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Rhizobacteria in Mycorrhizosphere Improved Plant Health and Yield of Banana by Offering Proper Nourishment and Protection against Diseases

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Abstract The corporate R&D banana orchards of *Musa paradisiaca* (dwarf Cavendish AAA, var. *shrimanti*) on a medium black alluvial soil with low nutrients harboured diversified species of vesicular-arbuscular mycorrhizal (VAM) fungi. These fungi infected the roots severely (69.2%), showed elevated (69.8 g^{-1} soil) spore density, increased soil bacterial density (245×10^8 cfu g^{-1}), produced siderophores (58.2%) and reduced nematode population (2.3 g^{-1}) in the mycorrhizosphere of plants for integrated plant nutrition management (IPNM) system as compared to traditional treatment of applying chemical fertilisers alone and other test treatments. The interactions of plant roots with native VAM and local and applied rhizobacteria in the matrix of soil conditioner enabled proper nourishment and protection of crop in IPNM treatment as compared to traditional way. Hence, exploitation of plant growth promoting rhizobacteria through judiciously designed IPNM system revealed the (a) relatively increased banana productivity (21.6%, 76 MT ha^{-1}), (b) least occurrence of fusarial wilt and negligible evidence of Sigatoka, (c) saving of 50% chemical fertilisers and (d) permitted control over soil fertility in producer's favour over traditional cultivation practices. These findings are discussed in detail.

Keywords Relative banana productivity · Rhizobacteria · VAM · Soil conditioner · IPNM · Sustainable production practices (SPP) · mycorrhizosphere

Introduction

Banana, grown on 45,000 ha of land in Jalgaon district, Maharashtra State of India providing 2.25 million MT yield annually, serves a major source of income for farming

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community in Tapati river basin. However, the last decade witnessed a decline in banana yields as a result of inappropriate cultivation practices. Sword suckers preferred as plantation material provided a vector for root parasites and consequent performance disabilities. Improper plant nourishment and soil-borne diseases were the major causes coming in the way of optimal productivity [1]. Furthermore, control of soil erosion and eradication of diseases were cost-prohibitive.

Since 1996, severe attacks of *Karpa* disease were encountered. This fungal infestation due to *Mycosphaerella fijiensis* (yellow spot Sigatoka) and *Fusarium oxysporium* var. *cubense* (fusarial wilt) caused considerable damage to banana crop [2]. Well-adapted varieties of the region (viz. *bhusawal*, *shrimanti*, *padalse* and *shendurni*) fell prey to these diseases. While exploring the possibility of integrated plant nutrition management (IPNM) with biotech inputs such as soil conditioner, consortium of biofertilisers and reduced chemical fertilisation, we observed that in spite of the surrounding heavily infected banana farms, the experimental trial plots on research farm of Bajirao Agro-Tech Ltd (BAT), Balwadi showed resistance to virulent fungi, by virtue of more greenery, vibrant good health, robustness and productivity. To examine, how the rhizobacteria in the mycorrhizosphere of banana responds to the plant and its health upon adoption of the sustainable production practices (SPP) through IPNM was explored during July 1999 to October 2000. Its results are discussed in the present article.

Materials and Methods

Soil Type and Plantation Design

Plantation of banana (*Musa paradisiaca*, dwarf Cavendish AAA, variety *shrimanti*) was undertaken on pre-ploughed medium black, alluvial soil (Table 1), which had the history of intensive cultivation of banana for the past 20 years by traditional farming. Each plot of 49 production units with a spacing of 1.5×1.5 m^2 for each treatment was replicated four times as per randomised block design on approximately 0.3 ha of research farm.

Table 1 Analytical profiles of soil prior to and after banana plantation at a gross.

Parameter	Soil contents (before)	Soil contents (after)
pH	7.6	7.5
EC (mS cm^{-1})	0.46	0.56
Water holding capacity (%)	46.3	58.7
Density (g cm^{-3})	1.48	1.12
Organic carbon (%)	0.13	0.43
N (kg ha^{-1})	113.6	194.4
P_2O_5 (kg ha^{-1})	15.3	20.0
K_2O (kg ha^{-1})	513	1,084
Iron (μg g^{-1})	1.8	2.8
VAM spore density (g^{-1})	4.8	18.0
Bacterial density (cfu g^{-1})	19×10^6	43×10^6
Siderophore units (%)	0.4	1.5
Nematode population (g^{-1})	1.0	0.6

Chemical and Biotech Inputs

Five treatments were given: (a) control i.e. no application of any input, (b) chemical (traditional) fertilisers alone, N at 900 kg ha^{-1}, P_2O_5 at 350 kg ha^{-1} and K_2O at 900 kg ha^{-1}; single super phosphate (SSP) and muriate of potash (MOP) were applied as a basal dose, while urea was applied in four splits (0, 1.5, 3.0 and 4.5 months after plantation), (c) consortium of biofertilisers (Biofert, fresh inocula of *Azotobacter chroococcum* at 20 l ha^{-1} and *Aspergillus awamorii* at 20 l ha^{-1} for N_2 fixation and PO_4 solubilisation, respectively) prepared as per Phirke [3], (d) soil conditioner (SC, properly engineered fluffy compost with C/N-ratio 15.4, N 1.92 [% *w*/dry *w*], P_2O_5 0.7 [% *w*/dry *w*] and K_2O 3.12 [% *w*/dry *w*]) at 10 MT ha^{-1} produced from banana orchard waste [4–6] and (e) IPNM system i.e. soil conditioner at 10 MT ha^{-1}, consortium of biofertilisers (fresh inocula of *A. chroococcum* at 20 l ha^{-1} and *A. awamorii* at 20 l ha^{-1}) and half dose of recommended chemical fertilisers (N at 450 kg ha^{-1}, P_2O_5 at 175 kg ha^{-1} and K_2O at 450 kg ha^{-1}). The nutrients were judiciously blended on the basis of pre-determined site-specific requirement to yield maximum tonnage of banana. SSP and MOP were applied in two splits (first as a basal dose and the second at 2 months after plantation), while urea was applied through drip irrigation on daily basis up to 6 months. Two months later, fresh sword suckers replaced percent mortality. Drip irrigation was resorted for measured irrigation depending upon the need as a function of soil moisture [7]. The rest of the post-plantation care was as per routine production practices used by commercial producers [8].

Parameters Monitored

Growth profiles (height, girth and chlorophyll content) were monitored after the emergence of peduncle at 8 months (shooting stage) [9, 10]. After the harvest, quality and quantity of banana were evaluated and average sword suckers, recyclable pseudostem waste per production unit and water content of biomass (% *w*/*w*) were recorded as a function of treatments. Total banana production per hectare was the product of an average bunch weight and 4,000 production units.

Soil Analysis for Fertility and Plant Protection Capacity

Three rhizospheric soil samples, collected 25-cm deep at the base of the pseudostem, nearby the populated hairy roots of banana plants at shooting stage constituted each sample. Such randomly selected three samples from a replication were thoroughly mixed to get a composite sample for each replication.

These samples were analysed for VA-mycorrhizal spore density and nematode population per gram by wet sieving and decantation technique [11] and identification of VAM spores was done [12]. The total viable count was taken by standard serial dilution and pour plate technique on nutrient agar [13]. Siderophores from rhizospheric air-dried soil were extracted by preparing 10% (*w*/*v*) soil suspension, shaking it on a rotary shaker (180 rpm, 30°C, 2 h) and centrifuging (5,000 rpm, 20 min, [14]). The supernatant containing siderophores was deferrated [15] and estimated [16].

Hairy, absorbing root samples were similarly collected, cleared with 10% KOH solution for 40 min to remove phenolic compounds and stained with trypan blue and percent mycorrhizal infection of the roots (mycorrhization) was determined by root slide technique [17].

Physico-chemical soil analysis prior to and after plantation; was done at a gross [18]. The macro-elements were analysed from a composite foliar sample from the randomly selected

three plants. Third healthy leaf laminae from the apex were chosen for analysis. These were washed, dried and dehydrated in temperature controlled and fan ventilated oven at 65°C+1 for 24 h. Then, those were mixed in equal gravimetric parts, homogenised and digested in perchloric acid [19]. Nitrogen content was determined by Kjeldahl procedure, P content by vanado-molybdo-phosphoric acid colorimetric method and K by flame photometry [20].

Statistical Analysis of Data

All data were analysed statistically and tested for its significance. Means were compared by Student's *t*-test, and for each treatment, relationships of percent mycorrhization were established with other sets of observed features by calculating correlation coefficients ($p<0.05$).

Results and Discussion

Rationale behind Experimental Treatments

Among the five treatments, (a) control treatment served as a natural negative comparison, receiving none of the inputs and representing native common microflora of the soil, (b) chemical treatment served as a positive comparison, receiving recommended total nutrient dose through chemical fertilisers alone for traditional Indian system of banana cultivation [1, 21], (c) Biofert treatment served as a test application of consortium of biofertilisers, (d) SC treatment served as a test amendment for organic carbon incorporation and (e) IPNM treatment served as a test SPP receiving balanced nutrition; pre-determined by site-specific nutrient budgeting through optimised practices [4].

Metamorphosis of Soil Fertility and Protection Capacity

The soil used for banana cultivation in the present study was nitrogen and phosphorus-deficient, potassium-rich, with low organic carbon content and moderate water holding capacity (Table 1). Besides this, it was also low in VAM spore density, bacterial density, nematode population and percent siderophore units. The SC applied was well-matured, particulate, moist, earthy odorous compost with high humus content. The consortium of biofertilisers used was freshly produced, well-adapted to regional black clay alluvial soil and was in actively multiplying state. However, as a result of addition of biotech inputs and improved cultural practices, a series of events seem to have taken place. Firstly, improvement in soil fertility level was observed due to increase in beneficial microbial and VAM count, which in due course promoted siderophore production. Secondly, as siderophores chelate, solubilise and transport iron, banana plants benefited in respect of iron nutrition and consequently relief from harmful fusarial wilt/ *Karpa* /nematode infestation. As in any other biosystem, proper iron nutrition of banana crop was helpful in enhancing chlorophyll content and improving the yield of banana.

Implications of VAM

The five treatments affected their respective mycorrhizosphere, resulting into varied effects on vigour, nutrient uptake, biomass and yield. Shooting stage was purposefully chosen for investigations as the plants exhibit maximum nutrient uptake from soil to impart distinct

physiological and morphological changes during this period [22]. Diversified species of VAM fungi were found in all the treatments and their replicates. Among those, species like *Glomus mosseae*, *Glomus fasciculatum*, *Glomus intraradices*, *Glomus macrocarpum*, *Gigaspora margarita*, *Sclerocystis* sp. and *Scutellospora* sp. were prominent. These VAM fungal species found in this banana orchard, representing the typical soil type of Tapati basin, were well-adapted to humid sub-tropical climate and could survive, flourish and protect the plants in these soils by tolerating frequent/longer droughts (unpredictable annual rainfall 45–70 cm, temperature range 10–47°C), soil salinity due to irrigation with salty ground water, toxicity of heavy metals unavoidably augmented through the application of high dose of phosphoric and potassic fertilisers and varying seasonality of banana plantation [23].

Improvement in Plant Protection Capacity of Soil

It is obvious from Table 2 that mortality was high in control as well as chemical treatment plots indicating imbalanced nutrition in soil as against effectiveness of treatments in other plots [8]. Those treated with soil conditioner and biofertilisers, individually or in combination (IPNM), showed more root ramification, which helped the plants to increase their mycorrhizospheric zone for soil-microbe–plant interaction and more nutrient/moisture absorption [13]. Well-drained soil and drip irrigation helped the plants to keep their mycorrhizospheric zone considerably aerated and moist enough to solve problems related to soil health [7].

Siderophore content of rhizospheric soils were specifically determined at shooting stage as applied and native microflora growing in the vicinity of roots exhibited optimal metabolism, growth and proliferation for imparting protection to plants against potent soil-borne pathogens, maintaining adequate moisture, mobility of soil solution and nutrient uptake [24]. These observations, summarised in Table 2, are due to inherent property of SC to promote siderophorogenesis. Siderophores, iron-chelating plant protectants, produced by almost all the rhizospheric organisms selectively avail iron by active transportation to both rhizospheric organisms and plants, by their capability of chelation. This chelated iron is selectively deprived to phytopathogens, thus suppressing them and improving the plant health [25].

Nitrogen fixing *A. chroococcum* is reported to produce hydroxamate type of siderophores [26]. Native soil microflora, including VAM, produced siderophores for optimal iron nutrition and plant protection from potent soil pathogens [27]. The cell walls of mycorrhizal fungi provided a suitable surface for the attachment and colonization of *A. chroococcum* [28]. The hydroxamate types of siderophores are reported to retard the growth of *F. oxysporium* [29]. Secondly, in this region, the crop was attacked by the nematode *Radopholus similis* and *Helicotylenchus* species [8]. That such infection is combatable through IPNM has been shown by the present studies. The increased chlorophyll content (Table 2) kept the plants presumably at higher energy level, rendering them resistant to pests and nematodes. Well-fed (healthy) banana plants showed higher siderophore contents, no physiological disorder, delivered early (after 6–7 months) inflorescence and earlier fruit maturity.

Improvement in Soil Nutrients Sustainability

Table 2 has clearly indicated that bacterial density, mycorrhizal spore density and percent root colonisation were highest in the IPNM treatment and they were superior to that of

Table 2 Vital profiles of mycorrhizosphere and VA-mycorrhizal banana plants.

Treatment	Bacterial density $\log_{10}[\times 10^6]$ cfu g^{-1}	VAM spores (g^{-1} soil)	Root mycorrhization (%)	Siderophore units (%)	Nematodes (g^{-1} soil)	Plant mortality (%)	Height (cm)	Girth (cm)	Chlorophyll ($\mu g\ g^{-1}$)
Control	7.83	54.4 a	58.8 a	07.1 a	6.2 a	25 a	155	52.3 ab	553 a
Chemical	7.78	45.9	41.4	18.4 a	6.7 a	27 a	163 ab	59.7 c	523 a
Biofert	8.89	55.7 b	62.3 bc	64.7 bc	4.8 b	8 b	162 a	50.4 a	655 b
SC	8.89	55.6 ab	59.2 ab	68.2 b	4.1 b	11 bc	164 ab	54.3 b	705 bc
IPNM	10.39	69.8	69.2 c	58.2 c	2.3	11 c	165 b	61.7 c	723 c

Each value represents the mean of four replications. Each mean within a column, not followed by the same letter, is statistically different ($p<0.05$) as per Student's *t*-test

control, increasing with biotech inputs. The various characteristics were found declined in chemical treatment plots. This resulted in considerable decrease in nematode population in IPNM plots vis-à-vis increase in chemically treated plots. The two trends of increased VAM spore density and decreased nematode population resulted in increased plant survival rate in IPNM system [30]. Increased survival and sparing the former from replacement of mortality (and consequent expenses) has brought immediate benefit to farmers.

SC Promotes the Concept of Mycorrhizosphere

The major substrate for microbial activity in the plant mycorrhizosphere was exogenous organic carbon (supplemented through SC application and indigenous addition of organic matter), compounded with endogenous secretions by the microbes/plant roots [31]. The mycorrhizosphere implied that mycorrhizae significantly influenced the microflora, root physiology and exudation(s) in the colonised plants, besides providing nutrition for plant growth promoting rhizobacteria (PGPR) [32]. This led to both qualitative and quantitative changes in the mycorrhizospheric soil of the mycorrhized plants, compared to the rhizospheric soil of less-mycorrhized plants [33]. The bacterial density in the mycorrhizospheric soil of mycorrhized plants was greater than in the rhizospheric soil of less-mycorrhized plants. The effect of mycorrhizae on the bacterial density was positive and varied qualitatively and quantitatively as a function of mycorrhizal symbiosis [34].

Implications of Mycorrhizosphere on Morphological Parameters of Banana

Among morphological profiles, values of all the treatments including chemical and IPNM was found higher than control, indicating the phototrophic competition among the appropriately treated plants for more exposure to sunlight for enhanced photosynthesis [1, 2]. Girth of the pseudostem was not found significantly affected, showing almost all treatments achieved their minimal horizontal growth required for projecting the weight of the plant upward [8]. Chlorophyll content was found higher in each biotech treatment and it was significantly different from control and chemical treatments (Table 2). This indicated more greenery, robustness and health in each biotech treatment as an effect of interactions of plant growth promoting rhizobacteria with the roots of adequately nourished plants [3, 4, 23].

Table 3 Physiological and productivity profiles of VA-mycorrhized banana plants.

Treatment	N (% *w/w*)	P (% *w/w*)	K (% *w/w*)	Average bunch weight (kg)	Healthy suckers/ production unit	Recyclable biowaste (kg)	Water contents in biomass (%)
Control	1.3 a	0.15 a	1.7 a	16.9 a	7.6 a	31.6	88.0 a
Chemical	3.5 b	0.29 b	4.6 b	18.6 bc	8.6 a	37.4 ab	87.8 b
Biofert	1.8 a	0.21 ac	2.2 a	18.1 b	10.8 b	36.2 c	87.6 b
SC	3.0 c	0.20 a	3.8 c	17.5 a	12.1 c	36.7 ac	87.3 a
IPNM	3.3 bc	0.26 bc	4.2 bc	19.0 c	11.7 bc	40.3 b	86.9 ab
Interpretative values on dry weight basis for foliar analysis of 8-month-old banana [19]							
Low	2.5–3.4	0.15–0.19	3.0–3.7				
Sufficient	3.5–4.5	0.2–0.4	3.8–5.0				
High	>4.5	>0.4	>5.0				

Each value represents the mean of four replications. Each mean within a column, not followed by the same letter, is statistically different ($p<0.05$) as per Student's *t*-test

Table 4 Average days for flowering, hands, fingers, length, girth and finger weight.

Treatment	Days for flowering	Number of hands/bunch	Number of fingers/bunch	Length of fingers (cm)	Girth of fingers (cm)	Finger weight (g)
Control	273.6	5.8	45.6	14.5	9.8	85.8
Chemical	364.2 ab	10.2 a	139.3 a	20.3 a	11.9 ab	124.4 a
Biofert	361.7 ac	8.6 b	118.4 b	20.9 ab	11.3 a	126.3 ab
SC	358.4 cd	9.2 b	123.1 b	21.4 bc	12.2 bc	127.7 b
IPNM	360.8 bcd	10.7 a	145.8 a	22.1 c	12.8 c	132.6

Each value represents the mean of four replications. Each mean within a column, not followed by the same letter, is statistically different ($p<0.05$) as per Student's *t*-test

Implications of Mycorrhizosphere on Banana Productivity

Table 3 summarised the N, P, K uptake, average bunch weight, functional sword suckers which can be propagated for new plantation and recyclable biowaste. P uptake was found to increase with severity of VAM infection, while K uptake increased with increase in nitrogen, so does nitrogen uptake due to *Azotobacter* as compared to control. Average bunch weight was also highest in IPNM treatment. This is in accordance with the findings that increase in the uptake of other nutrients moves to the root surface, primarily by diffusion [34]. Subsequently, in a comparative experiment on the effectiveness of P supply on banana growth, Linn and Fox [35] demonstrated that the intensity of mycorrhization decreased with an elevated P level. This appeared as a feedback effect natural controlling mechanism. DeClerck et al. [36] found a significant rise not only in P content but also in K content in mycorrhizal banana plants. Present findings supported the conclusions of Harinikumar and Bagyaraj [37] that application of half the recommended level of chemical fertilisers together with soil conditioner (organic matter) favoured the build up of native VAM population in sub-tropical soils. Therefore, their active association with roots has afforded more uptake of moisture, increasing percent water and nutrient uptake. Percent root colonisation was moderately positive with spore density, nutrient uptake, chlorophyll content and recyclable biowaste, while moderately negative with nematode population and percent

Table 5 Quality, quantity and productivity profiles of VA-mycorrhized banana plots.

Treatment	Occurrence of fusarial wilt	Evidence of Sigatoka	Quality of fingers	Production (MT ha^{-1})[a]	Relative banana productivity (%)[b]
Control	+++++	+++++	Poor	67.6	–
Chemical	++++	++++	Moderate	74.4	15.5
Biofert	+++	+++	Superior	72.4	12.7
SC	+++	+++	Superior	70.0	13.9
IPNM	+	+	Excellent	76.0	21.6

+ Severity of occurrence of pathogen, by visual observation of evidence of Sigatoka and finding the infection of red fungus in the pseudostem longitudinally

[a] Production (MT ha^{-1})=average bunch weight per plant (kg)/1,000×4,000 plants (10% of total production units 4,400 ha^{-1} with 1.5×1.5 m spacing were considered as practical failure due to unavoidable cultural and environmental factors)

[b] Relative banana productivity={(difference between the average weight of dry biomass of treatment and that of control)/average weight of dry biomass of the same treatment}×100

mortality of plants [23]. Although no correlation was established with height, girth, average bunch weight and suckers, cumulatively IPNM treatment showed better nourishment.

Mechanism of Operations in Mycorrhizosphere

Mycorrhization selectively altered pressure on the population of soil microbes, some of which engaged in controlling root pathogens [38, 39]. Mycorrhizal fungi have attributes of penetrating along with the root system and therefore providing increased absorptive surface [34]. This ramification with root system permitted enhanced nutrient moisture uptake from the soil rhizosphere and thereby minimised transplantation shock [36].

The 25-cm-deep root zone from the surface [1], 100–150 cm around a banana plant [2], supplemented with organic carbon (SC), PGPR, both applied and native, including mycorrhizae [40–42], cumulatively constituted its mycorrhizosphere. Drippers maintained requisite moisture in this rhizosphere [7]. Exogenously introduced biofertilisers, along with native beneficial population, proliferated in the matrix provided by SC and helped the plant symbiotically. They grew on the mycoplane as well as rhizoplane to interact with VAMs and roots. VAMs produced extracellular hyphae [40], which extended several centimeters into the surrounding matrix of rhizospheric soil and exhibited symbiotic association with these soil microbes. These hyphae-associated microbes frequently produced sticky exudates (long chain polysaccharides), which caused soil particles to adhere, creating small aggregates that imparted structure to soil, allowing for improved aeration, water percolation and stability [41, 42].

From Table 4, it is clear that hands/bunch, fingers/bunch, length and girth profiles of fingers and overall productivity were inferior in control, better with chemical fertiliser application and best with biotech inputs, increasing in a statistically significant manner with IPNM [22, 23]. These observations have a ramifying effect in the immediate future by way of more income to farmers and in the long term by way of sustainability of soil productivity. The latter contention is amplified by observations summarised in Table 5.

Reducing trend of fusarial wilt [43] and incidence of Sigatoka [44] were found in treatments of biotech inputs in general and IPNM in particular. This fungal infestation of air-borne *M. fijiensis* (Sigatoka) and soil-borne *F. oxysporium* var. *cubense* (fusarial wilt) caused considerable damage to banana crop [1, 2]. The variety *shrimanti* is also susceptible to these diseases [2, 8]. Siderophorogenesis in mycorrhizosphere selectively deprived iron to potent phytopathogens like *F. oxysporium* [28, 29], thus suppressing them and improving the iron nutrition [25]. Increased bacterial density, spore density and percent mycorrhization in the treatments introduced with rhizobacteria, predominated in the mycorrhizosphere and proliferated in the root zone, keeping the soil-borne fusarial pathogen restricted and retarded [43–45]. The adequate nourishment of banana plants through mycorrhizosphere treated with biotech inputs (as discussed earlier) provided robust health and systemic resistance against air-borne *Mycosphaerella* species to remain robust in spite of the infestation [8, 45].

The developed robustness in plants treated with biotech inputs and IPNM showed the improving trend in the quality of fingers and overall production per hectare. These speak volumes of the effect of native and introduced rhizobacteria and local VAM in IPNM, traditional farming and typical soil systems for commercial cultivation of banana [3, 23].

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