

## RESEARCH ARTICLE

### EFFECT OF TWO BACTERIAL ISOLATES ON SELECTED IMPROVED RICE VARIETIES UNDER *IN VITRO* AND GREENHOUSE CONDITION

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#### ABSTRACT

Sri Lankan rice cultivation has been heavily dependent on the large-scale use of inorganic fertilizers since the introduction of new improved rice varieties to farmers in the 1960s. Excessive use of chemical fertilizers is adverse on human health and the environment. Plant growth-promoting bacteria are recognized as an eco-friendly approach to replace inorganic fertilizer. However, there are a few numbers of reports on efficient biofertilizers for Sri Lankan conditions. Therefore, the objective of this study was to determine the effect of two selected local bacterial isolates (I-I and I-II) under *in vitro* and greenhouse condition, on commonly grown improved rice varieties in the low country of Sri Lanka. The experiment was carried out in a Completely Randomized Design with three replicates. Bacterial isolates ( $10^6$  colony forming units / millilitre) were inoculated on 2-day old sterile rice seedlings. Sri Lankan improved rice variety Bg 300 inoculated with I-I and I-II reported significant low number of days to flowering (DF) as  $74 \pm 0$  and  $75 \pm 0.11$  in contrast to that of control plants ( $79 \pm 0.16$ ) and both isolates I-I and I-II significantly increased root length as  $10 \pm 0.4$ , and  $12 \pm 0.03$  cm respectively, over control plants ( $7 \pm 0.2$  cm) under *in vitro* condition. Above experiment was extended under greenhouse condition with three improved rice varieties of Bg 300, Bg 379/2 and At 308 in Faculty of Agriculture, University of Ruhuna, Mapalana, Sri Lanka (in ecological zone WL2 of Low Country Wet Zone) and with Bg 300 in Beliatta, Sri Lanka (in ecological zone IL1b of Low Country Intermediate Zone) under greenhouse condition with four replicates. The bacteria inoculated plants of Bg 379/2 reported significantly lower number of DF of  $112.5 \pm 0.86$  and  $114 \pm 0.28$  in contrast to that of control plants ( $118.5 \pm 1.22$ ). The plants of Bg 300 and Bg 379/2 with I-I, produced the highest number of grains per first panicle (GP) as  $134 \pm 7.35$  and  $157 \pm 11.2$  respectively over those of the control plants of Bg 300 and Bg 379/2 ( $105.75 \pm 6.98$  and  $132 \pm 2.94$  respectively). Bacteria inoculation did not affect DF and GP in rice variety At 308 indicating the different responses of variety on inoculation in the experiment at WL2. Bacterial isolate I-I inoculated plants showed significantly higher GP in contrast to control in greenhouse at IL1b. There is a potential for tested bacteria to be developed as a biofertilizer through subsequent field experiments in the future.

Keywords: Inorganic fertilizer, Low Country Wet Zone of Sri Lanka, Plant growth promoting bacteria, Rice, Yield

#### INTRODUCTION

Rice (*Oryza sativa*) is the major cereal crop, which provides staple food for more than half of the world population. Chemical fertilizers are an indispensable component in modern

commercial agriculture. Use of chemical fertilizers exceeding the recommendation can cause many problems in the environment and human health. In order to overcome these issues, use of microorganisms as an alternative to chemical fertilizers supplying

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nutrients to plants have emerged as a sustainable solution.

Use of plant growth promoting bacteria (PGPB) has been a practice since decades. As reported by Smith (1992), mixing "naturally inoculated" soil with seeds became a recommended method of legume inoculation in the USA. The first patent ("Nitragin") was a *Rhizobium* sp. based inoculation (Nobbe and Hiltner 1896). Russia had used non-symbiotic, a rhizosphere bacteria in the 1930s and 1940s in commercial agriculture. Although application of PGPB on rice was not intensively reported as legumes, it has been proven that *Bacillus* (Xiao *et al.* 2020), *Pseudomonas* (Ghaffari *et al.* 2018), *Enterobacter* (Mitra *et al.* 2018), and *Streptomyces* (Gopalakrishnan *et al.* 2013) positively affected rice growth and yield. In Vietnam, application of a biofertilizer named BioGro based on various PGPR strains resulted in increase in rice growth and yield (Nguyen *et al.* 2003). The *Azolla-Anabaena* symbiosis is most important in terms of biological nitrogen fixation (Ito and Watanabe 1995; Roy *et al.*; 2016; Singh *et al.* 1988).

Ye *et al.* 2019 had reported that increasing nitrogen fertilizer levels result in a higher number of grains per panicle of rice. Supplementation of nitrogen to rice through PGPB must be a cost-effective strategy. However, there are limited records available on the evaluation of responses of rice varieties for local PGPBs in Sri Lanka. Most of the biofertilizers are inefficient under different environmental conditions and response of rice varieties to biofertilizer is variable, which must be due to their genetic differences (Malusa *et al.* 2016). The evaluation of response on commonly grown Sri Lankan rice varieties for the inoculation of PGPB is an essential step in developing biofertilizers for the future. Therefore, the objective of this study was to determine the effect of inoculation of two bacterial isolates on rice under *in vitro* and greenhouse conditions on the selected growth and yield parameters, which may be helpful in developing an

efficient biofertilizer in the future through subsequent repeated field experiments.

## MATERIALS AND METHODS

The experiment on determination of rice growth promoting activity of two bacterial isolates under *in vitro* condition.

Two bacterial isolates (I-I and I-II), in which phosphorus solubilizing ability under *in vitro* condition had been confirmed previously were used for this experiment. Improved rice variety Bg 300 was used for the inoculation. The experiment was carried out under *in vitro* condition at the Faculty of Agriculture, University of Ruhuna, Sri Lanka.

The bacterial isolates from glycerol stocks were streaked on Luria Broth (LB) medium petri plates. The plates were incubated at room temperature (30 °C) for one day. A toothpick of culture from each plate was inoculated to 50 ml of liquid LB and incubated in a shaker at 125 rpm at room temperature (30 °C) for 1 day. Seeds were washed under tap water and dehusked carefully. Next, seeds were surface sterilized using 5% commercial Clorox for 5 minutes followed by rinsing with sterile distilled water five times. The seeds were germinated on wet sterile tissue paper on petri dishes at room temperature. Five days old seedlings were inoculated with respective bacterial isolate at mid-log phase of growth for 20 minutes. Germinating seeds without bacterial inoculation were maintained as the negative control. The inoculated and non-inoculated rice seedlings were transferred to autoclaved mud containing, quarter filled glass bottles of 12.5 cm in height and 5.8 cm in diameter with a lid. The mud was sandy clay loam soil from the field of Faculty of Agriculture, University of Ruhuna. The soil pH was 6.47 and electrical conductivity was 0.17ms cm<sup>-1</sup>, while the organic matter was 4.68%. The bottles were kept at room temperature, in a Completely Randomized Design (CRD) with four replicates per each treatment. Bottles were watered with autoclaved distilled water when required. When plants were 4 weeks old, a sterilized polythene cover was placed to facilitate plant growth replacing the lid. Days

to flowering, plant height at flowering stage, and root length were measured.

Experiment on determination of rice growth promoting activity of isolates under closed greenhouse condition at the Faculty of Agriculture University of Ruhuna, Mapalana (in agro-ecological zone WL2 of Low Country Wet Zone) and Beliatta (in agro-ecological zone IL1b of Low Country Intermediate Zone). An open greenhouse was used in Beliatta.

Bacterial isolates I-I and I-II and three improved rice varieties (Bg 300, Bg379/2 and At 308) were grown for the experiment. Bacteria culture, rice seed sterilization and bacteria

inoculation were similar to those of *in vitro* experiment. Mud for experiment was obtained from Faculty of Agriculture, University of Ruhuna field and a farmer field in Beliatta, (which were sandy clay loam soil and clay loam soil respectively). The experiment in two locations were laid as CRD with four replicates in the greenhouse of each location. The inoculated and non- inoculated seedlings were maintained in sterilized mud filled cups (of 4 cm in diameter and 3 cm in height) for 2 weeks followed by transfer to pots of 22 cm in diameter and 20 cm in height as one plant per pot. The pots contained unsterilized mud from the relevant field. Pots were watered at regular intervals. DF and GP and plant height at flowering stage were measured. Greenhouse temperature was recorded during the experimental period. Inorganic fertilizer was not supplied.

### Statistical Analysis

Data was analyzed using SAS software for two way ANOVA and, Duncun's Multiple

range Test (DMRT) for mean separation.

## RESULTS AND DISCUSSION

### Experiment 1

In the *in vitro* experiment, the plants of Bg 300, inoculated with I-I and I-II reported significantly low DF as  $74 \pm 0$  and  $75 \pm 0.11$  in contrast to control plants ( $79 \pm 0.16$ ). The inoculated rice plants of I-II reported higher plant height at flowering stage ( $46 \pm 2.5$  cm) in contrast to control plants ( $38 \pm 0.5$  cm). Root length had been increased by I-I and I-II as  $10 \pm 0.4$ , and  $12 \pm 0.03$  cm respectively, over control plants with  $7 \pm 0.2$  cm (Table 1). De Souza *et al.* (2013) reported that most of the microorganisms isolated from the rhizosphere of rice produced indole compounds such as IAA. Furthermore, bacterial secretion of indolic compounds helps to increase root surface and length. Longer roots facilitate high nutrient and water uptake to the plants (Persello-Cartieaux 2003). According to our results, significantly higher root length in bacteria inoculated rice seedlings in contrast to non-inoculated bacteria could be due to the production of phytohormones by the inoculated bacteria. Furthermore, significant reduction of days to flowering and increase of plant height in inoculated rice seedlings also could be due to the production of phytohormones by the inoculated bacteria. This implies that the two isolates promote plant growth in rice variety Bg 300 under *in vitro* condition.

Average monthly temperatures from August to December 2018 were  $33.23^\circ\text{C} \pm 1.59$ ,  $34.16^\circ\text{C} \pm 3.79$ ,  $35.26^\circ\text{C} \pm 3.26$ ,  $33.8^\circ\text{C} \pm 1.6$  and  $32.4^\circ\text{C} \pm 1.79$  in the greenhouse (Figure 1). The bacteria inoculated plants of Bg379/2 reported significant lower number of days to flowering (DF) of  $112.5 \pm 0.86$  and  $114 \pm 0.28$  in contrast to control plants of  $118.5 \pm 1.22$  in green house

**Table 1: Effect of bacteria inoculation on plant growth parameters of rice variety Bg 300 under *in vitro* condition**

Character	Treatment Level		
	I-I	I-II	Negative control
Days to flowering	$74 \pm 0^b$	$75 \pm 0.11^b$	$79 \pm 0.16^a$
Plant height at flowering (cm)	$43.5 \pm 0.76^{ab}$	$46 \pm 2.50^a$	$38 \pm 0.50^b$
Root length (cm)	$10 \pm 0.40^a$	$12 \pm 0.03^a$	$7 \pm 0.20^b$

at Faculty of Agriculture, University of Ruhuna.

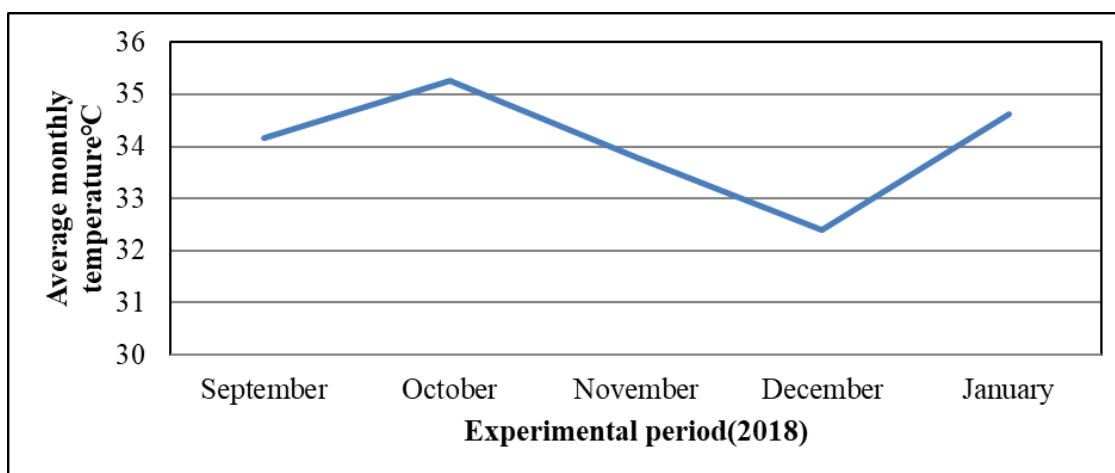
Ye *et al.* 2019 reported that supply on inorganic phosphorus could induce early flowering. The early flowering may have resulted due to the phosphorus solubilizing ability of the isolates. The plants of Bg 300 and Bg 379/2 with I-1, produced the highest number of grains per first panicle (GP) as  $134 \pm 7.35$  and  $157 \pm 11.2$  respectively over those of the control plants of Bg 300 and Bg 379/2 ( $105.75 \pm 6.98$  and  $132 \pm 2.94$  respectively) as indicated in Table 2. Bacteria inoculation did not affect DF and GP in At 308 suggesting the different responses of variety on inoculation. Phosphorus is an essential plant nutrient as it plays very

important role in plant growth. Phosphate solubilizing bacteria play an important role in soil Phosphorus dynamics and subsequent accessibility of phosphate to the plants, (Richardson 2001). Chen *et al.* 2006 showed that bacterial genera, including *Bacillus*, *Rodooccus*, *Antherobacter*, *Serratia*, *Gordonia* have the ability to solubilize phosphorous. Two bacterial isolates tested in this experiment were able to solubilize phosphorous. It could be another reason for the positive responses of inoculated plants than the non-inoculated plants.

Several studies have revealed that some PGPB can facilitate resistance to environmental stresses. During the environmental stress conditions endogenous

**Table 2: Effect of inoculation on number of grains per first panicle and days to flowering in three improved rice varieties under greenhouse condition at Faculty of Agriculture, University of Ruhuna**

Rice variety	Treatment	Number of grains per first panicle (GP)	Days to flowering (DF)
Bg 300	Isolate I-I	$134 \pm 7.35^a$	$114.5 \pm 1.25^a$
	Isolate I-II	$123.67 \pm 3.33^{ab}$	$116.75 \pm 1.84^a$
	control	$105.75 \pm 6.98^b$	$117.5 \pm 0.50^a$
Bg 379/2	Isolate I-I	$157 \pm 11.20^a$	$112.5 \pm 0.86^b$
	Isolate I-II	$134.67 \pm 5.62^a$	$114 \pm 0.28^b$
	control	$132 \pm 2.94^a$	$118 \pm 1.22^a$
At 308	Isolate I-I	$92.25 \pm 3.54^a$	$94.75 \pm 0.47^a$
	Isolate I-II	$94.25 \pm 9.12^a$	$94.5 \pm 0.47^a$
	control	$85.33 \pm 14.79^a$	$93.33 \pm 0.28^a$



**Figure 1: Variation of average environmental temperature during the experimental period in the greenhouse at Faculty of Agriculture, University of Ruhuna**

ethylene production is accelerated and increased ethylene level negatively affects plant growth and development (De Souza *et al.* 2015). The certain bacterial strains contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity which is one among the mechanisms that regulate the ethylene production of plants (Glick 2005). During the experiment period average temperature of the greenhouse was between

32.4<sup>0</sup>C-35.26<sup>0</sup>C, which must have exceeded the ambient temperature (Figure 1). Even under the high temperature conditions, bacteria inoculated plants performed better than non-inoculated plants (Table 2; Figure 2). Therefore, it can be suggested that the tested two bacteria isolates may have the ability to secrete ACC deaminase which needs further confirmation through evidences of molecular analysis for ACC deaminase gene. Variety

**Table 3: Effect of bacteria inoculation on plant growth parameters of rice variety Bg 300 under open-greenhouse condition at Beliatta**

Character	Treatment levels		
	I-I	I-II	Control
Plant height at flowering (cm)	91.5±2.02 <sup>a</sup>	94±3.78 <sup>a</sup>	83.66±4.4 <sup>a</sup>
Number of grains per first panicle (GP)	93.66±2.3 <sup>a</sup>	62.66±8.19 <sup>b</sup>	68.33±3.75 <sup>b</sup>



**Figure 2: Effect of inoculation on three improved rice varieties at vegetative stage under greenhouse condition at Faculty of Agriculture, University of Ruhuna**



**Figure 3: Effect of inoculation on improved rice variety Bg 300 at flowering stage under open-greenhouse condition at Beliatta**

and location interaction must be determined for I-I under field conditions for its utilization as a bio-fertilizer in the future.

According to results bacteria inoculation did not affect plant height at intermediate zone in Beliatta in contrast to control (Figure 3). Bacterial isolate I-I inoculated plants showed higher GP in contrast to those of control and bacteria isolate I-II inoculated plants (Figure 4). During the experiment period average

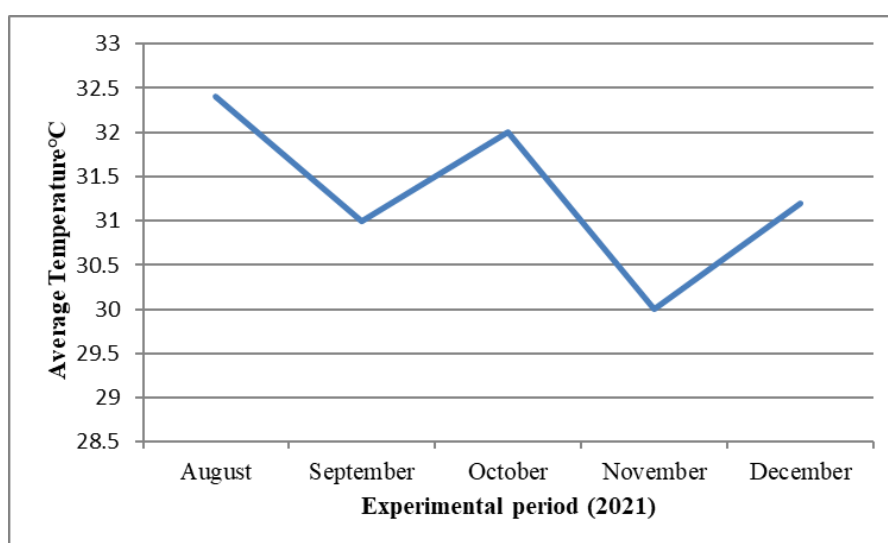
temperature of the greenhouse was between 30.2°C-32.4°C (Figure 5).

### CONCLUSION

The plants of Bg 300 inoculated with I-I and I-II reported significantly low number of DF and both isolates significantly increased root length under *in vitro* condition. Further inoculated rice plants of bacteria I-II produced significantly higher plant height at flowering stage in contrast to control plants in *in vitro*



**Figure 4: Effect of inoculation on panicle size of rice variety Bg 300 under open-greenhouse condition in Beliatta**



**Figure 5: Variation of average environmental temperature during the experimental period in the open-greenhouse in Beliatta**



experiment. Under greenhouse experiment at Faculty of Agriculture, University of Ruhuna, the bacteria I-I and I-II inoculated plants of Bg379/2 reported significantly lower number of DF. Further, bacteria I-I inoculated plants of Bg 300 and Bg 379/2 produced the highest number of grains per first panicle compared to the control plants of Bg 300 and Bg 379/2. In open greenhouse in Beliatta, bacterial isolate I-I inoculated Bg 300 plants showed higher GP in contrast to those of control and plants of bacterial isolate I-II.

Bacterial isolate I-I and I-II exhibit a potential in plant growth promotion and yield increase under *in vitro* and greenhouse conditions in the selected locations in low country wet zone and low country intermediate zone of Sri Lanka.

Further field experiments in both locations will be useful for identification of effect on environment on plant response to I-I and I-II for development of efficient biofertilizer in the future.

#### AUTHOR CONTRIBUTION

SG conceptualized and designed the experiment. LGIC performed the experiment, analyzed the data and drafted the manuscript. SG, IM, PG and GS supervised the experiment and revised the manuscript.

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