Biocatalysis and Agricultural Biotechnology 9 (2017) 67–73

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Cross-compatibility evaluation of plant growth promoting rhizobacteria of coconut and cocoa on yield and rhizosphere properties of vegetable crops

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ARTICLE INFO

Keywords:
Cocoa
Coconut
Cross-compatible PGPR
Vegetables
Vermicompost

ABSTRACT

Plant growth promoting rhizobacteria (PGPR), *Bacillus megaterium* TSB16 isolated from coconut and *Pseudomonas putida* KDSF23 from cocoa, were tested for cross-compatibility with vegetable crops in combination with coconut leaf vermicompost (CLV). The treatments included CLV @10 t/ha (T1), *B. megaterium* + CLV @ 6 kg /ha and 10 t/ha, respectively (T2), *P. putida* + CLV @ 6 kg /ha and 10 t/ha, respectively (T3) and recommended dose of NPK fertilizers @75:40:25 kg N, P_2O_5, K_2O + CLV @ 2.5 t/ha (T4). The results of the field trial indicated that the cumulative yield of tomato was significantly higher ($P < 0.05$) with chemical fertilizer application (278.7 g/plant) and *Pseudomonas putida* KDSF23+ CLV treatment (275.8 g/plant) compared to that in CLV application (239.5 g/plant). The yield of chilli was significantly higher in the plots that received chemical fertilizers. The soil N, P, K and organic carbon were highest in chemical fertilizer applied plots. However, a significant increase in population of rhizosphere microbial communities, particularly the plant-beneficial microbiota, and soil enzyme activities (phosphatase, dehydrogenase and urease) were recorded in the PGPR and CLV treated plots. The function-specific microorganisms *viz.* fluorescent pseudomonads, phosphate solubilizers, free-living nitrogen fixers and *Trichoderma* were 4.7–9.1, 3.5–3.8, 1.3–2.1 and 2.0–2.2 fold higher, respectively, in tomato and 1.5–1.6, 1.4–2.5, 1.3–1.4 and 2.9–3.4 fold higher, respectively, in chilli, in PGPR treated plots than that received chemical fertilizer. Our findings suggest that the PGPR strains isolated from the rhizosphere of coconut and cocoa can be utilized as a bioinoculant for vegetable production in organic agricultural systems indicating cross-compatible nature. They can also serve as a single bioinoculant for the main crop (coconut) and its intercrops (such as vegetables) in coconut based cropping system to reduce inorganic fertilizer application.

1. Introduction

On global scale, more than 175.7 million tonnes of chemical fertilizers are applied to soil annually (FAO, 2011) and have been found to be well correlated with the crop yield. However, they are one of the potent sources of environmental pollution that leads to resource degradation and negatively impact soil ecological functions (Kumar et al., 2014). Consequently, it is a great challenge to search for sustainable strategies to alleviate detrimental effects of intensive farming practices that consume chemical fertilizers. Effective biological technologies like the use of plant growth promoting rhizobacteria (PGPR) are being exploited for enhancing crop yield in modern agricultural systems in order to reduce the chemical fertilizer input. PGPR are soil- borne bacteria that have the ability to aggressively colonize the rhizosphere or plant roots stimulating the growth and yield of plants (Kloepper and Schroth, 1978). Studies have shown the positive effects of PGPR in increasing the growth of cereals (Lavakush et al., 2014), fruits (Kavino et al., 2010), vegetables (Gravel et al., 2007) and spices (Anandaraj and Sarma, 2003). Most of the research demonstrated the effectiveness of PGPR isolated from the rhizosphere of a particular crop on the yield and rhizosphere properties of the same crop. There are also reports of the effectiveness of PGPR isolated from one crop in improving the yield of other crops. Kandel et al. (2015) demonstrated that the diazotrophic endophytes of the eudicots poplar and willow colonized rice plants and enhanced plant growth by greater biomass, higher tiller number and taller plant stature than uninoculated control in N- limited conditions. Similarly, *Bacillus cereus,* isolated from annual crop, was capable of long-term colonization of cocoa foliage and reduced black pod rot disease severity caused by *Phytophthora capsici* in cocoa plants (Melnick et al., 2008). However, there is not much information available on cross-compatibility of PGPR isolated from plantation crops on annual or seasonal crops.

The coconut palm (*Cocos nucifera* L.) is a perennial tree crop grown

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http://dx.doi.org/10.1016/j.bcab.2016.11.006
Received 9 August 2016; Received in revised form 11 November 2016; Accepted 26 November 2016
Available online 30 November 2016

in over 90 countries in the tropics for diversified uses. Coconut, grown as a monocrop, is only marginally productive and profitable due to the poor utilization of resources in a coconut garden. The coconut farmers in India, particularly of Kerala State, are accustomed to grow a variety of annual and perennial crops in the interspaces of coconut palm to meet their various requirements. Cocoa, a plantation crop, is highly compatible and profitable mixed crop in coconut gardens. Vegetable crops are also grown predominantly as intercrops in coconut based homesteads of Kerala (Nayar, 2011). In our comprehensive study on agriculturally important microorganisms associated with coconut and cocoa, we isolated two plant growth promoting rhizobacteria, *Bacillus megaterium* TSB16 from rhizopshere of coconut and *Pseudomonas putida* KDSF23 from cocoa, with high potential for plant growth promotion (George, 2013; Thomas, 2013). It would be interesting to know if the same strains can be utilized as bioinoculants for the main as well as the intercrops. However, so far there have been no reports of PGPR isolated from plantation crops, particularly from coconut and cocoa, being used as bioinoculants for vegetable crops.

Therefore, a field experiment was conducted to study the effect of inoculation of PGPR, previously isolated from plantation crops, *viz.*, *Bacillus megaterium* TSB16 from the rhizosphere of coconut (George, 2013) and *Pseudomonas putida* KDSF23 from the rhizosphere of cocoa (Thomas, 2013), on yield of chilli and tomato cultivated as intercrops in coconut interspaces at ICAR-Central Plantation Crops Research Institute farm, (Kasaragod, Kerala, India) and its impact on nutrient status, microbial population and enzymes activities in rhizosphere soil.

2. Materials and methods

2.1. PGPR used in the study

Plant growth promoting rhizobacteria, *Bacillus megaterium* TSB16 and *Pseudomonas putida* KDSF23, used in this study were obtained from the glycerol stocks of these cultures maintained in the Microbiology Section, ICAR-CPCRI, Kasaragod, Kerala, India. These PGPR were originally isolated from the soils (5–15 cm depth) collected from the rhizosphere region of coconut palms and cocoa trees by standard procedures using the serial dilution technique. Ten-fold dilutions of soils collected from Kidu, Dakshina Kannada District, Karnataka State, India (Geographical location: 12°43′ N, 75°35′E; Cocoa var.: Criollo; Age: 12 years; Soil type: laterite; pH 5.3) were plated on King's B agar medium for isolation of *Pseudomonas putida* KDSF23 (Thomas, 2013). Standard heat treatment enrichment technique was used for obtaining *Bacillus megaterium* TSB16 (on nutrient agar medium) from coconut rhizosphere soil collected from Siddapura, Tumkur District, Karnataka State, India (Geographical location: 13°20′21″ N, 77°6′50″E; Coconut var.: Tiptur Tall; Age: 26 years; Soil type: sandy loam; pH 7.9) (George, 2013).

2.2. Preparation of talc-based formulations of PGPR

For the preparation of talc based biofertilizers of PGPR, *Bacillus megaterium* TSB16 and *Pseudomonas putida* KDSF23 were inoculated in to nutrient broth and King's B broth, respectively, and incubated at 33 °C for 48 h. Talc formulation of isolates were prepared by mixing the microbial culture with pre-sterilized talc powder under aseptic condition. The moisture content was maintained at 50% WHC, packed in polypropylene bag and sealed. The viable population of *Bacillus megaterium* TSB16 and *Pseudomonas putida* KDSF23 was 7×10^8 and 5×10^{10} cells per gram of carrier material, respectively.

2.3. Experimental site

Field experiment was conducted at the coconut farm (12″ 30″ N latitude and 75° 00′ E longitude with an altitude of 10.7 m above MSL) of ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala, India during December 2012 to March 2013. Mean maximum and minimum air temperature during the period were 31.2 and 23.6 °C, respectively, which is ideal for vegetable cultivation in this area. Average relative humidity during the cropping season was 61–94%. No rainfall was recorded in the growing season. The soil of the research site was sandy loam with pH 4.8, electrical conductivity of 15.1 μScm^{-1}, organic carbon content of 0.04%, and total nitrogen of 0.01%, 25.6 ppm available P and 17.4 ppm available K. The soil dehydrogenase, phosphatase and urease activities were 0.22 μg TPF/g soil/h, 17.8 μg PNP/ g soil/h and 27.4 μgNH_4/g soil/h, respectively. The population of bacteria, fungi, actinomycetes, *Trichoderma* species, fluorescent pseudomonads, phosphate solubilizers and free-living nitrogen fixers were found to be 40×10^5, 14×10^3, 16×10^4, 2×10^4, 7×10^2, 5×10^4 and 4×10^2 cfu (colony forming units)/g soil, respectively.

2.4. Experimental layout

The field experiment was conducted in an area of 81 m^2 within the interspaces of two rows of coconut palms (West Coast Tall variety, > 50 years age). The height of palms was more than 60 feet which allowed sufficient sunlight for cultivating intercrops. The 81 m^2 area was split into two plots of 40.5 m^2 each. The following four treatments were included for each vegetable crop:

1. T1 – Coconut leaf vermicompost (CLV) @10 t/ha
2. T2 -*Bacillus megaterium* @ 6 kg/ha + CLV @10 t/ha
3. T3 -*Pseudomonas putida* @6 kg/ha + CLV @10 t/ha
4. T4 –Recommended doses of NPK fertilizers @75:40:25 kg N, P_2O_5, K_2O + CLV @2.5 t/ha

The field experiment comprising the above four treatments was laid out in a randomized complete block design. Four replicates (one bed equal to one replicate) per treatment were maintained. The crops were irrigated with micro-sprinkler system ensuring proper moisture regime for the experimental plot. A basal application of coconut leaf vermicompost (CLV) produced in-house from coconut leaf substrate using local isolate of *Eudrilus* sp. at ICAR-CPCRI, Kasaragod, Kerala, India (Gopal et al., 2009) was applied @ 2.5 t/ha to the plot. Chemical fertilizers were applied at the recommended NPK dose (75 kg: 40 kg: 25 kg) for vegetables as per the package of practice of Kerala Agricultural University, Kerala, India. Talc formulation containing *Bacillus megaterium* and *Pseudomonas putida* was applied @ 6 kg/ha each, through soil inoculation.

2.5. Crop establishment and yield data

Healthy tomato and chilli seedlings raised on coir-pith compost in 100 cell seedling trays were obtained from Seed Production Centre, State Agriculture Department, Kasaragod, Kerala, India for the field studies. Tomato (var. Anagha) and chilli (var. Vellayani Athulya) seedlings were transplanted into the raised beds in their respective plots. A total of eighty tomato and ninety-six chilli seedlings were distributed equally within four treatments in the respective 40.5 m^2 plots. A replicate plot of tomato had five seedlings and chilli had six seedlings. Treatment application was initiated one week after the seedlings had established properly. For T1 treatment, CLV was added @ 10 t/ha in four split dose of 2.5 t/ha (about 1 kg per replicate plot). For T2 and T3 treatments, 3 g talc based PGPR formulation was mixed with 4 kg of soil and each bed received 1 kg of the PGPR-soil mix. The PGPR application was done twice during the field study, one week and one month after transplanting the seedlings. The CLV addition to T2 and T3 followed the same application method as in T1, the first two out of four splits coinciding with the PGPR application. The N, P and K in the chemical fertilizer treatment were supplied through the application of urea, rock phosphate and muriate of potash, respectively, after one week of planting the seedlings, in single dose as top dressing. The plots