Intercropping Transplanted Pigeon Pea With Finger Millet: Arbuscular Mycorrhizal Fungi and Plant Growth Promoting Rhizobacteria Boost Yield While Reducing Fertilizer Input

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Pigeon pea (Cajanus cajan) and finger millet (Eleusine coracana) are staple food crops for millions of the rural population in Asia and Africa. We tested, in field trials over three consecutive seasons at two sites in India, an intercropping and biofertilization scheme to boost their yields under low-input conditions. Pigeon pea seedlings were raised during the dry season and transplanted row-wise into fields of finger millet, and arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) were added alone or in combination to both pigeon pea and finger millet. Our major findings are (i) effects of the biofertilizers were particularly pronounced at the site of low fertility; (ii) dual inoculation of AMF + PGPR to finger millet and pigeon pea crops showed increased grain yields more effectively than single inoculation; (iii) the combined grain yields of finger millet and pigeon pea in intercropping increased up to +128% due to the biofertilizer application; (iv) compared to direct sowing, the transplanting system of pigeon pea increased their average grain yield up to 267% across site, and the yield gains due to biofertilization and the transplanting system were additive. These technologies thus offer a tool box for sustainable yield improvement of pigeon pea and finger millet.

Keywords: pigeon pea (Cajanus cajan), finger millet (Eleusine coracana), intercropping, arbuscular mycorrhizal fungi (AM fungi), plant growth promoting rhizobacteria (PGPR), biofertilizers

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR)—generally called “biofertilizers”—are two classes of microbes that are well-known for their broad spectrum of beneficial effects to plants, by mobilizing phosphate, producing plant growth hormones, alleviating drought by the production of ACC deaminase...
which helps improving crop nutrient uptake (Bender et al., 2016; Igiehon and Balabolka, 2017). Both AMFs and PGPRs have been successfully evaluated in many field crops, such as wheat and rice (Mäder et al., 2011). However, currently there is lack of comprehensive knowledge on whether application of AMF+PGPR could potentially reduce the mineral fertilizer inputs in finger millet and pigeon pea without compromising their yields.

Finger millet (Eleusine coracana) and pigeon pea (Cajanus cajan) are two important crops predominantly grown in marginal rainfed regions of Africa and Asia, particularly in India, and Latin America (Chandrashekar, 2010; Krishna, 2010; Rao et al., 2015; Gupta et al., 2017; Varshney et al., 2017). Both crops are rich in protein and minerals, and serve as staple food for millions of people (Chandrashekar, 2010; Gupta et al., 2017). In India, pigeon pea and finger millet are grown on about 3.38 and 2.5 million hectares, respectively. Their yields are reduced due to various biotic and abiotic stresses, and in particular to low soil fertility, which cannot be readily overcome by the application of mineral fertilizers because of their high cost (Varshney et al., 2012; Gupta et al., 2017).

Biofertilizers, such as AMF and PGPR, have been successfully applied to improve the grain yields and nutritional quality of food crops (Schutz et al., 2018), including pigeon pea and finger millet (Patro et al., 2008; Dutta et al., 2014; Sekar and Prabavathy, 2014; Gopalakrishnan et al., 2016). Biofertilizer can be complementary to other fertilization or plant nutrition strategies, they can help reduce chemical fertilizers and increase the efficiency of nutrient uptake and soil organic matter stabilization. Our study examines to what extent biofertilization is effective in mono- or mixed culture of the two crops in order to reduce the quantity of mineral fertilizers while safe-guarding farmers practice of cultivating pigeon pea and finger millets as intercrops. In particular, biofertilization with AMF in the intercropping system might lead to sustainable crop production through improved soil fertility via a common mycorrhizal network. Under rainfed conditions, a transplanting system of pigeon pea cultivation is gaining importance due to its yield advantage compared to direct sowing (Ashok et al., 2010; Murali et al., 2014). In the transplanting system, pigeon pea seedlings are pre-cultured in polybags, a type of pot alternative, and well-watered before the start of monsoon, a seasonal rainfall pattern found in tropics. They are transplanted in the field after about 6 to 7 weeks at the start of the monsoon season. Polybag transplanting systems in pigeon pea have been successfully tested both under mono- and intercropping systems (Ashok et al., 2010; Murali et al., 2014; Praharaj et al., 2015). Under such a scenario, the use of biofertilizers, particularly the AMF, may provide an additional benefit, by forming a common mycorrhizal network from the start. Inoculating pigeon peas during pre-culturing in polybags may allow to reduce the amount of AMF inoculum, which is often considered to limit the application of AMF in arable crops.

Our study was aimed at addressing four hypotheses (i) compared to application of 50% recommended dose of fertilizer (RDF) and farm yard manure (FYM), application 50% RDF + FYM + biofertilizer has a potential to significantly improve the grain and straw yields of pigeon pea and finger millet; (ii) under pigeon pea-finger millet intercropping system, application 50% RDF + FYM + biofertilizer, has potential to improve the total grain and straw yields of pigeon pea and finger millet on par with the 100% RDF + FYM; similarly compared to application of FYM alone (0% RDF), application of FYM + biofertilizer has a potential to marginally improve the total grain and straw yields of pigeon pea and finger millet; (iii) under pigeon pea (transplanted system)-finger millet intercropping system, application (placement) of AMF biofertilizers to the seedlings of pigeon pea in polybags is sufficient to obtain the grain and straw yields on par with the yields obtained when AMFs applied to both the crops; (iv) under pigeon pea (transplanted system)-finger millet intercropping system, application (placement) of AMF biofertilizers to the seedlings of pigeon pea in polybags would result better grain and straw yields compared to application of biofertilizers under direct sown pigeon-finger millet intercropping system.

**MATERIALS AND METHODS**

**Field Site, Rainfall Pattern, and Soil Properties**

Main field trials were conducted at two field sites in South India at Bangalore (Karnataka) and Kolli Hills (Tamil Nadu) over two seasons (July 2014 to January 2015 and July 2015 to January 2016), with a third trial at both locations during July 2016 to January 2017 for selected treatments. The geographic location of the two sites, and their climatic conditions and soil properties for the three cropping seasons are given in Supplementary Table 1. In each season, trials were established on fields with a cropping history of either cassava or finger millet as pre-crops, or remained fallow in the season before the trials were established. While rainfall distribution was normal for the season 2014-15, extremely strong monsoon rainfall caused heavy flooding in the season 2015-16. In the season 2016-17, there were severe drought spells after the crop establishment.

**Plants: Variety and Provider**

At Bangalore, for all three seasons of the field trials, GPU-28 (finger millet—*Eleusine coracana*) and BRG-2 (pigeon pea—*Cajanus cajan*) varieties, were used. The seeds were obtained from National Seed Project at GKVK, Bangalore, and had a germination percentage of more than 95%. At Kolli Hills, Suratai Kelvaragu (finger millet) and SA-1 (pigeon pea) varieties were used, except for the season 2015/16, where Vamban-3 (seed provided by Tamil Nadu Agricultural University) was sown instead of SA1 with the objective to reduce the relatively long duration of SA-1 compared to BRG-2. However, due to uneven flowering of Vamban-3 (National Pulses Research Centre, Vamban) at Kolli Hills, we reverted to SA-1 variety for the third
Microbial Inoculants: Strains, Provider, and Multiplication

Two species of AMF inoculants viz., *Rhizophagus fasciiculatus* (AMFfm) and *Ambispora leptoticha* (AMFpp) were selected from finger millet (Rao et al., 1983a) and pigeon pea (Reddy and Bagyaraj, 1991), respectively. PGPR strain (*Pseudomonas* sp. MSSRFD41 isolated from finger millet rhizosphere) selected for both finger millet and pigeon pea (Sekar and Prabavathy, 2014), and *Rhizobium* commercial product for pigeon pea alone were used for this study. The two AMFs were multiplied in a vermiculite based carrier material (substrate) using Rhodes grass (*Chloris gayana*) as host plant for 40 to 45 days. The inoculum was prepared by allowing the grass to dry after which the roots were chopped into pieces (ca., 0.5–1 cm), and mixing homogenously in the same substrate in which the grass was grown. Dried (ca. 5% moisture w/w) inoculum thus prepared was evaluated for the infective propagules through MPN method. The harvested AMFpp inoculum consisting of 24 spores per g substrate was applied at the rate of 444 kg inoculum per ha for finger millet as a band application. The PGPR strains were multiplied in King’s B medium and a liquid culture consisting of 1 x 10⁶ CFU per ml of *Pseudomonas* sp. The liquid culture was prepared by dissolving the pellet using sterile water and then mixing with PEG, glycerol and PVP (3% v/v). MSSRFD41 was applied as seed coating at the rate of 5 ml per kg seed. Additionally, a band application (along the planting rows) was applied at the rate of 49.51 (consisting 1 x 10⁶ CFU per ml). All control treatments for PGPR were treated with “cell free” broth with same volume as with cells (*Pseudomonas*) and all AMF controls were treated with vermiculite alone. Rhizobium (consisting of 1 x 10⁶ CFU per ml) was applied as seed inoculation at a rate of 10 ml per kg of all treatments with pigeon pea.

Establishment of Field Trials

Experimental Design in Seasons 2014-15 and 2015-16

The experiment was laid out with a plot size of 6.6 x 3.9 m in a randomized block design (RBD) with a total of 20 treatments: T1: Sole crop of finger millet at 100% RDF; T2: Sole crop of finger millet at 50% RDF; T3: T2 + AMF; T4: T2 + PGPR; T5: T2 + AMF+PGPR; T6: Sole crop of pigeon pea at 100% RDF; T7: Sole crop of pigeon pea at 50% RDF; T8: T7 + AMF; T9: T7 + PGPR; T10: T7 + AMF+PGPR; T11: Finger millet + Pigeon pea (8:2) inter cropping +100% RDF; T12: Finger millet + pigeon pea (8:2) inter cropping +50% RDF; T13: T12 + AMF; T14: T12 + PGPR; T15: T12 + AMF+PGPR; T16: Finger millet + Pigeon pea (8:2) inter cropping + 50% RDF + AMF; T17: Finger millet + pigeon pea (8:2) + 50% RDF + AMF+PGPR; T18: Finger millet + Pigeon pea (8:2) + No fertilizer + No biofertilizer (absolute control); T19: Finger millet + pigeon pea (8:2) + No fertilizer + AMF; T20: T19 + PGPR; (T1 - T20) (see Table 1 for further details). Each treatment was replicated four times (=80 plots) per site for seasons 2014-15 and 2015-16 laid out in randomized block design (Supplementary Figure 1). The experimental design included (i) three mineral fertilizer levels (100% RDF, 50% RDF, or 0% RDF)—to test the potential of biofertilizer reduce the mineral fertilizer and to compare with the yield obtained at 100% fertilizer dose recommended to farmers; (ii) three cropping systems (finger millet and pigeon pea mono and intercropping), and (iii) two to four levels of biofertilization. For two mineral fertilizer levels (50% RDF and 0% RDF), the combined microbial inoculants [AMF application to Finger millet (AMFfm) Pigeon pea (AMFpp) and PGPR], were tested against a treatment without biofertilizers. At 100% RDF we tested only the no biofertilizer treatments (T01, T06, and T11). In addition, at 50% RDF (T02, T03, T05, T07, T08, T09, T10, T12, T13, T14, and T15), we tested the application of PGPRs and AMF separately and in combination. Additional treatments were T16 – 50% RDF + AMFpp; T17 – 50% RDF + AMFpp + PGPR were used to test the “placement effect” (effect of placing the AMFpp or AMFpp+PGPR at the time of raising pigeon pea seedling in polybag in comparison with applying AMFfm alone or AMFfm+PGPR additionally in field as band application); T18 – 0% RDF + no inoculation (absolute control); T19 – 0% RDF + AMFpp; and T20 – 0% RDF + AMFpp+AMFpp+PGPR.

We used following treatments for verifying each of the four hypotheses indicated above: hypothesis 1: T02, T05, T07, T10, T12, and T15; hypothesis 2: T11, T12, T15, T18, and T20; hypothesis 3: T13, T15, T16, and T17; hypothesis 4: T12, T15, T12d, and T15d.

Experimental Design in Season 2016-17

In the third cropping season (2016-17), the performance of biofertilizer (AMF + PGPR) application on the transplanted vs. direct-sown pigeon pea in the intercropping system was tested (Table 1), using six treatments (T11, T12, T15 with transplanting as in the previous years, and, T11d, T12d, T15d with direct-sown
TABLE 1 | An overview of the 20 treatments in cropping seasons 2014-15 and 2015-16 and the six treatments in season 2016-17.

| Cropping season | Trt. Nr. | FM planting system | PP planting system | Crop | Min. Fert. | Bio. Fert. applied to PP | Bio. Fert. applied to FM |
|-----------------|----------|--------------------|--------------------|------|-----------|-------------------------|-------------------------|
| 2014-15 & 2015-16 | T01      | DS                 | FM                 | 100  | NA        | No                      | No                      |
|                 | T02      | DS                 | FM                 | 50   | NA        | AMFpp                   | No                      |
|                 | T03      | DS                 | FM                 | 50   | NA        | PGPR                    | AMFpp                   |
|                 | T04      | DS                 | FM                 | 50   | NA        | AMFpp                   | PGPR                    |
|                 | T05      | DS                 | FM                 | 50   | NA        | AMFpp                   | PGPR                    |
|                 | T06      | TP                 | PP                 | 100  | No        | No                      | No                      |
|                 | T07      | TP                 | PP                 | 50   | No        | No                      | No                      |
|                 | T08      | TP                 | PP                 | 50   | AMFpp     | No                      | No                      |
|                 | T09      | TP                 | PP                 | 50   | PGPR      | No                      | No                      |
|                 | T10      | TP                 | PP                 | 50   | AMFpp     | No                      | No                      |
|                 | T11      | DS                 | TP+F+F           | 100  | No        | No                      | No                      |
|                 | T12      | DS                 | TP+F+F           | 50   | AMFpp     | No                      | No                      |
|                 | T13      | DS                 | TP+F+F           | 50   | AMFpp     | PGPR                    | No                      |
|                 | T14      | DS                 | TP+F+F           | 50   | PGPR      | AMFpp+PGPR              | No                      |
|                 | T15      | DS                 | TP+F+F           | 50   | AMFpp     | PGPR                    | AMFpp+PGPR              |
|                 | T16      | DS                 | TP+F+F           | 50   | AMFpp     | PGPR                    | AMFpp+PGPR              |
|                 | T17      | DS                 | TP+F+F           | 50   | AMFpp     | PGPR                    | AMFpp+PGPR              |
|                 | T18      | DS                 | TP+F+F           | 0    | No        | No                      | No                      |
|                 | T19      | DS                 | TP+F+F           | 0    | AMFpp     | AMFpp                   | AMFpp                   |
|                 | T20      | DS                 | TP+F+F           | 0    | AMFpp     | AMFpp                   | AMFpp                   |
| 2016-17         | T11      | DS                 | TP+F+F           | 100  | No        | No                      | No                      |
|                 | T12      | DS                 | TP+F+F           | 50   | No        | No                      | No                      |
|                 | T15      | DS                 | TP+F+F           | 50   | AMFpp     | AMFpp                   | AMFpp                   |
|                 | T11d     | DS                 | DS+F+F           | 100  | No        | No                      | No                      |
|                 | T12d     | DS                 | DS+F+F           | 50   | No        | No                      | No                      |
|                 | T15d     | DS                 | DS+F+F           | 50   | AMFpp     | AMFpp                   | No                      |

Pigeon pea was directly sown at the time of transplanting the polybag seedlings and sowing of the finger millet. Two pigeon pea seeds were sown per seeding hole and later thinned out to have one seedling per hole as before. Sowing in the field was done with the same spacing as in the main trials (see above). Both transplanted and direct sown system resulted in total 24 plots per site. All other operations, such as field preparation and plant protection measures, were the same as in the main trials in previous seasons.

All pigeon pea plants were inoculated with rhizobium. All control treatments for PGPR were treated with “cell free” broth with same volume as with cells (Pseudomonas). Similarly, all AMF control plots were amended with vermiculite alone, the carrier material for AMF propagation. A dose of 7.5 t per ha farmyard manure was applied to all plots.

Field Preparation and Inputs
The land was prepared by passing a disc plow followed by a cultivator twice to remove weeds and to crush the soil clods. All plots received a blanket application of FYM at a dose of 7.5 t per ha prior to sowing. The 100% RDF for finger millet and pigeon pea were applied at the rate of 50:40:25 and 25:50:25 NPK (Urea: Single Superphosphate; Muriate of Potassium) kg per ha, respectively. Using custom-made wooden markers, furrows or lines were opened at a row spacing of 30 cm for finger millet and 60 cm for pigeon pea in the main field and as per the plan of layout (Figure 1). In each plot, the crops were planted row-wise, with 22 rows of finger millet in monoculture (Figure 1A), 11 rows of pigeon pea in monoculture (Figure 1B) and 16 rows of finger millet plus 4 rows pigeon pea in the intercropping system (Figure 1C). Finger millet and pigeon pea harvest area were marked in mono and intercropping system (Figure 1). After 20 days of finger millet sowing and transplanting of pigeon pea, thinning and gap filling of finger millet was done manually to maintain target plant density. Weeds were controlled by a manual hoeing 30 days after planting, followed by a hand weeding on 40 days to keep the plot weed free and for better soil aeration. At Bangalore, pigeon pea was protected against pod borer (Helicoverpa armigera) incidence through prophylactic measures twice at fortnightly intervals during flowering at pod development stage. At Kolli Hills, neem oil was sprayed against the blister beetles, Mylabris spp. (Meloidae: Coleoptera). The crops were grown for about 4 months (finger millet) and pigeon pea grown for 8 and 5 months for SA-1 and Vamban-3 varieties respectively.
Harvest, Analyses, and Report

After attaining maturity, finger millet and pigeon pea straw and grains were harvested from their respective harvest area marked in each plot (Figure 1). The harvested grains and straw were sun-dried, and their weights were recorded. A subsample of the sun-dried material was oven dried for 24 h at 80°C. Oven dried weight straw and grains were used for calculating the dry matter yield expressed in metric tons per ha. Number of tillers in finger millet were counted in 0.6 m row length (8 plants) and the average per plant was calculated. Seed weights of harvested crops were measured (in grams) in 1,000 and 100 randomly selected seeds of finger millet and pigeon pea, respectively.

Statistical Analyses

Statistical analysis was performed using JMP software v.11. Two-way ANOVA was performed to find if there was any interaction effect between crop and biofertilizer. We found no interaction effect, indicating that the effect of biofertilizers for pure crop (for both finger millet and pigeon pea) was similar. Therefore, we compared the means of the biofertilizer treatment (AMF+PGPR) to the treatments without biofertilizers (No) across each year and site. A t-test was performed to find whether the means differed significantly between the “No” and “AMF+PGPR” treatments. In the intercropping system an additional t-test for the total mean of both the crops was performed. A multi-axis panel figure was constructed using two or more individual graphs.

RESULTS

Effect of Biofertilizers at 50% RDFs

Across the two sites (Bangalore and Kolli Hills) and seasons (2014-15 and 2015-16), at 50% RDF, there was a general trend of improved grain yields of finger millet and pigeon pea when inoculated with both AMF+PGPR compared with inoculation with either AMF or PGPR alone. During 2014-15, under finger millet monocropping system at Kolli Hills site, the combined inoculation of AMF+PGPR increased the grain yield of finger millet by +126% as compared to uninoculated control (Figure 2). At the Bangalore site, there was a trend of increased grain yields of finger millet and pigeon pea in the mono-
and intercropping systems in response to the biofertilizers, although there was significant effect only during 2014-15 under intercropping system (Figure 3). However, at the Kolli Hills site, as compared to the uninoculated control, inoculation of AMF+PGPR improved the grain yields of finger millet and pigeon pea both in monoculture and in the intercropping system. For intercropping yield increase due to inoculation was +126% and +128% during 2014-15 and 2015-16, respectively (Figure 3). Relatively lower grain yield of the pigeon pea during 2015-16 may be caused by a change in variety and flooding (see section Materials and Methods).

**Effects of Biofertilizers at Three RDFs (0, 50, and 100%) Under Intercropping**
At 0% RDFs and 50% RDFs, across the sites and seasons, there was a trend of improved grain yields of finger millet and pigeon pea, due to application of AMF+PGPR (Figure 4). At Bangalore, the inoculation of AMF+PGPR increased the combined grain yields of finger millet and pigeon pea by +12 and +13% at 0% RDFs and 50% RDF, respectively. At Kolli Hills during 2014-15 at 0% RDF, the grain yields of pigeon pea and finger millet were +69% higher when inoculated with AMF+PGPR as compared to uninoculated control. There was a general trend that grain yields
of finger millet and pigeon pea at 50% RDF plus AMF+PGPR were on par (sometimes slightly higher) with the ones obtained at 100% RDF without inoculation. At the Bangalore site, during 2015-16, the combined grain yields of finger millet and pigeon pea at 100% RDF (without AMF+PGPR) was identical with the grain yield obtained at 50% RDF with AMF+PGPR. Similarly, during 2014-15, at Kolli Hills site, the combined grain yields of finger millet and pigeon pea at 50% RDF plus AMF+PGPR was +14% higher than the grain yields obtained at 100% RDF without AMF+PGPR.

“Placement Effect” of the Biofertilizers at 50% RDF

An overview of the “placement effect” (for definition see Materials and Methods section) is presented in Figure 5. In brief, the “placement effect,” as we hypothesized in this study, is to find whether application (placement) of AMF biofertilizers to the seedlings of pigeon pea grown in the polybags would be sufficient to obtain the grain and straw yields of pigeon pea (transplanted) and finger millet on par with the yields obtained when AMFs applied to both the crops. There was a trend showing that grain yields of pigeon pea and finger millet due to application of AMFpp alone was on par with the grain yields obtained when both AMFpp+AMFfm were...
applied (Figure 5). A similar trend was observed in the grain yields obtained when AMFpp was applied in combination with PGPR. At Bangalore site, during 2014-15, the grains yields of finger millet and pigeon pea obtained by applying AMFpp+PGPR during preculturing pigeon pea (polybag) and in addition applying the AMFfm as band to the finger millet did not significantly increase the grain yields of finger millet and pigeon pea compared to applying the AMFpp+PGPR to the pigeon pea during the polybag alone. Similarly, in the same year, at Bangalore site, applying AMFpp alone to the pigeon pea during the preculturing (polybag) stage had the same grain yields of finger millet and pigeon pea when AMFfm was added as band in addition to the AMFpp. In contrast to the Bangalore site, at Kolli Hills (with inherently poor soil), in the first year, application of AMFpp and AMFfm during preculturing and band application, respectively, showed higher values (+34%) than application of AMFpp alone during preculturing. A similar trend was also observed in the second year although the effect was not significant. At Kolli Hills site, there were no significant “placement effect.”

**Effect of Biofertilizers in Transplanted vs. Direct Sown Pigeon Pea Intercropping System**

As expected, the transplanted system showed distinctly higher grains yields of pigeon pea than the direct-sown system at both sites, both under AMF+PGPR and non-inoculation treatments. Inoculation with AMF+PGPR increased pigeon pea yield in the transplantation system at both sites; the effect of transplanting system on finger millet was significant only in Kolli Hills in the direct-sown system (Figure 6).

**Effect of Biofertilizers on the Straw Yields and Other Growth Parameters of Finger Millet and Pigeon Pea**

In general, there were no significant changes in the straw yields of both the crops due to inoculation at the Bangalore site for both seasons (Supplementary Figure 2). At Kolli Hills, for both seasons, the straw yields of pigeon pea in monocropping were significantly improved due inoculation as compared to no inoculation. Similarly, during the season 2014-15, the combined straw yields of pigeon pea and finger millet in the inoculating system were significantly higher in inoculated treatments as compared to non-inoculated treatments. At 50% RDF, across site and year, the combined straw yield of pigeon pea and finger millet in the inoculated treatments were on par with the yields obtained at 100% RDF without inoculation (Supplementary Figure 3) (for example, see data for Bangalore and Kolli Hills during 2014-15). On the other hand, the finger millet and pigeon pea combined straw yields did not differ significantly between the treatments, particularly during the season 2015-16 at Kolli Hills. In general, the straw yields were not affected by the placement of the biofertilizer (Supplementary Figure 4), i.e., either in polybag alone or application as both band as well as in polybags. Similarly, inoculation did not significantly improve the straw yields under transplanting system (Supplementary Figure 5). Improved crop yields, particularly the pigeon pea grains, may be due to relatively better establishment of pigeon pea seedling during the preculturing stage in polybags (Supplementary Figure 6), although the results were not consistent across sites and year. Similarly, the higher grain yields in finger millets and pigeon pea may be due to improved number of tillers and number of branches per plant respectively (Supplementary Figure 7). Inoculation with AMF+PGPR caused a slightly increase in seed weights in finger millet and pigeon pea (Supplementary Figure 8).

**DISCUSSION**

Here we show that biofertilizer application can be useful for improving grain yields under 0% RDF (organic farming) and 50% RDF (reduced fertilizer input) for monocultures and intercropping of pigeon pea and finger millet. For pigeon pea, this is true both when sown directly-a common practice adopted by marginal farmers-or when transplanted in polybags-a labor intensive improved system (Fehle, 2016) (Figure 7). Specifically,
we show that total grain yield of pigeon pea and finger millet under intercropping system can be systematically improved by applying biofertilizers (AMF and PGPR) separately or together in pigeon pea-finger millet mono as well as in intercropping system. We found that application of biofertilizers + 50% RDF improved grain and straw yields. Reduced mineral fertilizer input through supplementing with biofertilizers could minimize certain detrimental effect, such as leaching of nitrogen to nearby water bodies, potential negative impact on microbial diversity, including mycorrhizal fungi. Furthermore, on economical view point, attaining grain yield equivalent to 100% RDF using 50% RDF plus biofertilizers shows potential savings for the farmers on the cost of mineral fertilizer input despite cost for purchasing biofertilizers need to be accounted. Improved yields obtained for pigeon pea and finger millet in this study indicates there is a great potential in systematically (through biofertilizers) reducing the existing large yield gap between potential yield (ca. 2.5 and 3.5 t per ha for pigeon pea and finger millet, respectively) and average yields (ca. 0.8 and 1.5 t per ha for pigeon pea and finger millet, respectively) obtained on farmer's fields in Asia (Ashok et al., 2010; Varshney et al., 2012). Below we discuss scenarios under which biofertilizers either in combination with transplanting or intercropping system affects grain yields of pigeon pea and finger millet.

**Biofertilizer Effect on Grain Yield**

Various studies have shown that application of single microbial species or consortia improve crop growth, including finger millet and pigeon pea (Mäder et al., 2011; Gupta et al., 2015; Schutz et al., 2018), although the outcome may also depend on the host genotype. Our results corroborates with Mäder et al. (2011) showing that combined application of AMF+PGPR improves grain yield than application of single microbial inoculant. Although it was beyond our scope to understand exact mechanism for improved grain yields in pigeon pea and finger millet due to application of biofertilizers, earlier studies have shown that better phosphorus uptake via AMF, crop tolerance to biotic and abiotic stresses via PGPRs, regulation of plant hormones are among the common mechanism through which biofertilizers help to increase crop growth (see reviews (Aroca and Ruiz-Lozano, 2009; Reddy,
In our case the improved finger millet and pigeon pea growth may be primarily due to the choice of AMFpp and AMFfm which were originally isolated from the rhizosphere of pigeon pea and finger millet respectively via screening (Rao et al., 1983a,b; Reddy and Bagyaraj, 1991). Similarly the PGPR strain MSSRFD41 was isolated from finger millet and may have been co-evolved as better symbiont for the host (Sekar et al., 2010, 2018). The observed variation in the microbial performance across the sites and years could be attributed to the differences in the soil physio-chemical properties and climate (Supplementary Table 1).

Furthermore, improved crop yields, particularly the pigeon pea grains, may be caused by better establishment of pigeon pea seedling during the pre-culturing stage in polybags, although the results were not consistent across sites and season (Supplementary Figure 6). Similarly, the higher grain yields in finger millets and pigeon pea may be explained by higher number of tillers and number of branches per plant, respectively (Supplementary Figure 7). Inoculation seems to have only a slight effect on the increase in seed weights in finger millet and pigeon pea (Supplementary Figure 8).

In general, we observed that the straw yields were only marginally improved through biofertilizer application as compared to grain yields (see Supplementary Figures 2–5). We are aware that crop residue (or straw) is major factor in the soil nutrient cycles (Correia et al., 2005) and for livestock (Chandrasekharaiyah et al., 2003). Nevertheless, considering the significance of our work, especially from farmers view point, here we primarily report only the grain yields. Furthermore, reporting additional data such as root biomass/architectures/microbial diversity is beyond scope of this study, although this would have allowed us to better interpret our results.

**Effect of Transplanting and Biofertilizer on Grain Yield**

Transplanting system is common in several crops, including finger millet and pigeon pea (Ghosh et al., 2007; Praharaj et al., 2015; Thilakarathna and Raizada, 2015), primarily due to yield advantage associated with better establishment of root and resistance to pest and diseases (Ashok et al., 2010; Mallikarjun et al., 2015; Praharaj et al., 2015). However, benefits of transplanting method in combination with biofertilizers has not been established yet. Here we show that pre-colonized pigeon pea under transplanted system results in higher grain yields of pigeon pea and finger millet, particularly under monocropping system, which may be attributed to the better root-growth, nutrient uptake and improved soil structure via the AMFs (Cartmill et al., 2012). Due to less labor and input costs, direct sown crops is common farming practice among marginal farmers but in recent times transplanting system of pigeon pea is being advocated due to higher yields attainable particularly under delayed monsoon and avoiding pest infestation during early stages of direct sown crops (Praharaj et al., 2015). Our study shows that transplantation benefits in pigeon pea can be further improved by application of biofertilizers. Improved yield of pigeon pea and finger millet due to biofertilizers under transplanting system could serve as criteria for the farmers to consider adopting a labor intensive system (Fehle, 2016). Inoculation of pigeon pea during pre-culturing stage would reduce the labor cost and quantity of inoculum required of applying biofertilizer in standing crops. Furthermore, it would be practically easier to apply any bio-fertilizer in small polybags than applying large fields.

**Effect of Intercropping and Biofertilizers on Grain Yield**

Intercropping is considered a productive system through improved soil biodiversity (Li et al., 2007), nutrient acquisition (Brooker et al., 2015), particularly N and P. On the other hand, intercropping can be less productive due competition for resources such as light and nutrient. It is known that beneficial microbes play a key role in below-ground resource-sharing but their role in intercropping is less explored primarily due to technical challenges such as tools required to accurately measure the nutrient and water sharing between the plant and the symbionts. We observed, compared to no biofertilizer, application of AMF+PGPR improved the combined grain yields of finger millet and pigeon pea although the results were not always same across season and site, which may be due to “unequal return of investments” between pigeon pea and finger millet via the common mycorrhizal network (Walder et al., 2012), a possibility that needs to be investigated. Improved growth of finger millet under intercropping system may have been due to possible “bioirrigation” via the common mycorrhizal network (Saharan et al., 2018), although this needs to be further verified using stable isotopes and by measuring appropriate physiological parameters under field conditions.

**CONCLUSIONS**

Our comprehensive field study conducted for three successive cropping seasons and at two geographical locations clearly shows the potential of a combined application of AMF (Ambispora leptoticha and Rhizophagus fasciculatus) + PGPR (Pseudomonas sp. strain MSSRFD41) to considerably reduce the mineral fertilizer input without jeopardizing the yields in pigeon pea and finger millet. The use of biofertilizers turned out to be efficient not only in systems with reduced mineral fertilizer input, but also in systems with addition of only farmyard manure. Thus, biofertilization is a sustainable and viable technology both in low-input and organic farming systems, particularly in transplanted pigeon pea-finger millet systems. Biofertilizers in combination with transplanting may offer an efficient cropping system of pigeon pea and finger millet because yield increase was found to be additive. Our comprehensive analysis would form a basis to improve the yield and productivity of finger millet and pigeon pea, particularly for marginal farmers of Southern India. Nevertheless, our results obtained in experimental fields need
to be interpreted with caution when recommending the biofertilizer application to marginal farmers, particularly with different soil and environmental conditions not tested in this work.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study are available on request to the corresponding author.

**AUTHOR CONTRIBUTIONS**

PM, MN, TB, AK, SJ, VP, MT, and EA designed the study. SJ, VP, PY, BM, MT, EA, MS, and MN conducted the experiment. SJ, VP, PY, BM, MT, EA, and MS provided data. MN and PM analyzed the data. MN wrote the manuscript. DB provided AMF cultures. VP and SJ provided PGPR culture. All authors read and contributed in the revision of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00088/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.