BIOLOGICAL CONTROL OF BROWN ROT DISEASE AND ENHANCED CROP PRODUCTION IN TEA BY A FLUORESCENT *PSEUDOMONAS* STRAIN

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ABSTRACT

A fluorescent Pseudomonas strain isolated from soil under tea cultivation and designated as RRLJ 134 exhibited in vitro antibiosis against Fomes lamoensis, the causal organism of brown rot disease of tea on three different synthetic media. Dressing of two year old tea cuttings with this strain enhanced the shoot height, root length, number of buds, leaves and chlorophyll content of the newly emerged leaves in the nursery condition. Also, a statistically significant increase in fresh and dry weight of root, shoot, leaf and a bud was noted against control. The application of this strain also showed significant reduction in the number of infected tea cuttings in soil amended with F. lamoensis under nursery condition. The result indicates the possible use of this strain as a bio-control agent of brown rot disease of tea besides enhancing the crop production.

Keywords: *biological control, brown rot disease, fluorescent Pseudomonas, Fomes lamoensis, tea.*

INTRODUCTION

Brown rot disease of tea, caused by the fungus *Fomes lamoensis,* is a primary root disease prevalent in low elevation tea growing areas. In North East India, sandy soil with low pH coupled with warm, rainy and/or humid weather favors this disease. The disease development is rapid (Ann *et al.*, 1999) and once established, it spreads to adjacent plants through root-to-root contact (Ann *et al.*, 1999a; Bolland, 1984). After the removal of diseased plant, it can survive in the roots remaining under the soil for more than 10 years (Chang, 1996). It can also remain viable as mycelium in the rhizosphere for several months.

Some measures for controlling the disease are flooding and fumigation with ammonia of fields and application of fungicides. But the recurrent rate after termination of treatments and their economic feasibility are still a problem. Moreover, continuous application of synthetic compounds deteriorates the soil fertility and crop production. Hence, nowadays interest has been shifted to environmentally safe and economically viable alternatives for crop improvement. Use of bio-agents such as plant growth promoting rhizobacteria (PGPR) serves as an ideal alternative, as their application results in physiological and bio-chemical stimulation of plant roots resulting in rapid emergence, higher chlorophyll content and enhanced stature (Lynch, 1976). Growth promotion can also depend on suppression of either deleterious microorganisms of the soil, that reduce plant growth and development, and/or of soil borne pathogens that cause diseases such as damping-off, rots and wilts (Ogoshi *et al.*, 1997). Among the PGPR strains, *Pseudomonas* and *Bacillus* genera received much attention.

Hence, keeping in view of the above facts, the present investigation was carried out to see the efficacy of a fluorescent *Pseudomonas* strain RRLJ

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134 on the growth and control of brown rot disease of tea.

MATERIALS AND METHODS

Organisms

The Rhizobacterial strains designated as RRLJ 134 was isolated from the tea growing soil (pH 5.0) of a tea plantation in Dooars region of West Bengal, India and identified as fluorescent *Pseudomonas* species through various morphological, physiological and biochemical test as described by Cappuccino and Sherman (1983) and Bergey's Manual of Systematic Bacteriology (Holt, 1984). The fungal pathogen *Fomes lamoensis* (culture no. 4140) was procured from Indian Type Culture Collections (ITCC) of Indian Agricultural Research Institute (IARI), New Delhi, India.

Soil

The experiment was conducted at Regional Research Laboratory, Jorhat campus on sandy-loam soil with pH 4.63, total nitrogen of 0.015 per cent, organic carbon 0.268 per cent and have no previous history of any pesticide or synthetic agrochemical application.

Plant

Tea (*Camellia sinensis* L. (O) Kuntze) cuttings (2 year old clone of TV 23) obtained from a local supplier was used in the studies.

In vitro antibiosis

Dual culture test, as described by Dileep Kumar and Dube (1992) was used to examine the antagonism of the strain RRLJ 134 against *F. lamoensis* on Nutrient Agar (NA), King's medium B (KB) and Potato Dextrose Agar (PDA) medium. For this a loopful of the strain RRLJ 134 was streaked on one side of the Petri dishes containing KB, NA and PDA (2.0 cm inside from the periphery) and a mycelial disc of *F. lamoensis* (≈ 6.0 mm), cut from an actively growing culture, was placed on the opposite end at a distance of 5.0 cm from the bacterial streak and allowed to grow at 28 ± 2° C. The zone of inhibition (in cm) produced by the strain against the test fungal pathogen was measured after seven days of growth. To check whether the inhibition was due to siderophore, the experiment was repeated in KB medium amended with 30μg $FeCl_3$.

Bacterization of tea cuttings

Two years old tea cuttings were bacterized with a patented technology developed by Bezbaruah and Dileep Kumar (1998) with the following modifications. The bacteria grown in KB medium was scrapped with a help of a sterile glass rod and mixed with moist sterile soil to make a paste (had approximately 1.0 x 10^7 cfu /gm soil). The surface sterilized root zone of the tea cuttings was dipped in this paste for 12h and 5g of this soil paste was attached around the bottom portion of the cutting before planting. Plant treated with moist soil without any bacteria served as the control. Both treated and control seedlings were planted in polythene bags filled with field soil and maintained under the nursery condition.

Growth promotion and disease suppression studies

The experiment was conducted in complete randomized design with four different treatments (50 plants per treatment) under nursery condition. The first treatment contained non-bacterized tea cuttings (as described above) planted in polythene bags containing field soil infested with 25 ml of homogenized mixture of mycelial culture of *F. lamoensis* grown in potato dextrose broth

for two weeks (pathogen alone). The second set contained tea cuttings bacterized with RRLJ 134 planted in the polythene bags containing field soil without *F. lamoensis* (only bacteria). The third treatment had bacterized cuttings planted in soil infested with *F. lamoensis* (bacteria + pathogen) and the last set contained non-bacterized cuttings planted in pathogen free soil (no bacteria + no pathogen, i.e., control). The collar region of each cutting from second and third sets were drenched three times at an interval of 15 days with 20 ml of the RRLJ 134 solution (adjusted to have approximately 1.0 x 10^7 cfu/ml). The observation on different growth parameters such as root length, shoot height, number of leaves, fresh and dry weight of root, shoot, leaf and a bud along with disease incidence were recorded at an interval of 15 days up to 90 days.

RESULTS

RRLJ 134 exhibited a zone of inhibition against *F lamoensis* in all the test media (Table 1). Best inhibition zone was noted in potato dextrose agar. Addition of iron into the KB medium had no effect on the extent of inhibition.

Table 1: *In vitro* antibiosis as zone of inhibition produced by RRLJ 134

Fungal pathogen	Inhibition zone (in cm) after 7 days of growth on		
	PDA	KB	NA
Fomes lamoensis	2.23	1.00	2.10

36% disease incidence was recorded in case of *F. lamoensis* challenged inoculated tea cuttings followed by 16% and 11% in case of treatment *F. lamoensis* + RRLJ 134 and RRLJ 134 respectively over control and consequently 56% disease control was recorded in case of *F. lamoensis* infested tea cuttings treated with RRLJ 134.

Bacterization of RRLJ 134 resulted a statistically significant increment of the shoot height, root length and number of leaves over the control (Table 2). Maximum shoot height, root length and number of fresh leaves were recorded from the tea cuttings treated with RRLJ 134 followed by *F. lamoensis* + RRLJ 134. All the treatments were found to significantly differ from each other in case of their effect on all the parameters recorded.

Table 2: Effect of different treatments on root, shoot and number of leaves of tea cuttings

Treatments	Root length (cm)	Shoot height (cm)	No. of leaves
Fomes lamoensis	12.54^{d}	30.75^{d}	3.40^{d}
RRLJ 134	22.13^{a}	45.11^{a}	15.00^{a}
F. lamoensis +RRLJ 134	19.85^{b}	35.50^{b}	10.00^{b}
Control	15.00^{c}	34.87^{c}	8.50^{c}
S.Ed.±	0.12	0.24	0.36
CD-5%	0.25	0.50	0.75

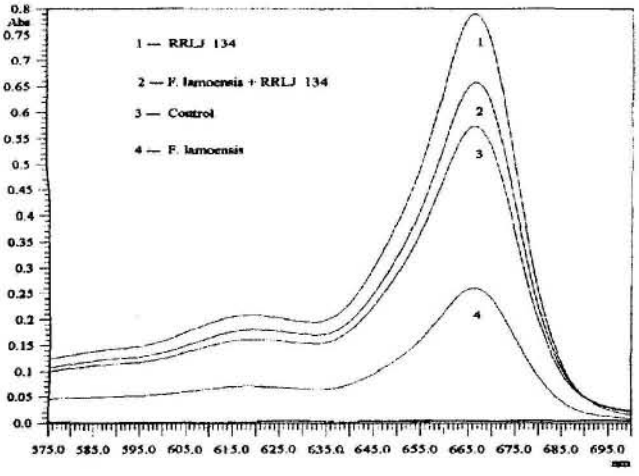
An enhanced fresh and dry weight of shoot, root and leaf & a bud was also recorded with the treatments over the control (Table 3).

Table 3: Effect of different treatments on fresh and dry weight of root, shoot and leaf & a bud of tea cuttings.

Treatments	Root		Shoot		Leaf & a Bud	
	Fresh wt. (g)	Dry wt.(g)	Fresh wt. (g)	Dry wt.(g)	Fresh wt. (g)	Dry wt.(g)
Fomes lamoensis	0.30^{d}	0.18^{d}	3.89^{d}	1.59^{c}	0.65^{c}	0.22^{b}
RRLJ 134	0.91^{a}	0.58^{a}	8.11^{a}	2.86^{a}	0.89^{b}	0.29^{a}
F. lamoensis +RRLJ 134	0.87^{b}	0.45^{b}	6.50^{b}	2.47^{ab}	1.13^{a}	0.21^{b}
Control	0.77^{c}	0.34^{c}	6.02^{c}	2.18^{b}	0.60^{c}	0.20^{b}
S.Ed.±	0.04	0.02	0.19	0.22	0.06	0.02
CD-5%	0.09	0.04	0.39	0.46	0.12	0.05

Here also, the highest increment was noted with RRLJ 134 except fresh weight of leaf & a bud, which was found to be highest with *F. lamoensis* + RRLJ 134 treatment. Statistical analysis revealed that all the treatment differ significantly in their effect except shoot dry weight, fresh and dry weight of leaf & a bud. Enhanced chlorophyll content in the newly emerged leaves was also noted in the bacterized cuttings (Fig. 1). Highest chlorophyll content was recorded in RRLJ 134 alone and least was noted with cuttings grown in *F. lamoensis* alone soil. Tea cuttings treated with RRLJ 134 and grown in *F. lamoensis* infested soil showed improved chlorophyll content over pathogen alone as well as the control plants.

Fig.1. Absorption spectra of chlorophyll of tea leaves with different treatments



DISCUSSION

The *in vitro* antibiosis studies had confirmed that the strain RRLJ 134 have the ability to control the growth of the test fungus in three media. The amendment of iron into the medium did not influence the inhibition capacity, proves that even though the strain produced siderophore, the inhibition was not siderophore mediated. Further studies showed that the strain RRLJ 134 was able to produce a phenazine type antibiotic in KB media with antifungal activity (Mishra and Dileep Kumar, unpublished data). Siderophore and/or antibiotic mediated antibiosis is reported in *Pseudomonas* species by many workers (Reddy *et al.*, 2003; Dekaboruah and Dileep Kumar, 2002; Dileep Kumar *et al.*, 2001; Dileep Kumar, 1998; Ogoshi *et al.*, 1997). The results confirmed the ability of this rhizobacterial strain in controlling the disease besides growth promotion in tea. Biological control of plant diseases through plant growth promoting rhizobacteria, particularly with *Pseudomonas* species reported earlier by different workers (Reddy *et al.*, 2003; Ogoshi *et al.*, 1997). Earlier from our laboratory, we reported a strain belongs to *Proteus* genera producing siderophore, was able to control the termite infestation in tea plants besides enhanced biomass production (Bezbaruah *et al.*, 1996).

We also developed a methodology for growing this organism in a synthetic medium containing urea, molasses, KH_2PO_4 and $MgSO_4.7H_2O$. Spraying of 10 per cent aqueous broth of the RRLJ 134 grown in this medium for seven days at the collar region of tea plants (100ml/plant) previously infested with *F. lamoensis* curtailed the disease incidence upto 50 per cent under nursery condition (data not shown). The efficacy of this formulation in field condition is under evaluation.

From the above findings it can be concluded that the strain RRLJ 134 can be utilized for the crop improvement and/or suppression of brown rot in tea after successful field trails.

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