FULL TEXT

ABSTRACT
The System of Rice Intensification (SRI) is an innovative method of rice (Oryza sativa) cultivation that combines many farm practices. Though the benefits of SRI are obvious, the underlying principles in enhanced yields are not yet scientifically analyzed. Two important components of SRI are keeping the rice field moist without flooding and frequent weeding out practices that enhance the aerobic conditions which in turn improve soil biological activity including enhanced root growth and activity of aerobic soil organisms. We have taken up the present investigations, with the premise that soil microorganisms especially fluorescent pseudomonads (FLPs), whose role in enhancement of plant growth is unequivocal, may contribute to the enhanced growth and yield of rice cultivated under SRI. The results of present investigations revealed that rice cultivated under SRI harboured more the population of FLPs in rhizosphere than non-rhizosphere soil. Screening of rhizospheric FLPs isolates has revealed that many of the isolates possessed the ability of producing growth promoting substances like IAA, GA, siderophores, ‘p’ solubilization. Some selected strains have also shown resistance towards heavy metals, salts and pH. They have also exhibited significant antifungal activity and enhanced the seed germination and efficient root colonization. Further, artificial inoculations have also clearly shown to enhance the growth in terms of height, dry weight of shoot and root. Thus, the results substantiate the role of FLPs for the enhanced growth and yield of rice cultivated under SRI.

Keywords: Fluorescent pseudomonads, Rice, System of Rice Intensification (SRI).

INTRODUCTION
System Rice Intensification (SRI) has emerged in the 1980’s as a synthesis of locally advantageous rice production practices encountered in Madagascar by Fr. Henri de Laulanie, a Jesuit Priest. But it is Dr. Norman Uphoff from, CIIFA, Ithaca, USA, brought this method to the notice of outside world. Today SRI is being adopted in many countries including India and the response from farmers has been overwhelming seeing the benefits of the method notwithstanding the constraints. SRI is not a fixed package of technical specifications, but a system of production with four main components viz, soil fertility management, planting method, weed control and irrigation management. Several field practices have been developed around these components. One of the key inputs of the SRI is the use of organic nutrients and aeration of soil through frequent intercultivation with weeder which are better at promoting the abundance and diversity of microorganisms that may improve the enhanced growth and production (Ranamukhaarachchi and Sanjeewanie Ginigaddara 2009).

Considerable attention has been paid to fluorescent pseudomonads (FLPs) as plant growth promoting rhizobacteria (PGPR) due to their effective seed bacterization, aggressive root colonization (Manoharachary and Tilak 2012) and their ability to control soil borne pathogens. They are equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion through production of a variety of antibiotics, chitinolytic enzymes, siderophores, HCN and growth promoting hormones like IAA, GA. Inorganic phosphate...
solubilization by FLPs has also been reported (Srivastava et al., 2004). Recently, Ali et al. (2009) reported that FLPs also confer thermotolerance to the crop plants. The present investigations are targeted to assess the role of FLPs isolated from rhizosphere of rice cultivated under SRI for its enhanced plant growth and productivity.

**MATERIALS AND METHODS**

**Samples collection:** Five locations (Mulugu, Hasanparthy, ARC, KU Campus, Paluvalpula) cultivating rice under SRI and representing most of the agro-edaphic conditions of Warangal district of A.P. India were selected. Plants in the age group of 30 to 40 days, grown in these soils were selected for collecting rhizosphere and non rhizosphere soil samples. Samples of soil from the field were carried to laboratory in an ice-box and stored in a refrigerator and isolation and enumeration of FLPs and non-FLPs were made within 12 hrs of sample collection.

**Isolation of fluorescent pseudomonads:** Soil serial dilution technique followed by spread plate technique was adopted. Specific dilutions (ranging from 10^-2 to 10^-6) were standardized and used for isolations. Number of viable colonies were counted and recorded as number per gram of sample taken. King’s B medium was employed for fluorescein detection of FLPs. The colonies without fluorescein were treated as non-FLPs.

**Studies on PGPR traits**

i. **Indole acetic acid (IAA) production:** IAA production was tested by Salkowski colorimetric technique (Glickmann and Dessaux 1995).

ii. **Gibberellic acid (GA) production:** GA production was determined by Cho et al. (1979).

iii. **Siderophore production:** Deferrated glassware and iron deficient MM9 medium was used in these experiments. Presence of siderophores was tested and confirmed by FeCl3 test (Jalal and Dick 1991), and the assay was performed by spectrophotometry (Meyer and Abdullah 1978).

iv. **Phosphate solubilization:** Molybdophosphoric acid blue method (Koening and Johnson 1942) was adopted for estimation phosphate solubilization.

v. **Hydrogen cyanide (HCN) production:** Production of HCN by an isolate was identified by the method of Castric (1977).

vi. **Ammonia production:** Bacterial isolates were grown in test tubes containing peptone water (10.0 g peptone, 5.0 g NaCl, 1 l DW, pH 7.0) and the production of ammonia was estimated (Dye 1962).

vii. **β-1, 3-glucanase assay:** β-1, 3-glucanase activity was determined as per the method outlined by Lim et al. (1991) and expressed as 1 nmol of glucose released per minute per mg of protein.

viii. **Protease production:** Production of protease was tested by spot inoculation (Maurhofer et al., 1995).

ix. **Chitinase production:** Chitinase production by the FLFs was determined by the method suggested by Lim et al. (1991).

**Tolerance of isolates against environmental factors**

i. **Heavy metals:** Tolerance of different selected isolates of FLFs to different heavy metals was tested by agar dilution method (Ahmad and Yadav 1988).

ii. **Salt tolerance:** Salt tolerance was determined by inoculating the test isolates into tubes containing their respective media added with sodium chloride solution giving final concentrations of 2, 4, 6 and 8%.

iii. **pH tolerance:** The pH of the mediums was adjusted using 1N NaOH ranging from 4.5 - 9.5.

**In-vitro antifungal interaction between bacterial and fungal strains:** Antagonism on agar plates was studied by modified method of Fokkema (1973).

**Seed germination assay:** Liquid cultures of selected isolates were raised in nutrient broth and a population approximately 1.3 - 2.6 x 10^7 cell / ml was obtained. Seeds of rice were surface sterilized (70% ethanol, 1% HgCl2) and submerged in liquid cultures of isolates separately for 1 hr. Then the seeds were dried in a laminar air-flow and placed on 0.8% water agar (10 seeds / plate). Plates were incubated at 28°C and seed germination and radicle length was recorded at 72 hrs. A simultaneous control was maintained with seeds without any rhizobacterial coating.

**Root colonization:** Colonization of roots by bacterial isolates was determined by the method described by Misaghi (1990). Number of colony forming units (CFU) were enumerated and expressed as number per gram of sample.
Formulation of inoculum: The carrier (soil: lignite: 1:1) based bacterial cultures were prepared as per the method described by Tilak et al. (2010).

Soil used for the experiments was collected from soil profile at Kakatiya University (pH 7.0, 0.10% organic matter, 0.56 mhos/cm electrical conductivity (EC), > 50 Kg/ha available phosphorus, 467 Kg/ha available potassium) and sieved through a 2 mm sieve, autoclaved for 2 hrs at 121°C before mixing with sterile sand. Plastic pots (10x11cm) were filled with sterile sand and soil (1:3) and surface sterilized seeds were sown in pots and after germination thinned down to one. The results are subjected to statistical analysis, wherever required.

RESULTS AND DISCUSSION

The results of present investigations reveal that, in general, the rhizosphere of rice cultivated under SRI supported more FLPs than the non-rhizosphere soil. The ratios of FLPs and non FLPs in rhizosphere soils ranged from 0.03 to 0.14 that varied with both the type of soil and age of the plant. In case of non-rhizosphere soil, the ratio ranged between 0.003 to 0.19 (Table 1). Thus a wide variation exists in the distribution of pseudomonads in rhizosphere and non-rhizosphere soils. It is also evident from the table that the population of both types of pseudomonads varied with the type of soil. This differential distribution of FLPs can be explained in terms of different edaphic factors and variation within the practice of SRI. The results also reveal that in rhizosphere soils of rice grown in all soils, the maximum population of both types of pseudomonads was supported at the age of 30 days of plant growth. It may be due to the vigorous growth and metabolism of plant at this age, during which many metabolites are likely to be released which serve as the nutrients for rhizospheric organisms. Subsequently, the populations decreased slowly and steadily. Interestingly, such type of tendency was not observed in case of non-rhizosphere soil.

Pseudomonads are reported to be dominant in the rhizosphere of rice and their inoculation can increase growth and yield production (Kloepper et al., 1989). An enumeration of rice rhizosphere from different cultivars revealed the occurrence of Pseudomonas sp, mainly of P. putida, P. aeruginosa, P. fluorescens and P. citchari. Reddy and Rao (2009) using RAPD analyzed the genetic diversity of the FLP isolates of rice and found that the isolates were genetically diverse.

A total of 250 isolates obtained with different colony morphology were screened for biochemical traits that are expected to be involved in plant growth promoting (PGP) and plant health promoting activities. Twenty isolates (8.0 %) out of 250 isolates proved positive for at least two traits examined. Out of 20 isolates, eight were able to produce detectable

### TABLE 1: Distribution of fluorescent pseudomonads in rhizosphere and non-rhizosphere soils of rice (SRI) grown in different localities of Warangal

<table>
<thead>
<tr>
<th>Place</th>
<th>Age of the crop (DAS)</th>
<th>Rhizosphere</th>
<th>Non-rhizosphere</th>
<th>Ratio</th>
<th>Rhizosphere</th>
<th>Non-rhizosphere</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mulugu</td>
<td>30</td>
<td>3.00*</td>
<td>35.43</td>
<td>0.08</td>
<td>1.4</td>
<td>12.35</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.05</td>
<td>62.5</td>
<td>0.03</td>
<td>3.3</td>
<td>38</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.95</td>
<td>49.5</td>
<td>0.03</td>
<td>3.5</td>
<td>37.5</td>
<td>0.093</td>
</tr>
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<td>Hasanparthy</td>
<td>30</td>
<td>1.7</td>
<td>15.25</td>
<td>0.11</td>
<td>2.67</td>
<td>52.6</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.2</td>
<td>17.12</td>
<td>0.12</td>
<td>4.12</td>
<td>42.26</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>3.06</td>
<td>43.2</td>
<td>0.07</td>
<td>3.26</td>
<td>36.15</td>
<td>0.09</td>
</tr>
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<td>ARC</td>
<td>30</td>
<td>6.6</td>
<td>48.5</td>
<td>0.13</td>
<td>6.5</td>
<td>33.5</td>
<td>0.19</td>
</tr>
<tr>
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<td>60</td>
<td>6.67</td>
<td>45</td>
<td>0.14</td>
<td>4.75</td>
<td>28.75</td>
<td>0.16</td>
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<td></td>
<td>90</td>
<td>5.45</td>
<td>39.25</td>
<td>0.13</td>
<td>2.8</td>
<td>23</td>
<td>0.12</td>
</tr>
<tr>
<td>KU Campus</td>
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<td>3.05</td>
<td>43.20</td>
<td>0.07</td>
<td>0.19</td>
<td>27.5</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.3</td>
<td>24.25</td>
<td>0.13</td>
<td>0.34</td>
<td>17</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.2</td>
<td>15.05</td>
<td>0.14</td>
<td>0.28</td>
<td>58.75</td>
<td>0.004</td>
</tr>
<tr>
<td>Paluvalpula</td>
<td>30</td>
<td>3.55</td>
<td>24.75</td>
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<td>1.4</td>
<td>46.5</td>
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<td>2.7</td>
<td>17</td>
<td>0.05</td>
<td>2.5</td>
<td>53.76</td>
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<td>1.85</td>
<td>25.75</td>
<td>0.07</td>
<td>1.5</td>
<td>11.75</td>
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</table>

Values are expressed in colony forming units (10⁴ CFU/g)
amounts of IAA in the culture broths (Table-2). Significant amount of GA was also produced by five isolates. Siderophore production in iron free medium was observed in 13 isolates. Production of HCN and ammonia were observed in nine and eleven isolates respectively. Similarly, protease production in nine isolates; β-1, 3 glucanase production in eleven isolates; production of chitinases in only four isolates were observed. Solubilization of inorganic phosphates (tri and di calcium) by FLPs was observed in eight isolates. Among all, Pf – 3, Pf – 6, Pf – 8 isolates were found showing positive for more number of traits tested. They have shown the relative efficacy ratios of 0.66, 0.77 and 0.77 respectively. These strains, here after referred to as RPF-3, RPF-6 and RPF-8, were further evaluated for other growth promoting attributes.

In the present investigations, resistance of selected FLPs towards different pH, heavy metals and sodium chloride concentration was studied and the results are presented in the Tables 3 and 4. The two isolates RPF-3 and RPF-8 did not show any growth at pH 4.5 and 9.5. However RPF-6 was able to tolerate even pH 4.5 and 9.5. For all the three isolates, pH 7.0 appears to be ideal for growth. All the three isolates were completely inhibited in presence of mercury and cobalt and exhibited a significant resistance to molybdenum. The resistance power of the FLP isolates decreased with increase in concentration (upto 6%) of NaCl and all the three isolates were completely inhibited at 8% concentration.

FLPs have been widely tested for biocontrol activity against fungal pathogens. They produced highly potent broad-spectrum antifungal molecules against a variety of phytopathogens, thus acting as effective biocontrol agents (Srivastava and Shalini 2008). In the present study, an attempt was made to evaluate the three strains of FLPs for antifungal activity against selected four phytopathogenic fungi viz Fusarium oxysporum, Curvularia lunata, Colletotrichum falcum, Macrophomina phaseolina and the results are presented in Table-4. It is evident from the results that these strains caused inhibition zones ranging from 0.2 to 2.1 cm (diameter) for different phytopathogenic fungi. However, the antifungal activity varied both with the FLP strains as well as test fungi. RPF-6 showed more inhibitory activity against all the four pathogenic fungi. In case of fungi, the inhibitory effect of all the three isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>IAA like acid</th>
<th>Gibberelic acid</th>
<th>Siderophores</th>
<th>HCN</th>
<th>Ammonia</th>
<th>β-1,3 glucanase</th>
<th>Protease</th>
<th>Chitinase</th>
<th>P</th>
<th>Relative solubilization</th>
<th>Relative efficacy</th>
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<td>RPF1</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>RPF2</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>RPF3</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>RPF4</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>RPF5</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>RPF6</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>RPF7</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>0.22</td>
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<tr>
<td>RPF8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>--</td>
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</tr>
<tr>
<td>RPF9</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>0.22</td>
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<tr>
<td>RPF10</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
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<td>--</td>
<td>+</td>
<td>--</td>
<td>0.33</td>
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</tr>
<tr>
<td>RPF11</td>
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<td>--</td>
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<td>--</td>
<td>+</td>
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<td>--</td>
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<td>--</td>
<td>0.22</td>
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<tr>
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<td>+</td>
<td>--</td>
<td>+</td>
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<td>--</td>
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<td>--</td>
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<td>+</td>
<td>--</td>
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<td>--</td>
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<tr>
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<td>+</td>
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<td>--</td>
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<td>+</td>
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<td>--</td>
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<td>--</td>
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<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>--</td>
<td>+</td>
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<td>--</td>
<td>--</td>
<td>+</td>
<td>0.33</td>
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<td>+</td>
<td>--</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>0.22</td>
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</tr>
<tr>
<td>RPF20</td>
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<td>+</td>
<td>--</td>
<td>+</td>
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<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>0.44</td>
<td></td>
</tr>
</tbody>
</table>

% of isolate | 40 | 25 | 65 | 45 | 55 | 55 | 45 | 20 | 40

+ = Positive; -- = Negative
was more pronounced on \textit{C. falcatus}. \textcite{Pal et al. (2001)} reported that \textit{P. fluorescens} and \textit{Bacillus} sp. have reduced the root rot, collar rot and stem rot of maize caused by \textit{Rhizoctonia solani} and \textit{Fusarium} wilt of cotton caused by \textit{F. oxysporum} sub sp. \textit{vasinfectum}. inoculation with three isolates of FLPs enhanced the seed germination and root elongation over the control (Fig. 1). The highest enhancement was observed with RPF-6 isolate. Significant numbers of FLPs in all the three zones of root indicate a successful colonization and perhaps with a significant physiological functions. Interestingly, the population of FLPs was more in rhizosphere followed by rhizoplane and least in endorhizosphere (Table 5). The PGP ability of FLPs is a function of good root colonization and production of growth hormones and seed treatment PGPR strains improved seed germination, seedling vigor, seedling emergence and seedling stand over the control in maize. 

In the present investigations, an effort was made to evaluate the influence of inoculations of present FLPs on the growth parameters of rice and the results revealed that there is a pronounced enhancement in all the four parameters of rice plants inoculated with FLPs. However, enhancement effect varied with the FLPs isolates and also with the parameter. In general, enhancement effect is more on plant height and shoots dry weight than the root parameters (Table 6, Fig.2). \textcite{Cakmakci et.al., 2006}

\begin{table}[h]
\centering
\caption{Resistance of fluorescent pseudomonads to different heavy metals and NaCl concentrations}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Isolate} & \textbf{Control} & \textbf{Hg} & \textbf{Co} & \textbf{Zn} & \textbf{Cu} & \textbf{Mo} & \textbf{Control} & \textbf{2} & \textbf{4} & \textbf{6} & \textbf{8} \\
\hline
RPF3 & 1.82 & NG & NG & 0.4 & 0.47 & 1.72 & 1.46 & 1.42 & 0.74 & 1.34 & NG \\
RPF6 & 1.17 & NG & NG & 0.32 & 0.52 & 1 & 1.28 & 1.08 & 0.01 & 0.21 & NG \\
RPF8 & 1.29 & NG & NG & 0.48 & 0.49 & 1.16 & 1.62 & 1.32 & 0.18 & 0.56 & NG \\
Control & 1.01 & NG & NG & 0.2 & 0.32 & 0.9 & 1.25 & 1.42 & 0.21 & 0.4 & NG \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Evaluation of antifungal activity of fluorescent pseudomonads against four pathogenic fungi}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Isolate} & \textbf{Fusarium oxysporum} & \textbf{Curvularia lunata} & \textbf{Colletotrichum falcatus} & \textbf{Macrophomina Phaseolina} \\
\hline
RPF3 & 0.4 & 0.9 & 1.7 & 0.5 \\
RPF6 & 1.6 & 2 & 2.1 & 0.5 \\
RPF8 & 0.2 & 0.9 & 2 & 0.8 \\
LSD 0.05 & 0.0428 & 0.0041 & 0.0118 & 0.001 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Effect of selected pseudomonad isolates on seed germination and root colonization of rice}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Isolate} & \textbf{No. of seeds sown} & \textbf{No. of seeds germinated} & \textbf{% of germination} & \textbf{Root length (cm)} & \textbf{Rhizosphere} & \textbf{Rhizoplane} & \textbf{Endorhizosphere} \\
\hline
RPF3 & 40 & 32 & 82 & 0.6 & 6.1 & 6.2 & 9.6 & 8.4 & 0.8 & 0.04 \\
RPF6 & 40 & 36.1 & 90.25 & 2.1 & 12 & 12.6 & 5.3 & 5.4 & 0.7 & 0.26 \\
RPF8 & 40 & 34.2 & 85.5 & 1.8 & 10 & 9.5 & 2.6 & 3.8 & 1.6 & 0.7 \\
Control & 40 & 20.1 & 50.25 & 1.1 & -- & -- & -- & -- & -- & -- \\
\hline
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Showing the germination of rice seeds}
1. Control (un inoculated)
2. Inoculated with RPF3
3. Inoculated with RPF6
4. Inoculated with RPF8
\end{figure}
TABLE 6: Influence of inoculations of fluorescent pseudomonads on growth of rice cultivar under green house conditions (45 DAS)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Plant height (cm)</th>
<th>Shoot dry weight (g)</th>
<th>Root length (cm)</th>
<th>Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPf-3</td>
<td>15.5</td>
<td>10.02</td>
<td>3.7</td>
<td>2.1</td>
</tr>
<tr>
<td>RPf-6</td>
<td>6.4</td>
<td>7.05</td>
<td>3.3</td>
<td>1</td>
</tr>
<tr>
<td>RPf-8</td>
<td>11.5</td>
<td>7.6</td>
<td>6.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Control</td>
<td>6.3</td>
<td>5.86</td>
<td>4.8</td>
<td>1.02</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.003</td>
<td>0.020</td>
<td>0.016</td>
<td>0.010</td>
</tr>
</tbody>
</table>

* Values are mean of three replicates and significant at p<0.05

It can be concluded from the present investigations that FLPs owing to their growth promoting traits contribute at least partly, towards enhanced growth and yield of rice cultivated under SRI. However, field trials are required to validate the results under green house conditions.

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