oryza sativa · Compost · Hosts · Mass multiplication

Introduction
The basic objective of farming is to generate high quality food with higher productivity to fulfill the demand of increasing populations in the most sustainable way causing least possible damage to ecosystems. Sustainable development is the only solution to encompass soil and crop productivity, while maintaining ecosystems and biodiversity (Muthukumar and Udaian 2002a; Ijdo et al. 2010). One potential way to reduce the negative impact of chemical fertilizers is the use of plant growth promoting rhizobacteria (PGPR). Among microbial communities, endomycorrhizal fungi form a mutualistic association between roots of most plants and fungi of order Glomales involves a large number of events modifying the morphological as well as biochemical processes of the two symbionts (Kapoor et al. 2008). Establishment of a symbiotic association between AMF and host plants increases the nutrient bioavailability through mobilization of key nutrients to the crop plants by increasing the nutrient bioavailability through mobilization of key nutrients to the crop plants and reinstating the soil fertility, reducing the negative impact of chemical fertilizers on our environment (Rashid et al. 2016; Wood, 1991).

The broad application of AMF has been restrained by difficulties in obtaining large quantities of pure inoculum and the commercial exploitation is still in its infancy (Kadian et al. 2013). Quantitative and qualitative populations of AMF depend on different cultivation practices adopted for plant growth, environmental conditions, type of suitable substrate and host (Kadian et al. 2018). Selection of suitable substrate for mass production of AMF is important (Ijdo et al. 2010). The use of waste substrates along with traditional substrates (soil–sand mixture) is the classical, cheapest and most-preferred method for mass culture of AM fungi. The inoculum obtained after the process should be first screened in pots and later under field condition for various crops as it is very important that inoculum should be able to colonize plant roots effectively, multiply in the...
substrate and be able to enhance plant growth (Kapoor et al. 2008; Tanwar et al. 2013a, b).

Approximately, 80% of the world’s rice (Oryza sativa L.) is cultivated in developing countries. Rice straw is a waste obtained after the harvest and is mainly consisting of stems, roots and some spikes. Rice straw is rich in polysaccharides, lignin, silica content, nitrogen, cellulose and K, Ca, P, Fe, Mg, Na, and Mn (Sarnklong et al. 2010). Despite this, it is frequently burned producing soil degradation and air and water pollution (Guzmán et al. 2015).

A more practical approach to use of rice straw waste along with appropriate hosts is needed for higher yield and mass multiplication of AM fungi at low cost. Thus, the project was undertaken to select suitable form (dry and compost) and concentration (0, 25, 50 or 100 g/pot) of rice straw waste with appropriate hosts (S. bicolor, H. vulgare and T. aestivum) to choose the best host–substrate combination for maximized mass production of selected AM Fungi (G. mosseae) associated with agricultural crops.

Materials and methods

Soil characteristics

The present study was carried out in a polyhouse of Botany Department, Kurukshetra University, Haryana, India. The characteristics of the soil were: sand—64.2%, silt − 21.81%, clay—3.90%, starting EC—0.25 dS/m, pH 6.8, total N—0.042%, organic carbon—0.06%, available P—0.0018 kg/m2, S—14.80 ppm and K—0.022 kg/m2.

AM fungus isolation and production of starter inoculum

The AM fungi, Glomus mosseae, is an important dominant AM fungi associated with most of the agricultural crops (Kadian et al. 2013; Yadav et al. 2013a, b; Tanwar et al. 2013a, b). Starter inoculum of the selected AM spores was multiplied on maize (Zea mays L.) for 2 months using the funnel technique method of Menge and Timmer (1982). Rice straw used as substrate was dried (grounded to make a fine powder) or composted (placed in nylon net bags and buried for 3 months). Sorghum (S. bicolor), barley (H. vulgare) and wheat (T. aestivum) were selected as host plants and tested with substrate.

Experimental setup

The concentrations of substrate (0, 25, 50 or 100 g/pot) were mixed to earthen pots (25.4 x 25 cm) and thoroughly mixed with sterilized sand: soil in a ratio 1:3 (w:w) to make final volume of 2 kg. To each pot, 200 g of AM inoculum consisting of chopped AM-colonized root pieces of maize, along with soil containing about 350–420 AM spores 100/g, was added.

Ten healthy seeds of S. bicolor, H. vulgare and T. aestivum were sown in each pot above the inoculum. After 15 days of growth, these plants were thinned to five plants per pot. Plants were watered regularly on alternate days and 100 mL/pot of Hoagland’s solution (without KH₂PO₄) (Hoagland and Arnon 1950) was poured to each pot at 15-day intervals.

Harvest and analysis

Plant vegetative growth was observed after 90 days of plantation. Plants were manually uprooted to measure their height and root length. The plants were washed in running tap water. Roots and shoots were separated to note their fresh weight and then placed in an oven to dry at 70 °C until a constant dry weight was obtained. The percent of AM root colonization and AM spore counts was calculated using the method of Philips and Hayman (1970) and Gerdemann and Nicolson (1963).

Statistical analysis

The present experiment was a 4 × 2 × 3 factorial in a completely randomized design with each treatment replicated five times. The statistical analysis of data was subjected to analysis of variance (ANOVA) in SPSS (ver. 11.5, SPSS Inc., Chicago, IL, USA). Means were separated using Dunn’s multiple range test.

Results and discussion

The stability of plant growth is considered as a vital factor for endomycorrhizal fungal production. It is evident from the results that although all the host plants inoculated with G. mosseae produced fungal spores and colonized roots that were characterized by the presence of extramatrical hyphae, intraradical hyphae, arbuscules and vesicles yet substantial differences were obtained with different substrate types as well as various host plants.

When barley was used as a host and rice straw as substrate, results of the study depicted that host plant greatly influenced sporulation and root colonizing ability of G. mosseae. 50 g dry substrate resulted in the greatest plant growth in terms of plant height, while the plant biomass, root length and maximum spore number and root colonization were found to be maximum at 100 g (Tables 1 and 2). Barley acts as a suitable host for increasing AM inoculum density due to its short life span, better root architecture, colonization level and tolerance to low levels of soil phosphorus.
(Ijdo et al. 2010). But the application of compost substrate at 100 g resulted in maximum plant height, spore number and root colonization (Tables 1 and 2). Labidi et al. (2007) noticed an increased the AM fungal population using *Acacia tortilis* leaves compost to eroded soil. Muthukumar and Udaiyan (2002b) also reported an enhancement in the AM spore population when they used compost as a substrate.

Likewise, wheat plant inoculated with AM fungi using different concentrations (0, 25, 50 or 100 g/pot) of substrates showed stimulatory effect on plant growth, biomass and mycorrhization. Application of 100 g dry substrate significantly resulted in maximum plant height, while maximum AM spore number and root colonization were observed with 50 g dry substrate (Tables 3, 4). Generally, with an increase in the growth period after infection, root colonization of host also increases. However, this increase in colonization and period does not have greater bearing on AMF spore production. Again in wheat, compost substrate, irrespective of the

### Table 1 Efficacy of various forms of rice straw substrate and their different concentrations on plant growth parameters of *H. vulgare* for inoculum production of *G. mosseae*

| Type of rice straw substrate | Conc. of each substrate (g/pot) | Plant height (cm) | Fresh shoot weight (g) | Dry shoot weight (gm) | Root length (cm) | Fresh root weight (g) | Dry root weight (g) |
|-----------------------------|---------------------------------|-------------------|------------------------|-----------------------|------------------|----------------------|---------------------|
| Dry                         | 0                               | 19.6f             | 2.728e                 | 0.62h                 | 11.8 h           | 2.36f                | 0.93g               |
|                            | 25                              | 24.0e             | 2.946d                 | 0.93g                 | 14.0f            | 2.68d                | 0.97f               |
|                            | 50                              | 30.0c             | 3.038 h                | 1.25f                 | 17.2e            | 2.73c                | 0.98e               |
|                            | 100                             | 25.6e             | 3.244 g                | 1.35e                 | 22.4b            | 2.94b                | 1.02de              |
| Compost                    | 0                               | 29.0d             | 3.268f                 | 1.62d                 | 12.4 g           | 2.49e                | 1.03d               |
|                            | 25                              | 32.4c             | 3.384c                 | 1.78c                 | 19.6d            | 2.74c                | 1.21c               |
|                            | 50                              | 35.4b             | 3.430b                 | 1.85b                 | 21.2c            | 2.94b                | 1.26b               |
|                            | 100                             | 38.2a             | 3.930a                 | 1.95a                 | 25.4a            | 3.02a                | 1.41a               |
| LSD (P ≤ 0.05)             |                                 | 1.6905            | 0.4045                 | 1.0261                | 1.4012           | 0.2101               | 0.2104              |
| ANOVA F(7,15)              |                                 |                   |                        |                       |                  |                      |                     |
| *S* type                   |                                 | 67.238            | 5.237                  | 23.765                | 3.366            | 5.559                | 21.650              |
| *S* conc.                  |                                 | 34.221            | 72.634                 | 50.872                | 168.006          | 181.194              | 120.144             |
| *S* type × *S* conc.       |                                 | 54.309            | 123.263                | 33.548                | 238.428          | 32.761               | 33.767              |

*S* substrate

*Values in a column followed by same letter are not significantly different, least significant different test, P ≤ 0.05

### Table 2 Effect of rice straw substrate (Dry and Compost) on colonization and spore population of *G. mosseae* in *H. vulgare*

| Type of rice straw substrate | Conc. of each substrate (g pot⁻¹) | Pattern of colonization | AM spore number/10 g of soil | % Root colonization |
|-----------------------------|---------------------------------|-------------------------|-----------------------------|---------------------|
| Dry                         | 0                               | + – +                   | 41.6h                       | 30.4g               |
|                            | 25                              | + ++ ++                 | 191.0f                      | 71.0e               |
|                            | 50                              | + +++ +++               | 200.0e                      | 91.8c               |
|                            | 100                             | + +++ +++               | 221.0c                      | 93.4b               |
| Compost                    | 0                               | + ++ +                  | 70.60g                      | 51.6f               |
|                            | 25                              | + ++ +                  | 211.0d                      | 83.0d               |
|                            | 50                              | + ++ +                  | 258.4b                      | 95.0ab              |
|                            | 100                             | + +++ +++               | 291.0a                      | 96.8a               |
| LSD (P ≤ 0.05)             |                                 |                         | 21.0561                     | 9.5192              |
| ANOVA F(7,15)              |                                 |                         | 180.012                     | 77.680              |
| *S* type                   |                                 |                         | 38.468                      | 45.107              |
| *S* conc.                  |                                 |                         | 16.772                      | 12.762              |

Each value is a mean of five replicates, *S* substrate, AM arbuscular mycorrhizae, - absent, + scantly, ++ moderate, +++ abundant

*Indicates the level of significance at (P ≤ 0.05) level

Means followed by same letter/s within a column are not significantly different over one another (least significant different test, P ≤ 0.05)
concentration, was always proved to be stimulatory over the unamended control. The maximum spore number and root colonization were recorded in the rhizosphere of the wheat plants supplemented with 100 g compost substrate. The variation in the capacity of host plant for mass multiplication of mycorrhizal fungi might be due to the specific variation in host plant root type, its anatomy and morphology, nutrient and endogenous hormone level characteristics and the environmental interaction (Liu and Wang, 2003).

In Sorghum, 50 g of rice straw substrate, irrespective of the concentration, resulted in maximum plant height, maximum spore number and root colonization (Tables 5 and 6). The massive and dense root system of Sorghum showed good results than other two host plants. Likewise,

| Table 3 | Efficacy of various forms of rice straw substrate and their different concentrations on plant growth parameters of T. aestivum for inoculum production of G. mosseae |
|---------|---------------------------------------------------------------------------------|
| Type of rice straw substrate | Conc. of each substrate (g/pot) | Plant height (cm) | Fresh shoot weight (gm) | Dry shoot weight (gm) | Root length (cm) | Fresh root weight (gm) | Dry root weight (gm) |
| Dry     | 0                                   | 27.4g | 3.604f | 1.00g | 09.20h | 1.47h | 0.484h        |
|         | 25                                  | 32.4e | 3.948d | 1.20d | 19.22c | 3.94d | 2.184b        |
|         | 50                                  | 40.8c | 8.908a | 3.26b | 13.30e | 3.83e | 1.250e        |
|         | 100                                 | 52.2a | 8.438b | 3.40a | 10.36g | 2.00f | 0.684f        |
| Compost | 0                                   | 28.0g | 3.826e | 1.02f | 12.11f | 1.64g | 0.666g        |
|         | 25                                  | 34.2d | 4.508c | 1.05e | 15.34d | 4.00c | 2.136d        |
|         | 50                                  | 45.2b | 3.282g | 1.23c | 22.27b | 4.04b | 2.154c        |
|         | 100                                 | 31.8f | 3.146h | 1.21d | 28.42a | 4.81a | 2.942a        |
| LSD (P ≤ 0.05) |                                   | 6.1225 | 0.3494 | 1.1593 | 1.4131 | 0.5946 | 0.4677 |
| ANOVA F(7,15) |                                    | 45.556 | 127.228 | 166.780 | 44.876 | 81.690 | 50.280 |
| S type  |                                    | 182.580 | 218.726 | 213.342 | 162.884 | 673.94 | 553.28 |
| S conc. |                                    | 24.776 | 55.541 | 77.245 | 31.601 | 121.55 | 12.566 |

Each value is a mean of five replicates, ± : standard deviation, S substrate,

*Indicates the level of significance at (P ≤ 0.05) level

Means followed by same letter/s within a column are not significantly different over one another (least significant different test (P ≤ 0.05))

| Table 4 | Effect of rice straw substrate (Dry and Compost) on colonization and spore population of G. mosseae in T. aestivum |
|---------|---------------------------------------------------------------------------------|
| Type of rice straw substrate | Conc. of each substrate (g pot⁻¹) | Pattern of Colonization | AM spore number | % Root colonization |
|         |                                    | Mycelium | Vesicles | Arbuscules |                                    |                      |
| Dry     | 0                                   | +        | –        | +         | 60.4h | 32.8f        |
|         | 25                                  | ++       | ++       | ++        | 95.8e | 55.0d        |
|         | 50                                  | +        | +        | +         | 202.4c | 100.0a       |
|         | 100                                 | +        | –        | +         | 82.2f | 50.0e        |
| Compost | 0                                   | +        | +        | +         | 077.6 g | 26.00g       |
|         | 25                                  | +        | +        | +         | 235.2b | 71.8c        |
|         | 50                                  | +        | +        | ++        | 109.0d | 86.8b        |
|         | 100                                 | +        | +++      | ++        | 302.8a | 100.0a       |
| LSD (P ≤ 0.05) |                                   | 15.7951 | 7.3281 |                |                      |
| ANOVA F(7,15) |                                    | 12.453 | 22.45 |                |                      |
| S type  |                                    | 253.182 | 93.525 |                |                      |
| S conc. |                                    | 13.801 | 17.228 |                |                      |
| S type × S conc. |                                | 15.7951 | 7.3281 |                |                      |

Each value is a mean of five replicates, ± : standard deviation, S substrate, AM arbuscular mycorrhizae, - absent, + scanty, ++ moderate, +++ abundant

*Indicates the level of significance at (P ≤ 0.05) level

Means followed by same letter/s within a column are not significantly different over one another (least significant different test (P ≤ 0.05))
Kadian et al. (2018) reported significant increment in the root colonization and AM spore number while using sorghum as host and chickpea husk as substrate. Such difference may be due to the environmental conditions and other biotic factors. Moreover, the suitability of these species could be due to the production of a wide variety of water soluble and volatile organic compounds that may act as stimulant, attractants, nutritional sources, and even as genetic regulatory signals for AM fungi for better colonization (Douds et al. 2010). Chaiyasen et al. (2017) have also reported that AMF root colonization is directly proportional to root growth of host plant. As this waste enhances the nutrient uptake, thereby improving the root system, which favorably enhance the spore population in its surroundings and promotes plant growth (Lin et al. 2012).

Table 5 Efficacy of various forms of rice straw substrate and their different concentrations on plant growth parameters of S. bicolor for inoculum production of G. mosseae

| Type of rice straw substrate | Conc. of each substrate (g pot⁻¹) | Plant height (cm) | Fresh shoot weight (gm) | Dry shoot weight (gm) | Root length (cm) | Fresh root weight (gm) | Dry root weight (gm) |
|-----------------------------|-----------------------------------|-------------------|-------------------------|----------------------|-------------------|-----------------------|---------------------|
| Dry                         | 0                                 | 71.0d             | 11.68f                  | 3.90g                | 0.993h            | 2.04f                 | 0.57h               |
|                             | 25                                | 81.0c             | 16.00d                  | 4.03e                | 19.25e            | 4.93b                 | 2.26b               |
|                             | 50                                | 86.4b             | 13.42e                  | 3.96f                | 14.52f            | 4.28c                 | 1.35e               |
|                             | 100                               | 97.0a             | 09.26g                  | 2.54h                | 11.74g            | 1.42g                 | 0.63g               |
| Compost                     | 0                                 | 55.2g             | 16.10d                  | 4.24d                | 20.24d            | 1.73h                 | 0.70f               |
|                             | 25                                | 60.5f             | 22.34c                  | 8.27c                | 22.40c            | 4.21e                 | 2.07d               |
|                             | 50                                | 68.4e             | 23.80b                  | 8.96b                | 25.26b            | 4.26d                 | 2.12c               |
|                             | 100                               | 47.0h             | 26.04a                  | 9.81a                | 28.16a            | 4.99a                 | 3.03a               |

LSD ($P \leq 0.05$)

ANOVA $F_{(7,15)}$

| S type | 97.969 | 23.787 | 66.043 | 30.752 | 332.756 | 136.289 |
|--------|--------|--------|--------|--------|---------|---------|
| S conc. | 887.54 | 459.55 | 327.21 | 87.324 | 543.034 | 54.105 |
| S type × S conc. | 23.776 | 12.654 | 4.537 | 16.443 | 23.650 | 11.543 |

Each value is a mean of five replicates, ±: standard deviation, S substrate, AM Arbuscular mycorrhizae, - absent, + scanty, ++ moderate, +++ abundant

*Indicates the level of significance at ($P \leq 0.05$) level

Means followed by same letter/s within a column are not significantly different over one another (least significant different test ($P \leq 0.05$))

Table 6 Effect of rice straw substrate (Dry and Compost) on colonization and spore population of G. mosseae in S. bicolor

| Type of rice straw substrate | Conc. of each substrate (g pot⁻¹) | Pattern of Colonization Mycelium Vesicles Arbuscules | AM spore number/10 g of soil | % Root colonization |
|-----------------------------|-----------------------------------|---------------------------------------------------|-----------------------------|--------------------|
| Dry                         | 0                                 | +                                                 | +                           | 051.0h             | 47.2h              |
|                             | 25                                | +                                                 | ++                          | 134.8d             | 62.4e              |
|                             | 50                                | +                                                 | ++                          | 300.8b             | 97.0b              |
|                             | 100                               | +                                                 | –                           | 68.40g             | 52.4 g             |
| Compost                     | 0                                 | +                                                 | +                           | 77.8f              | 60.4f              |
|                             | 25                                | +                                                 | +++                         | 212.4c             | 86.8c              |
|                             | 50                                | +                                                 | ++                          | 403.4a             | 101.4a             |
|                             | 100                               | +                                                 | ++                          | 116.4e             | 68.6d              |

LSD ($P \leq 0.05$)

ANOVA $F_{(7,15)}$

| S type | 9.408 | 6.651 |
|--------|-------|-------|
| S conc. | 50.460 | 76.485 |
| S type × S conc. | 6.454 | 23.763 |

Each value is a mean of five replicates, ±: standard deviation, S substrate, AM Arbuscular mycorrhizae, - absent, + scanty, ++ moderate, +++ abundant

*Indicates the level of significance at ($P \leq 0.05$) level

Means followed by same letter/s within a column are not significantly different over one another (Least significant different test ($P \leq 0.05$))
Conclusion

In summary, soil amendment with compost rice straw proved more suitable for AM fungal multiplication over dry substrate. *S. bicolor* acts as a most suitable host and produced the most inoculum followed by *T. aestivum*. Assessment of the effects of substrates and hosts on AM fungi production might be useful in developing a package of practices for agricultural crops, which can be explicitly used for the maintenance of sustainable plant productive systems. However, in the present investigation, all the three hosts screened are very common and easily available. Any of the host can be exploited by farmers with rice straw (in any form) for the mass production of *G. mosseae*, encouraging them to take full benefits of this classical, attractive and alternative cost-effective method.

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