



Pre-evaluation response of rhizosphere hybridization in acid lime

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ABSTRACT

Rhizosphere hybridization is a new concept to secure the plant growth-promoting microorganisms to produce better nursery plants. Taking into the objective of rhizosphere hybridization, the present investigation was conducted in the secondary nursery block of ICAR-Central Citrus Research Institute, Nagpur. As many ten treatments consisting of rhizosphere microbes in form of water extract of the rhizosphere of acid lime, neem, bamboo, banyan and microbial consortium were tested in acid lime seedlings (cv NRCC Acid lime-7) raised on a solarized but containerized soil completely randomized design with three replications during 2018–20. Results showed a significant response to different treatments on plant growth parameters. In our study, the effect of the combined rhizosphere of banyan (*Ficus benghalensis*), neem (*Azadirachta indica*) and bamboo (*Dendrocalamus strictus*) was significantly superior to the rest of the treatments in increasing the biometric (seedling height, seedling diameter and number of branches per seedling), number of leaves per seedling, root architecture (tap root length, tap root diameter and number of secondary roots per seedling) and microbial biomass (fungal count and bacterial count) response of acid lime seedling compared to control treatment. Rhizosphere soil treatment was found to be superior over rhizosphere water extract treatment. Among various rhizosphere water extract treatments, acid lime rhizosphere water extract performed better in biometric response of acid lime seedling. Microbial consortium was observed to be better in increasing the microbial biomass of the rhizosphere, which could probably translate its response at later stages but added an additional resilience to rhizosphere, thereby, potentially effective in reducing the mortality once planted in main field.

Key words: Biometric response, Microbial biomass, Microbial consortium, Pre-evaluation, Rhizosphere hybridization

Plants are often challenged by several environmental stresses due to climatic changes that negatively affect plant growth and productivity (Gao *et al.* 2007). The agriculturist demand sustainable solutions to combat climatic changes affecting crop yield. In this regard, rhizosphere security has become a pre-requisite to sustain the production by exploiting the microbiome profile of different crop species, hybridizing their rhizosphere would be a way forward approach of decoding the crosstalk between plants and microbes (Srivastava and Malhotra 2017). The portion of soil inhabited by roots, both horizontally and vertically, known as rhizosphere, influences the soil health, fruit yield and quality of citrus fruits (Srivastava and Singh 2008, Ngullie *et al.* 2015). Rhizosphere processes are one of the most important but least understood ways in which plants affect nutrient cycling (Srivastava and Singh 2015, Shao *et al.* 2018). To sustain rhizosphere properties, microbes play a significant role in biofertilization of crops (Srivastava *et al.* 2015). Plant growth-promoting rhizobacteria (PGPR) is

a heterogeneous group of naturally occurring soil bacteria that colonize plant roots and benefit plants by providing growth promotion directly or indirectly (Agrawal and Agrawal 2013). PGPR are known for their ability to induce plant defence/tolerance and antagonize plant pathogens. PGPR are considered as potential biocontrol agents which promotes plant growth (Planchamp *et al.* 2014).

PGPR can enhance plant growth directly by providing plants with nutrients such as nitrogen through the process of nitrogen fixation or by solubilizing phosphorus from soil bound phosphate (Berg 2009). PGPR also has the ability to synthesize several plant growth hormones such as auxins and cytokinins and improves plant abiotic stress tolerance via maintaining water availability in the rhizosphere (Yang *et al.* 2009). Banyan, neem and bamboo are claimed to have a long life. We hypothesize that an exceptional ability of these plants to survive and grow under rigorous conditions may be due to considerable plant roots association with rhizobacteria. So in this present study, we focused on the hybridization of rhizosphere to pre-evaluate the nursery-grown plant, under controlled screen house conditions.

MATERIALS AND METHODS

The present study was carried out in the secondary

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nursery block (21°9'2" N; 79°1'50" E) of ICAR-Central Citrus Research Institute, Nagpur. As many ten treatments consisting of rhizosphere microbes as soil and its water extract of acid lime (*Citrus aurantifolia* Swingle), neem (*Azadirachta indica*), bamboo (*Dendrocalamus strictus*), banyan (*Ficus benghalensis*) and microbial consortium (*Bacillus pseudomycoides* Nakamura (MF113272), *Acinetobacter radioresistens* Nishimura (MF113273), *Micrococcus yunnanensis* Cohn (MF113274), *Aspergillus flavus* Link (MF113270) and *Paenibacillus alvei* Cheshire and Cheyne (MF113275)) were tested in acid lime seedlings cv NRCC Acid lime-7, raised in solarized soil using the experimental design completely randomized design with three replications during 2018–20 (Table 1).

Rhizosphere soil collection: The rhizosphere soils (two kilograms for each plant) were collected from well-established plants of acid lime, neem, bamboo and banyan at 1–1.5 m distance from the trunk and a depth of 0–20 cm coinciding with a zone of maximum feeder roots and

microbial biomass.

Preparation of soil extract: Soil extract was prepared by mixing fertile rhizosphere soil with distilled water in the ratio of 1:1. The extract was kept untouched for 24 h to release the active PGPR to the water from the soil. The supernatant extract is filtered and collected for the application.

Each treatment had 30 number of seedlings were irrigated well before treatment application to improve its efficacy. Ten gram of rhizosphere soil and ten millilitre of rhizosphere water extract and microbial consortium was given as treatment application in acid lime seedling potting mixture. The details of treatments are T₁- Control, T₂- Microbial consortium, T₃- Rhizosphere water extract of acid lime, T₄- Rhizosphere soil of bamboo, T₅- Rhizosphere water extract of bamboo, T₆- Rhizosphere soil of neem, T₇- Rhizosphere water extract of neem, T₈- Rhizosphere soil of banyan, T₉- Rhizosphere water extract of banyan and T₁₀- Rhizosphere soil of bamboo, neem and banyan. All the other agricultural practices were remaining same

Table 1 Rhizosphere physico-chemical and microbial properties of different plant species used under the study

Geographical location	Neem (<i>Azadirachta indica</i>)	Banyan (<i>Ficus benghalensis</i>)	Bamboo (<i>Dendrocalamus strictus</i>)	Acid lime (<i>Citrus aurantifolia</i>)	Potting mixture (Soil, FYM, Sand)
	21°8'3" N; 79°1'3" E	21°9'8" N; 79°1'21" E	21°9'0" N; 79°1'54" E	21°9'3" N; 79°1'20" E	21°9'1" N; 79°1'52" E
Physical properties					
Munsell colour score	10 YR4/4	10 YR3/1	10 YR3/1	10 YR3/1	10 YR3/2
Sand (%)	42.2	44.1	42.2	45.2	46.3
Silt (%)	25.5	20.4	23.6	21.2	26.5
Clay (%)	32.3	35.5	34.2	33.6	27.4
Bulk density (mg/m)	1.71	1.38	1.43	1.6	1.79
Porosity (%)	36.9	48.2	40.3	42.2	32.4
Soil moisture (33 Mpa)	34.2	33.8	32.4	32.4	28.4
Soil moisture (1500 Mpa)	16.2	14.1	18.2	18.2	16.1
Soil AWC (%)	18.0	19.7	16.2	16.2	12.3
Chemical properties					
pH	6.8	7.1	7.1	7.2	6.9
EC (dS/m)	0.160	0.158	0.159	0.154	0.161
KMnO ₄ -N (mg/kg)	108.26	118.35	121.89	112.81	108.96
Olsen-P (mg/kg)	8.9	10	9.8	9.6	8.8
NH ₄ OAC-K (mg/kg)	201.6	220.4	216.4	208.3	204.5
DTPA- Fe (mg/kg)	11.9	12.5	13.6	13.2	11.8
DTPA- Mn (mg/kg)	10.35	12.27	11.54	11.82	10.54
DTPA- Cu (mg/kg)	1.99	1.98	2.07	1.94	2.01
DTPA- Zn (mg/kg)	0.68	0.94	0.85	0.76	0.65
Microbiological properties					
Bacterial count (10 ³ cfu/g soil)	27	26	31	42	23
Fungal count (10 ³ cfu/g soil)	9	23	13	10	14

for all treated seedlings. All the vegetative parameters like shoot length, shoot width, number of leaves/plants, number of branches, leaf length, leaf width, internode length, root length, root width and number of secondary roots were observed. The microbial count was performed by standard plate count technique (Wollum 1982). Ten grams of each pre-treated soil sample was weighed and added to the 90 ml sterile distilled water blank. The 1 ml suspension was transferred to 9 ml of water blank. Similarly, the serial dilution was made up to the highest dilution of 10^{-3} . 0.1 ml of each diluted suspension was spread on to prepared plates of nutrient agar and potato dextrose agar and was incubated at $28 \pm 2^\circ\text{C}$ for 48 h. The total microbial count was expressed as colony forming unit per gram (cfu/g soil) of soil sample.

Statistical analysis: The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTAT and WASP 2.0 applying completely randomized design (CRD) at 5% level of significance.

RESULTS AND DISCUSSION

The biometric response of acid lime seedlings by different treatments was observed to be significant (Table 2). Biometric parameters like shoot length, shoot diameter and number of branches varied from 61.77–92.17 cm, 5.35–8.38 mm and 4–9.83, respectively. In all the treated seedlings, the combined effect of all rhizosphere soil (bamboo, neem and banyan) i.e. treatment T_{10} was found to be superior over rest of the treatments. Treatment T_8 and T_4 was found statistically similar with T_{10} . Rhizosphere soil treatments were found better than rhizosphere water extract, but rhizosphere water extract was far better than control. Acid lime rhizosphere water extract was found superior among all the rhizosphere water extract. The microbe-microbe interaction of same species might have created protective phyto-microbiome matrix to upsurge the growth regulation

in acid lime seedling. Microbial consortium proved to be far superior to control in all the observations. The increased number of leaves, leaf phenotype and root architecture (Table 2) may help in the accumulation of more amount photosynthates which increased biometric response of acid lime seedlings. Cheke *et al.* (2018a) experimented with rhizosphere hybridization by taking rhizosphere soil of three species of *Ficus* along with sweet orange seedling. They observed that all the rhizosphere hybridized soil found superior over non rhizosphere soil treatment. The superiority of microbial consortium was well supported by the in-field studies of Srivastava *et al.* (2015) and Hota *et al.* (2020).

A significant variation in number of leaves/ seedling and root architecture was observed (Table 2). Data showed that number of leaves/ seedling varied from 60.18–99.05, leaf length from 45.44–61.5 mm, root length from 23.28–37.72 cm, root diameter from 5.27–8.75 mm and number of secondary roots/ seedling from 18.57–35.88 in response to different rhizosphere hybridization treatments. Among all the treatments T_{10} was found to be superior over rest of the treatments. Treatment T_8 and T_4 were significantly at par with T_{10} in maximum parameters except for root diameter and number of secondary roots. Microbial consortium played important role in up surging the leaf phenotype and root architecture parameters by providing plant growth-promoting micro-organism which helped supplementation of nutrients to applied seedlings (Srivastava *et al.* 2019). Rhizosphere hybridization increased the microbial biomass (Fig 1) which helped solubilisation of non-available nutrients into available form and increased the secondary root development, and ultimately strengthen the root architecture and leaf phenotype (Cheke *et al.* 2018b).

Rhizosphere hybridization significantly showed a large variation in microbial biomass (Fig 1). The population density of microbes (bacterial count and fungal count) was found to be superior in T_{10} treatment over control.

Table 2 Agronomic response of rhizosphere hybridization on biometric parameters of acid lime seedlings

	Seedling height (cm)	Seedling diameter (mm)	Number of branches/seedling	Number of leaves/ seedling	Tap root length (cm)	Tap root diameter (mm)	Number of secondary roots/seedling
T_1 - Control	61.7	5.3	4.0	60.1	23.2	5.2	18.5
T_2 - MC	83.2	6.5	7.0	88.4	32.8	7.5	30.8
T_3 - AIRW	76.9	6.9	8.1	89.0	31.6	6.9	31.1
T_4 - BbR	85.3	7.2	9.0	95.5	36.0	7.8	32.1
T_5 - BbRW	67.6	6.2	4.6	71.6	28.5	6.0	25.6
T_6 - NR	70.7	5.9	6.6	75.9	30.3	6.4	25.6
T_7 - NRW	65.6	6.0	5.1	65.8	26.4	5.8	22.3
T_8 - ByR	86.9	7.5	9.0	97.3	36.3	8.5	33.6
T_9 - ByRW	72.4	6.3	6.8	82.2	31.3	7.3	29.5
T_{10} - BbNByR	92.1	8.3	9.8	99.0	37.7	8.7	35.8
CD (P=0.05)	7.1	0.5	1.0	4.9	2.8	0.6	2.5

Note: MC- Microbial consortium; AIRW- Rhizosphere water extract of acid lime; BbR- Rhizosphere soil of bamboo; BbRW- Rhizosphere water extract of bamboo; NR- Rhizosphere soil of neem; NRW- Rhizosphere water extract of neem; ByR- Rhizosphere soil of banyan; ByRW- Rhizosphere water extract of banyan; BbNByR- Rhizosphere soil of bamboo, neem and banyan.

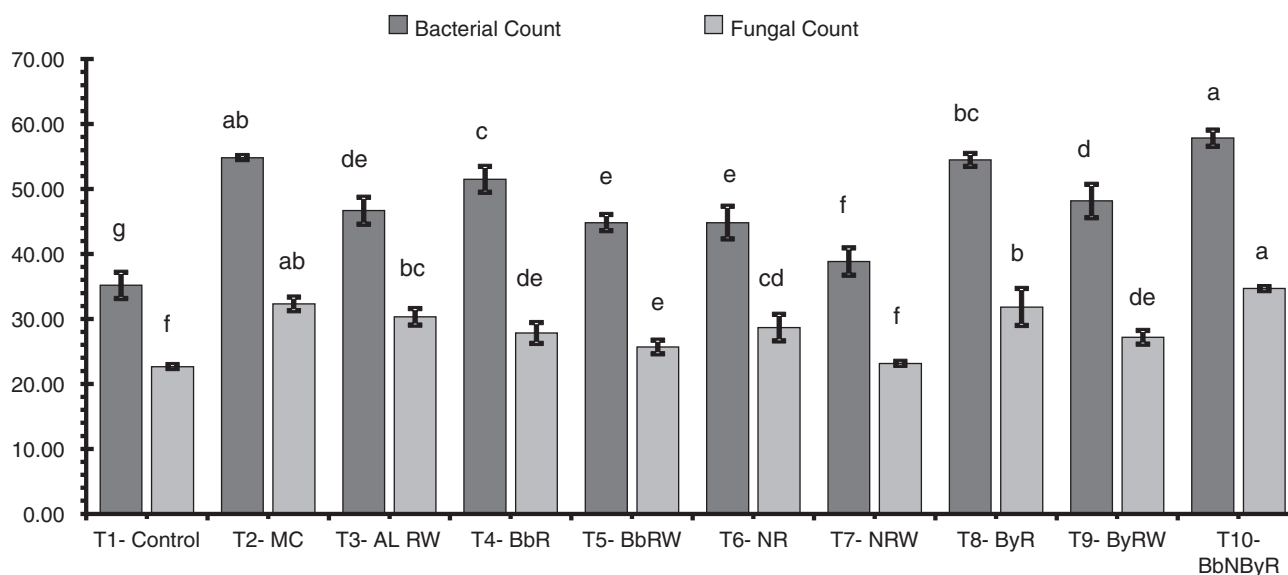


Fig 1 Response of rhizosphere hybridization on microbial count. Error bars indicate standard deviation. MC- Microbial consortium; ALRW- Rhizosphere water extract of acid lime; BbR- Rhizosphere soil of bamboo; BbRW- Rhizosphere water extract of bamboo; NR- Rhizosphere soil of neem; NRW- Rhizosphere water extract of neem; ByR- Rhizosphere soil of banyan; ByRW- Rhizosphere water extract of banyan; BbNByR- Rhizosphere soil of bamboo, neem and banyan

Treatment T₂ was found to be statistically analogous to T₁₀ in bacterial count and fungal count. Rhizosphere soil was attributed to the secretion of acidic root exudates, enzymes protons and mucilages. Root exudates can stimulate the microbial community in the rhizosphere by providing soil microbes with nutrients and easily degradable energy sources from root exudates and dead root cells (Kaksonen *et al.* 2006). Microbial diversity of *Ficus* (Rodge *et al.* 2016), Neem (Biswas *et al.* 2016) and Bamboo (Tu *et al.* 2014) were well studied to reciprocate their behaviour to conjugate and multiply in conducive environment. Thus, rhizosphere properties defined, in terms microbial pool have a strong effect on plant growth responses, depending upon diversity and evenness of rhizosphere of different tree species.

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