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...
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 nonsymbiotic, 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 Watanable 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⁻¹, 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 ±0 and 75±0.11 in contrast to control plants (79 ±0.16). The inoculated rice plants of I-II reported higher plant height at flowering stage (46 ±2.5 cm) in contrast to control plants (38 ±0.5 cm). Root length had been increased by I-I and I-II as 10 ±0.4, and 12±0.03 cm respectively, over control plants with 7 ±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°C±1.59, 34.16°C±3.79, 35.26°C±3.26, 33.8°C±1.6 and 32.4°C±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±0.86 and 114±0.28 in contrast to control plants of 118.5±1.22 in green house

<table>
<thead>
<tr>
<th>Table 1: Effect of bacteria inoculation on plant growth parameters of rice variety Bg 300 under in vitro condition</th>
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</thead>
<tbody>
<tr>
<td>Character</td>
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<td>------------------------------</td>
</tr>
<tr>
<td>Days to flowering</td>
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<tr>
<td>Plant height at flowering (cm)</td>
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<tr>
<td>Root length (cm)</td>
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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-I, produced the highest number of grains per first panicle (GP) as 134±7.35 and 157±11.2 respectively over those of the control plants of Bg 300 and Bg 379/2 (105.75±6.98 and 132±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

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>Treatment</th>
<th>Number of grains per first panicle (GP)</th>
<th>Days to flowering (DF)</th>
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</thead>
<tbody>
<tr>
<td>Bg 300</td>
<td>Isolate I-I</td>
<td>134±7.35a</td>
<td>114.5±1.25a</td>
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<tr>
<td></td>
<td>Isolate I-II</td>
<td>123.67±3.33b&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.75±1.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>105.75±6.98b</td>
<td>117.5±0.50a</td>
</tr>
<tr>
<td>Bg 379/2</td>
<td>Isolate I-I</td>
<td>157±11.20a</td>
<td>112.5±0.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isolate I-II</td>
<td>134.67±5.62a&lt;sup&gt;a&lt;/sup&gt;</td>
<td>114±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>132±2.94a</td>
<td>118±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>At 308</td>
<td>Isolate I-I</td>
<td>92.25±3.54a</td>
<td>94.75±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isolate I-II</td>
<td>94.25±9.12a</td>
<td>94.5±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>85.33±14.79a</td>
<td>93.3±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
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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 is negatively affects for 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°C-35.26°C, which must had 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

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

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.

REFERENCES
Glick BR 2005 Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiology Letters, 251(1), 1-7.
Richardson AE 2001 Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Functional Plant Biology, 28(9), 897-906.
Singh AL, Singh PK and Lata P 1988 Effects of different levels of chemical Nitrogen (urea) on Azolla and Blue-
green algae intercropping with rice. Fertilizer research, 17(1), pp.47-59.


