Potential of Rhizobacterial *Pseudomonas* and *Bacillus* spp. to Manage Papaya Ringspot Virus Disease of Papaya (*Carica papaya* (L.)

C. Ranasinghe, D.M. De Costa[1*], B.M.V.S. Basnayake[2], D.M. Gunasekera[2], S. Priyadharshani[2] and N.V.R. Navagamuwa[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Sri Lanka

ABSTRACT: *The present study was conducted to determine the potential of rhizobacterial species in managing Papaya Ringspot Virus Disease (PRSVD) and their ability to promote plant growth and yield and induce host plant resistance through the activity of defense-related enzymes. Twenty Pseudomonas spp. and four Bacillus spp. which were isolated from healthy papaya rhizosphere were applied by two methods, namely seed treatment and a root dip. The ability to reduce symptom development, promote plant growth and yield along with synthesis of peroxidase, phenylalanine ammonia lyase (PAL) and β-1, 3-glucanase under plant house and field conditions were evaluated. Molecular identification confirmed the presence of P. fluorescens, P. putida, P. aeruginosa, P. taiwanensis and several unidentified species of Pseudomonas among the Pseudomonas isolates used in the present study. Application of six selected bacterial isolates either by seed or root dip method reduced leaf symptom severity and increased the activity of peroxidase and PAL enzymes significantly (P<0.005), compared to the plants which were not treated with bacterial isolates. When the bacterial isolates were applied by the root dip method, there was no significant (P<0.005) difference on the activity of peroxidase, PAL and β-1, 3-glucanase among the isolates. Results revealed the scattered ability of the rhizobacterial isolates to promote plant growth, reduce symptom development of PRSVD and induction of defense-related enzymes, though all the desirable features were not possessed by any given isolate. Hence, application of rhizobacterial mixtures is encouraged.*

Keywords: *Plant growth promoting rhizobacteria, induced host plant resistance, peroxidase, phenylalanine ammonia lyase (PAL), β-1, 3-glucanase*

INTRODUCTION

Papaya (*Carica papaya* L.), belonging to the family Caricaceae, is one of the most important fruit crops in Sri Lanka at subsistence and commercial level. Papaya is cultivated throughout the tropical world and in the warmest parts of the sub tropics. Being a rich source of antioxidants, carotenes, vitamins, minerals and fiber, papaya is considered to be one of the nutritionally valuable fruit crops throughout the globe. Among numerous pests and diseases affecting the crop yield, papaya ringspot virus disease (PRSVD) caused by papaya ringspot virus (PRSV) is a biotic threat faced by papaya cultivations worldwide. PRSV affects the

[1] Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka

* Corresponding author: devikadecosta@gmail.com

[2] Plant Virus Indexing Unit, Department of Agriculture, Homagama, Sri Lanka

photosynthetic capacity of infected plants and subsequently reduces plant growth, produce deformed and inedible fruits and finally the death of the plant results in. When plants are infected by the PRSV at the seedling stage or within two months after transplanting, the trees will not produce mature fruits. If trees are infected at a later stage, fruit production is reduced and the quality of the fruit is reduced due to the development of oily-coloured rings spots on the peel and reduction of sugar concentration of the fruit tissues (Gonsalves, 1998). PRSV is transmitted by several species of aphids in non-persistent way (Maia *et al*., 1996). More often, roughing of infected plants, spraying the infected plants with systemic insecticides are the management measures practiced to manage PRSVD.

Considering the ineffectiveness, practical limitations and environmental and health hazards of roughing and spraying of systemic insecticides, biological control has been proposed as an alternative to manage PRSVD. Use of plant growth promoting rhizobacteria (PGPR) has become a novel trend in crop cultivation to promote plant growth and manage plant diseases as a non-chemical approach. PGPR are beneficial-free living soil bacteria that can promote the plant growth either directly or indirectly (Glick, 1995). In addition to plant growth promotion, application of PGPR has gained importance in crop protection as antagonists or biological control agents against a wide range of plant pathogens (Dey *et al*., 2014). In particular, the application of PGPR strains such as *P. fluorescens* 89B-27, *P. fluorescens* CHAO and *S. marcescens* strain 90-166P have greatly reduced viral diseases in a range of crops, namely cucumber, tomato, tobacco, chilli, cowpea, and soybean (Almaghrabi *et al*., 2014; Dey *et al.*, 2014; Gray and Smith, 2005).

Induction of host plant resistance is one of the mechanisms through which PGPR achieve protection against fungal, bacterial and viral plant pathogens (Halfeld-Vieira *et al*., 2006). PGPR-triggered induced host plant resistance strengthen the plant cell walls, alters host plant physiology and metabolic responses, leading to an enhanced synthesis of plant defense chemicals upon challenge by pathogens and/or abiotic stress factors (Ramamoorthy *et al.*, 2001). Therefore, the present study was conducted to isolate and identify potential PGPR for the management of PRSVD and to determine the efficiency of selected rhizobacterial isolates with reference to induction of host plant resistance in papaya for management of PRSVD and to determine plant growth and yield performances.

MATERIALS AND METHODS

Isolation of bacteria from rhizosphere

Soil adhered to the root system of healthy-looking plants, was collected at a depth of 10 cm from papaya cultivations at Puttlam, Kurunegala, Colombo and Nuwara Eliya districts. Soil suspensions were serially diluted and plated on Nutrient Agar (NA) and King's B (KB) (King *et al*., 1954) media and incubated at 28 ^{o}C for 2-3 days.

Identification of bacteria by biochemical and molecular methods

Well-separated bacterial colonies developed on NA and KB media were maintained as pure cultures and subjected to several biochemical assays (e.g. 3% KOH test, Gram staining, catalase test, gelatine liquefaction test, semi solid medium test, methyl red and VP test, starch hydrolysis test) and observed under UV light. Genomic DNA was isolated from the pure cultures of selected bacterial isolates and subjected to PCR using Ps-for 5'GGTCTGAGAGGATGATCAGT3' and Ps-rev 5'TTAGGTCCACCTCGCGGC3' primers

for amplification of a 990 bp long *Pseudomonas* specific gene region (Rajwar and Shagal, 2016). PCR products were subjected to DNA sequencing and DNA homology search using BLAST and FASTA.

Screening of bacterial isolates effective in reducing PRSVD and increasing of plant growth

A pot experiment was conducted at Plant Virus Indexing Centre, Homagama using papaya (var. red lady) to select promising rhizobacterial isolates in reducing the development of PRSVD. A total of 24 bacterial isolates (20 *Pseudomonas* spp. and 4 *Bacillus* spp. which were initially identified by morphological and biochemical tests) were used for screening. Two methods of application of bacterial isolates, namely seed treatment and root dipping were practiced. Prior to seed treatment with the bacterial isolates, papaya seeds were surface sterilized by a 1% sodium hypoclorite solution for 3 min, washed with distilled water and blot-dried. The surface sterilized seeds were soaked separately in the 24 bacterial suspensions, each having a cell concentration of $1x10^8$ cfu/mL for 18 h. Seeds soaked in bacterial suspensions were air-dried and seeded in polythene bags filled with sterilized potting medium (top soil and compost at 1:1 ratio) at a rate of one seeds/bag. The seeds soaked in sterile distilled water were served as the control treatment. Six weeks after sowing, the plants were transferred to larger pots, having the sterilized potting medium as above. At the time of transplanting and subsequently at two weeks and three weeks after transplanting the plants were treated as a soil drench with additional 250 mL volumes of the respective bacterial cell suspensions ($1x10^8$ cfu/mL).

In the root dip method, root systems of six weeks-old papaya seedlings were dipped separately in the 24 bacterial suspensions having a cell concentration of ($1x10^8$ cfu/mL) for 18 h prior to transplanting. Plant roots dipped in sterilized water for 18 h were served as controls. Two additional rounds of bacterial solutions, having cell concentrations of ($1x10^8$ cfu/mL) were added, two and three weeks after transplanting at a rate of 250 mL per pot. All the plants including controls were mechanically-inoculated with PRSV 15 days after transplanting. Each treatment combination (isolate x method of application) was replicated three times by maintaining three plants per replicate according to Complete Randomized Design (CRD).

Quantification of defense enzymes

A separate pot experiment was conducted using six selected bacterial isolates (five *Pseudomonas* spp. and one *Bacillus* spp.) at the Plant Virus Indexing Center to quantify defense-related enzymes in leaf tissues of papaya plants subjected to treatment with selected isolates of bacteria by two methods of application, namely seed and root dip treatments. Tender leaves (2^{nd} and 3^{rd} leaves from the top) were collected from the plants, 25-day after transplanting (i.e. 10-day after inoculation of PRSV and one day after the second application of the bacterial isolates to the transplanted plants). Collected leaves were snap frozen in liquid nitrogen and stored at -80 ^oC till used for enzyme extraction. Peroxidase, Phenylalanine Ammonia Lyase (PAL) and β-1,3- glucanase activity in leaf tissues of papaya were quantified as the representative defense-related enzymes synthesized due to induced host plant resistance, by methods described by Hammerschmidt *et al*., 1982; Dickerson *et al*., 1984 and Pan *et al*., 1991 respectively.

Field efficiency of selected PGPR isolates in managing PRSVD

A field experiment was done at the Plant Virus Indexing Centre to evaluate the field efficiency of six selected bacterial isolates, namely 1, 46, 53, 74 and 78 and B1 using papaya var. red lady, based on the findings of the pot experiment. Six weeks-old seedlings were transplanted in the fields in pits having dimensions of 2 x 2 x 2 m. Pits were filled with soil medium containing top soil :compost :cow dung at 1:1:1 ratio and allowed to have natural infection of PRSV. The two methods of treatment, seed and root dip were practiced as the way described in the pot experiment of the present study. Two weeks after field planting of the papaya seedlings, 250 mL of each bacterial suspension having a cell concentration of 1 x 10^8 cfu/mL was applied per pit. Then onwards, subsequent applications of the bacterial cell suspensions were done in monthly intervals until flowering stage of the plants. Each bacterial isolate x method of treatment combination was replicated three times according to a randomized complete block design.

Data collection

In the pot experiment, disease severity of papaya leaves were recorded from one week after inoculation of the virus till eighth week after inoculation at weekly intervals according to a disease scale developed in the present study. The disease severity was recorded according to a scale ranging from 0-7 where 0 = no symptoms and 7 = more than five leaves having severe symptoms. In the field experiment, disease severity of leaves was reported at monthly intervals, according to a disease scale ranging from 0 – 9 where 0 = no symptoms and 9 = all immature leaves showing severe mosaic symptoms, puckering and vein clearing. In the field experiment, disease severity of the fruits was recorded according to a scale developed in the present study. Accordingly, 0 = no symptoms, 1 = < 10% of the fruit surface has ringspot symptoms, 2 = 11-25% of the fruit surface has ringspot symptoms, 3 = 26-50% of the fruit surface has ringspot symptoms, 4 = 51-75 % of the fruit surface has ringspot symptoms, 5 = more than 75% of the fruit surface has ringspot symptoms. Ten fruits were used to record the disease severity per a combination of isolate x method of treatment.

As growth measurements, plant height and stem girth (15 cm above the soil level) were measured biweekly from the time of transplanting (six weeks) to 4 months after transplanting. Dry weights of the root and shoots were recorded. Activity of peroxidase, PAL and β-1,3- glucanase was quantified by the methods described by Hammerschmidt *et al*., 1982; Dickerson *et al*., 1984 and Pan *et al*., 1991, respectively.

Data analyses

Data on plant growth parameters were analyzed by analysis of variance using SAS software to determine significance or otherwise of method of application, treatments (isolates) and their interaction effects. Mean separation was done by Duncan's multiple range test. Disease severity data was analyzed by CATMOD procedure.

RESULTS AND DISCUSSION

Identification of bacterial isolates

Out of the bacterial isolates obtained from 100 soil samples used for isolation, 20 isolates were identified as *Pseudomonas* spp. (based on the growth on KB medium and biochemical

tests) and 4 isolates were identified as *Bacillus* spp. based on Gram staining, 3% KOH test and cell morphology. All the 20 isolates of *Pseudomonas* spp. resulted in a 990 bp PCR product when amplified by Ps-for and Ps-rev primers. DNA sequences of the 20 isolates of bacteria were highly homologous with different Pseudomonas spp. when subjected to DNA homology search as shown in Table 1. Accordingly, among the isolated *Pseudomonas* spp. of the present study, two isolates were highly homologous with *P. putida*, one with *P. flourescense*, three with *P. taiwanensis*, two with *P. aeruginosa* and 12 with different strains of *Pseudomonas* spp.

Table 1. Homology search results of *Pseudomonas* species isolated from papaya rhizosphere

Bacterial Isolate	Highly homology species	Strain	Accession No.	Max score	Total score	Query cover %	E value	% Identity
1	*Pseudomonas* sp.	CCUG 64384	LT601002	1327	1327	96	0.0	92
4	*Pseudomonas* sp.	P108(2011)	JF430834	1301	1301	96	0.0	94
6	*P. putida*	DLL-E4	CP007620	1411	6961	96	0.0	92
9	*Pseudomonas* sp.	UFSC-A611	MF572137	1716	1716	94	0.0	99
14	*P. taiwanensis*	TIL TAL 43	KT998859	1712	1712	94	0.0	99
33	*Pseudomonas* sp.	BJQ-B3	FJ600357	1351	1351	88	0.0	93
46	*P. fluorescens*	PML21	KX527632	1482	1482	95	0.0	95
49	Uncultured *Pseudomonas* sp.	Clone Filt 91	HM152678	1489	1489	91	0.0	96
50	*Pseudomonas* sp.	UFSCA 611	MF572137	1718	1718	95	0.0	99
53	*Pseudomonas* sp.	AR 470	LN829589	1029	1029	94	0.0	87
54	*Pseudomonas* sp.	P108 (2011)	JF430834	1317	1317	96	0.0	91
61	*P. putida*	Md1-34	MF581440	1696	1696	94	0.0	99
62	*P. taiwanensis*	TIL TAL 43	KT998859	1724	1724	93	0.0	99
66	*P. taiwanensis*	TIL TAL 43	KT998859	1714	1714	96	0.0	99
67	Uncultured *Pseudomonas* sp.	Clone Filt 34	HM152622	1703	1703	97	0.0	99
71	*Pseudomonas* sp.	UFSCA 611	MF572137	1716	1716	93	0.0	99
74	*Pseudomonas* sp.	F2-14	KT735211	1506	1506	90	0.0	96
75	*P. aeruginosa*	PCP18	HM439404	1387	1387	94	0.0	94
78	*Pseudomonas* sp.	UFSCA 611	MF572137	1681	1681	94	0.0	99
RS2	*P. aeruginosa*	AMU1	KF764698	1341	1341	91	0.0	93

Screening effective bacterial isolates to control PRSVD and promote plant growth

Severity of leaf symptoms

Severity of leaf symptoms differed significantly among plants treated with different treatments (Figure 1). However, no significant difference of leaf symptom severity was observed between the two methods of application. Isolates 1, 6, 46, 49, 50, 53, 74, 75, 78 and B1 resulted in disease severities lower than 4 under both treatment methods. Some of the bacterial isolates were very effective in reducing the severity of the leaf symptoms, when applied as a root dip than the seed treatment method (e.g. isolates 33, 54 and 71). The lowest recorded disease severity was given by isolate 6 when applied as a seed treatment. Isolates 4, 66, 67 and B3, when applied as a root dip treatment, were not effective in reducing the disease severity in comparison to the control treatment (Figure 1). Moreover, isolates 62 and 78 gave equal level of disease severity when treated by both methods of application. Therefore, a clear relationship between the method of application and the reduction of disease severity was not observed when all the bacterial isolates were considered (Figure 1).

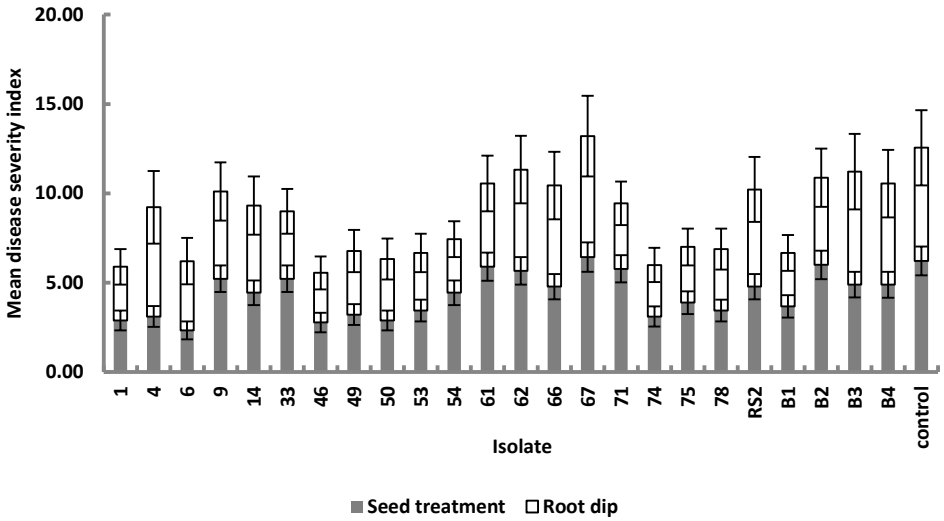

Figure 1. Severity of the leaf symptoms shown by PRSV-inoculated papaya plants which were treated with 24 isolates of bacteria as seed treatment or root dip treatment, in comparison to untreated control, at the 8th week after inoculation.

Growth performance

The interaction effect of method of application x treatment (bacterial isolate) (P<0.0021), method of application (P<0.0081) and bacterial isolates (P<0.0001) significantly influenced on plant height. Stem girth of the plants was significantly influenced by the method of application (P<0.0007) and the bacterial isolates (P<0.0001). Stem and root dry weights were significantly differed among the plants treated with different bacterial isolates (P<0.0003 and P<0.0001 respectively).

As shown in Table 2, plant height was significantly increased by the seed treatment of isolates 1, 6, 53, 54, 75, 78 and B1 in comparison to control treatment. However, root dip treatment of isolates 50 and 75 showed a significantly lower plant height than that of the plants of the control treatment. All the other isolates, when applied as a root dip treatment, had no significant difference on the plant height in comparison to that of the control treatment. There was no significant difference among the 24 isolates and the control treatment on stem girth, when the isolates were applied as a seed treatment. However, when the isolates 53 and 54 were applied as root dip treatments, stem girth of plants was increased significantly than that of the control. Significantly higher shoot dry weight, in comparison to that of the control was resulted in the plants treated with isolate 4 and 50. Root dry weight was significantly increased due to the treatments of nine bacterial isolates, namely 1, 6, 9, 66, 74, 75, 78, B1 and B3, when compared with that of the plants of control. Based on the performances of the above tested bacterial isolates on symptom severity on leaves, plant height, stem girth and root dry weight, isolates 1, 46, 53, 74, 78 and B1 were selected for further studies including defense-related enzyme assays and evaluation of field efficiency of the bacterial isolates. Isolate 46 was used in further studies as it was identified as the only *P. fluorescents* isolate in the present study.

Table 2. Plant height, stem girth, shoot dry weight and root dry weight of papaya plants treated with 24 different bacterial isolates under pot experimental condition

PGPR isolate	Plant height (cm)		Stem girth (cm)		Shoot dry weight (g)		Root dry weight (g)	
	Seed treatment	Root dipping	Seed treatment	Root dipping	Seed treatment	Root dipping	Seed treatment	Root dipping
1	69.0^{abc*}	67.7^{a}	6.7^{abcd}	6.4^{ab}	25.0^{ab*}	21.7^{b}	10.6^{abc}	10.8^{abcd}
4	65.8^{abcde}	61.3^{abc}	6.5^{abcde}	6.3^{abc}	19.5^{cdefgh}	40.3^{a*}	8.4^{ef}	10.1^{abcd}
6	70.6^{ab*}	68.0^{a}	6.3^{abcde}	6.5^{ab}	25.1^{a*}	21.9^{b}	10.6^{abc}	11.3^{ab*}
9	66.0^{abcde}	62.7^{abc}	5.8^{de}	5.7^{abcd}	16.4^{fghij}	17.9^{b}	10.8^{ab*}	10.0^{abcd}
14	66.3^{abcde}	63.0^{abc}	6.0^{cde}	5.8^{abcd}	14.7^{ij}	18.6^{b}	9.1^{bcdef}	9.7^{abcd}
33	62.6^{bcdefg}	64.1^{abc}	5.8^{de}	5.8^{abcd}	12.7^{j}	15.4^{b}	9.4^{abcdef}	9.3^{bcd}
46	58.5^{defg}	64.3^{abc}	5.8^{de}	6.0^{abcd}	19.6^{cdefgh}	18.7^{b}	9.2^{bcdef}	9.8^{abcd}
49	56.5^{fg}	64.6^{abc}	6.3^{abcde}	6.1^{abcd}	14.2^{ij}	17.1^{b}	9.5^{abcdef}	10.0^{abcd}
50	56.7^{fg}	43.5^{d*}	6.1^{cde}	5.4^{cd}	20.5^{bcdef}	13.9^{b}	10.7^{abc*}	9.8^{abcd}
53	72.3^{a*}	65.7^{ab}	7.2^{a}	6.7^{a*}	22.0^{abcd}	17.9^{b}	9.5^{abcdef}	11.0^{abc*}
54	69.8^{ab*}	65.6^{ab}	7.1^{ab}	6.7^{a*}	21.2^{abcd}	17.7^{b}	9.1^{bcdef}	10.6^{abcd}
61	62.1^{bcdefg}	61.2^{abc}	5.8^{de}	5.7^{abcd}	15.9^{ghij}	14.8^{b}	9.0^{bcdef}	9.2^{bcd}
62	57.8^{efd}	61.8^{abc}	5.7^{e}	5.5^{bcd}	12.6^{j}	13.0^{b}	8.2^{f}	8.6^{d}

66	60.8^{cdefg}	63.0^{abc}	5.7^{e}	6.0^{abcd}	15.4^{hij}	14.2^{b}	10.1^{abcde}	8.7^{d}
67	60.7^{cdefg}	61.7^{abc}	5.8^{de}	5.8^{abcd}	13.0^{ij}	17.3^{b}	8.4^{ef}	9.5^{bcd}
71	61.1^{cdefg}	58.3^{bc}	6.0^{cde}	5.7^{abcd}	20.0^{cdefg}	19.2^{b}	9.6^{abcdef}	9.9^{abcd}
74	63.5^{bcdefg}	68.0^{a}	6.2^{bcde}	6.5^{ab}	21.5^{abcd}	21.9^{b}	10.7^{abc*}	11.9^{a*}
75	66.5^{abcd*}	57.0^{c}	6.8^{abc}	5.2^{d}	17.6^{defghi}	16.3^{b}	10.3^{abcd}	10.4^{abcd}
78	72.7^{a*}	59.7^{abc}	6.6^{abcde}	5.6^{bcd}	23.0^{abc*}	18.7^{b}	10.3^{abcde}	10.8^{abcd}
RS2	66.3^{abcde}	62.4^{abc}	6.1^{cde}	5.9^{abcd}	16.9^{efghij}	15.0^{b}	8.8^{cdef}	8.8^{cd}
B1	67.6^{abc*}	66.6^{ab}	6.3^{abcde}	6.5^{ab}	21.1^{abcde}	18.1^{b}	11.2^{a*}	11.2^{ab*}
B2	65.0^{abcdef}	58.7^{bc}	5.8^{de}	5.6^{bcd}	14.7^{ij}	15.6^{b}	9.7^{abcdef}	10.0^{abcd}
B3	63.5^{bcdefg}	59.6^{abc}	5.8^{de}	5.5^{bcd}	15.5^{hij}	15.0^{b}	10.2^{abcde}	10.4^{abcd}
B4	55.5^{g}	59.1^{bc}	6.2^{bcde}	5.6^{bcd}	13.8^{ij}	10.4^{b}	8.6^{def}	10.0^{abcd}
Control	57.8^{efg}	59.7^{abc}	6.4^{abcde}	5.5^{bcd}	17.4^{defghi}	12.6^{b}	8.4^{ef}	8.6^{d}

Values with the same superscripts in each column are not significantly different at P=0.05.
** indicate the significantly higher values in comparison to the respective value in the control treatment.*

Activity of defense-related enzymes in papaya leaf tissues

Mean activity of peroxidase enzyme had a significant influence by the method of application (P<0.038) and the enzyme activity significantly differed among treatments (isolates) (P<0.0001). Activity of PAL and β-1,3-glucanase was significantly influenced by the bacterial isolates (P<0.0038 and P<0.0032 respectively), but not by the method of application. Findings revealed that application of all six bacterial isolates either by seed or root dip method increased the activity of peroxidase and PAL significantly in comparison to that of the control. However, with reference to β-1,3-glucanase, only the seed treatment of the bacterial isolates showed an increased enzyme activity than that in the plants in the control. The enzyme activity has no significant difference among the treatments when treated with the bacterial isolates as root dip (Table 3). The highest peroxidase and PAL activities were reported by isolate 78, irrespective of the method of application. When the bacterial isolates were applied by the root dip method, there was no significant difference among the bacterial isolates on the activity of PAL and β-1,3-glucanase.

Table 3. Yield for five picks and activity of peroxidase, PAL and β-1,3-glucanase in papaya leaf tissues collected at 10 days after inoculation of PRSV and one day after the second application of the bacterial isolates to the transplanted plants treated with five different bacterial isolates by two application methods under pot experimental condition

Isolate no.	Peroxidase (Changes in absorbance min^{-1} g^{-1} leaf tissue)		PAL (µg min^{-1} g^{-1} leaf tissue)		β-1,3- glucanase (mg min^{-1} g^{-1} leaf tissue)		Yield (kg) for five picks	
	Seed treatment	Root dipping	Seed treatment	Root dipping	Seed treatment	Root dipping	Seed treatment	Root dipping
1	18.46^{ab}	15.23^{ab}	371.09^{ab}	385.21^{a}	3.48^{a}	3.00^{a}	14.22^{a}	12.44^{a}
46	12.99^{cd}	10.33^{c}	364.77^{ab}	400.93^{a}	2.87^{ab}	2.85^{a}	14.00^{a}	11.00^{a}
53	18.07^{ab}	15.36^{ab}	377.35^{ab}	391.50^{a}	2.93^{ab}	2.87^{a}	11.77^{ab}	10.1^{a}
74	13.82^{bc}	11.08^{bc}	377.35^{ab}	366.34^{a}	2.94^{ab}	2.56^{a}	11.44^{ab}	10.67^{a}
78	18.89^{a}	18.65^{a}	427.66^{a}	419.80^{a}	3.07^{ab}	2.99^{a}	4.55^{c}	4.55^{b}
B1	15.72^{abc}	17.04^{a}	383.64^{ab}	367.91^{a}	2.42^{bc}	2.78^{a}	17.00^{a}	9.66^{a}
Control	8.89^{d}	7.76^{c}	322.22^{b}	295.59^{b}	2.05^{c}	2.03^{a}	5.66^{b}	9.55^{a}

Values with the same superscripts in each column are not significantly different at P=0.05

Field efficiency of selected bacterial isolates to manage PRSVD

Severity of leaf symptoms

Severity of leaf symptoms was significantly reduced by the six bacterial isolates under both application methods in comparison to the control (Figure 2a and 2b). It indicated the efficiency of all the selected bacterial isolates to reduce the symptom development on leaves under field conditions. However, there was no significant difference of the severity of symptoms of fruits of PGPR treated plants and the control under both methods of application (data not shown).

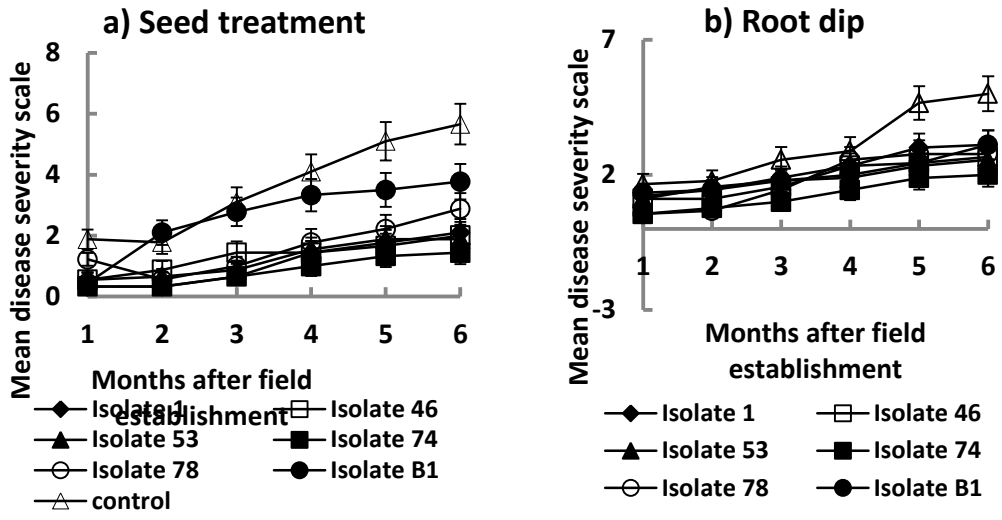

Figure 2. Severity of the symptoms on papaya leaves recorded over a six months period after transplanting of field plants treated with selected bacterial isolates by seed treatment (a) and root dip treatment (b)

Yield

Yield of papaya varied significantly by the treatment (isolates) and when the plants were treated with the seed treatment method (P<0.003). However, there was no significant difference among the isolates on yield when the plants were treated with the root dip method (Table 3). The plants treated with the isolates B1, 1 and 46 as a seed treatment, reported the highest yield. However, both methods of application of isolate 78 resulted in the lowest yield, which was significantly lower than that of the control treatment.

DISCUSSION

Bacterial genera belonging to *Pseudomonas, Bacillus, Azospirillum, Agrobacterium, Azotobacter, Arthrobacter, Alcaligenes, Serratia, Rhizobium, Enterobacter, Burkholderia, Beijerinckia, Klebsiella, Clostridium, Vario-vovax, Xanthomonas* and *Phyllobacterium* (Adesemoye *et al*., 2008) have been reported as PGPR. However, *Pseudomonas* and *Bacillus* sp. are the most widely used and studied genera as PGPR. Growth promotion and disease control ability of *Pseudomonas* and *Bacillus* spp. are complex and interrelated processes. It involves direct and indirect mechanisms such as synthesis of growth promoting hormones, production of siderophore, antibiotics, hydrogen cyanide and volatile compounds. Facilitation of solubilization of minerals and induction of host plant resistance are some other traits possessed by *Pseudomonas* and *Bacillus* spp. as PGPR. It has been reported the use of *Pseudomonas* and *Bacillus* spp. and their efficiency in promoting growth, controlling of fungal, bacterial and viral diseases in many agriculturally-important crops (Tahir *et al*., 2017; Wang *et al*., 2015; Saharan and Nehra, 2011).

Molecular identification confirmed the identity of the rhizobacterial isolates used in this study as *P. putida, P. fluorescens, P. aeruginosa,* P. *taiwanensis* and several other *Pseudomonas* spp. Previous reports have shown that *P. putida, P. fluorescens, P. aeruginoasa* and *P. taiwanesis* have the ability of producing fluorescent pigments and as promising candidates for plant growth promotion, insect pest management, control of fungal and viral pathogens of plants and to tolerate abiotic stress factors (Saharan and Nehra, 2011; Kupferschmied *et al.,* 2013; Vurukonda *et al.,* 2016).

Findings of the present study further confirmed the ability of the used bacterial isolates (*Pseudomonas* and *Bacillus* spp.) to increase root and shoot dry weight and plant growth. Moreover, application of some isolates of the bacteria used in the present study reduced the severity of PRSVD symptoms on leaves and fruits, indicating their ability to reduce the development PRSVD. Further, the findings of the present study are in agreement with the ability to increase the synthesis of some selected defense-related enzymes by the application of rhizobacterial isolates and the ability of PGPR to induce host plant resistance. For example, Altinok *et al*. (2013) and Moradi *et al*. (2012) have reported the ability of PGPR isolates alone or in combination, in increasing the activity of defense-related enzymes in crop plants such as eggplant and banana. Even though, none of the bacterial isolates reduce the PRSVD to zero level, the findings confirmed the potential of bacterial isolates used in the present study to be used for the management of PRSVD and growth promotion.

Findings of the present study clearly revealed that individual bacterial isolates confer one or several desirable properties in terms of growth promotion, induction of host plant resistance and protection of the plant from the viral disease. However, application of mixtures of PGPR isolates has shown synergistic actions in biological control of plant pathogens (Raupach and Kloepper, 1998). This is because, compared to the use of individual PGPR strains, mixtures of several strains may result in a more stable rhizosphere community, provide several mechanisms of biological control, and may suppress a broader range of pathogens (Pierson and Weller, 1994). Nevertheless, determining the most compatible combination/s of the PGPR isolates is challenging as all the time all combinations of PGPR may not successful in protecting the crop from incoming pathogens.

Future studies are needed to evaluate the effect of combined PGPR isolates and combined methods of application towards management of papaya ringspot disease and to determine the other benefits such as survival rate of PGPR isolates, growth promotion and induction of host plant resistance by the isolates which are indispensable for commercialization and sustainability.

CONCLUSIONS

The present study identified several rhizobacterial isolates of *Pseudomonas* and *Bacillus* spp., which have the ability to promote growth, reduce symptom development of PRSVD and enhance the activity of defense-related enzymes.

ACKNOWLEDGMENTS

Authors wish to acknowledge to the Council for Agricultural Research Policy, Sri Lanka for financial assistance and the staff of the Molecular virology division at Plant Virus Indexing Center, Homagama, for the support given on molecular studies.

REFERENCES

Adesemoye, A.O., Obini, M. and Ugoji, E.O. (2008). Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. Braz. J. Microbiol. *39*, 423-426.

Almaghrabi, O.A., Albishri, H.M. and Moussa, T.A.A. (2014). Enhancement of maize growth using some plant growth promoting rhizobacteria (PGPR) under laboratory conditions. Life Sci. J. *11*, 764-772.

Altinok, H.H., Dikilitas, M. and Yildiz, H.N. (2013). Potential of *Pseudomonas* and *Bacillus* isolates as biocontrol agents against Fusarium wilt of eggplant. Biotechnol. Biotechnol. Equip. *27*, 3952-3958.

Dey, R., Pal, K.K. and Tilak, K.V.B.R. (2014). Plant growth promoting rhizobacteria in crop protection and challenges. pp 31-58. In: Goyal A, Manoharachary, C (Eds.). A future challenge in crop protection against fungal pathogens. Springer, New York.

Dickerson, D.P., Pascholati, S.F., Hagerman, A.E., Butler, L.G. and Nicholson, R.L. (1984). Phenylalanine ammonia-lyase and hydroxy cinnamate CoA ligase in maize mesocotyls inoculated with *Helminthusporium maydis* or *Helminthusporium carbonum*. Physiol. Plant Pathol. *25*, 11-123.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. *4*, 109-117.

Gonsalves, D. (1998). A case study: control of papaya rings spot virus in papaya. Annu. Rev. Phytopathol. *36*, 415-437.

Gray, E.J. and Smith, D.L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol. Biochem. *37*, 395-412.

Halfeld-Vieira, B.A., Vieira, J.R., Rameiro, R.S., Silva, R.S. and Baract-Pereira, M.C. (2006). Induction of systemic resistance in tomato by autochrthonus phylloplace residence *Bacillus cereis*. Pesq. Agrop. Bras. *41*, 1247-1252.

Hammerschmidt, R., Nuckles, F. and Kuc, I. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum largenarium.* Physiol. Plant Pathol. *20*, 73-82.

King, E.O., Ward, M.K. and Raney, D.E. (1954). Two simple media for demonstration of pyocyanin and flourescin. J.Lab. Clin. Med. *44*, 301-307.

Kupferschmied, P., Maurhofer, M. and Keel, C. (2013). Promise for plant pest control: root-associated pseudomonads with insecticidal activities. Front. Plant Sci. *4*, 287.

Maia, I.G., Haenni, A.L. and Bernardi, F. (1996). Potyviral HC-Pro: a multifunctional protein. J. Gen. Virol. *77*, 1335-1341.

Moradi, H., Bahramnejad, B., Amini, J., Siosemardeh, A. and Haji-Allahverdipoor, K. (2012). Suppression of chickpea (*Cicer arietinum* L.) fusarium wilt by *Bacillus subtilis* and *Trichoderma harzianum*. Plant Omics. J. *5*, 68-74.

Pan, S.Q., Ye, X.S. and Kuc, J. (1991). A technique for detection of chitinase, beta-1,3-glucanse and protein patterns after a single separation using polyacrylamide gel electrophoresis or electro focusing. Phytopathol. *81*, 970-974.

Pierson, E.A. and Weller, D.M. (1994). Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathol. *84*, 940-947.

Rajwar, A. and Sahgal, M. (2016). Phylogenetic relationships of fluorescent pseudomonads deduced from the sequence analysis of 16S rRNA, *Pseudomonas*-specific and *rpoD* genes. Biotech. *6*, 1-10.

Ramamoorthy, V., Vishwanathan, R., Reghachander, T., Prakasan, V. and Samiyappan, R. (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protect. *20*, 1-11.

Raupach, G.S. and Kloepper, J.W. (1998). Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathol. *88*, 1158-1164.

Saharan, B.S. and Nehra, V. (2011). A critical review: plant growth promoting rhizobacteria. Life Sci. Medicine Res. *21*, 1-30.

Tahir, H.A.S., Gu, Q., Wu, H., Niu, Y., Huo, R. and Gao, X. (2017). *Bacillus* volatiles adversely affect the physiology and ultra-structure of *Ralstonia solanacearum* and induce systemic resistance in tobacco against bacterial wilt. Sci. Rep. *7*, 40481. Doi: 10.1038/srep40481

Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M. and SkZ, A. (2016). Multifunctional *Pseudomonas putida* strain FBKV2 from arid rhizosphere soil and its growth promotional effects on maize under drought stress. Rhizosphere, *1*, 4-13.

Wang, X., Mavrodi, D.V., Ke, L., Mavrodi, O.V., Yang, M., Thomashow, L.S., Zheng, N., Weller, D.M. and Zhang, J. (2015). Biocontrol and plant growth-promoting activity of rhizobacteria from Chinese fields with contaminated soils. Microb. Biotechnol. *8*, 404-418.