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Research Article
Phytobeneficial and Plant Growth-promotion Properties of Silicon-solubilising Rhizobacteria on the Growth and Control of Rice Sheath Blight Disease

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Abstract

Background: Silicon is an important element for plant development and increases plant resistance against biotic and abiotic stresses. This study aimed to screen and evaluate potential silicon-solubilising rhizobacteria (SSR) with plant growth-promoting properties and inhibitory activities against rice sheath blight pathogen *Rhizoctonia solani*. **Materials and Methods:** The SSR were isolated from the disease-free rice field usi1ng magnesium trisilicate media. All isolates were screened *in vitro* for plant growth-promotion properties: The production of IAA and phosphate solubilisation. The inhibitory activities against *R. solani*: Dual culture testing, the production of volatile compound and hydrogen cyanide. The potential SSR isolates were identified using VITEK 2 system. **Results:** A total of 31 potential SSR were isolated from rice rhizosphere soil. Eight most potential SSR isolates were selected out of 31 SSR isolates obtained for further screening of the diffusible antibiotics and extracellular metabolites production against *R. solani*. Five SSR isolates (SSR2, SSR13, SSR24, SSR25 and SSR26) were selected as potential plant growth promoters with inhibitory effects against *R. solani*. Under greenhouse conditions, rice plants treated with SSR13, SSR24 and SSR26 showed significantly reduction in rice sheath blight disease incidence with 33.33, 16.67 and 20.00%, respectively, compared to the controls (56.67%). Isolate SSR24 showed a significantly lower disease susceptibility index of only 6%, compared to the control at 59%. Rice plants treated with SSR13 showed the highest plant growth in treatment without *R. solani* infection. Isolates SSR13 and SSR24 were identified as *Serratia marcescens* and *Pseudomonas aeruginosa*, respectively. **Conclusion:** Isolates *Serratia marcescens* (SSR13) and *Pseudomonas aeruginosa* (SSR24) show the most potential to be developed as rice plant growth promoters and also to control of rice sheath blight disease caused by *R. solani*. This study helps to reduce chemical application in rice sheath blight management toward sustainability in rice production system.

Key words: Silicon-solubilizing rhizobacteria, *Rhizoctonia solani*, rice sheath blight disease, phytobeneficial, plant growth-promotion

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice sheath blight disease caused by *Rhizoctonia solani* is a devastating disease that reduces rice productivity. This fungus leads to the damping of seedlings, black lesions on roots and destroys the stem when plant parts come into contact with the soil[1]. Sheath blight disease is a major threat to worldwide rice production areas[2], especially those under intensive rice production systems. In Asia, sheath blight disease is estimated to reduce lowland rice yield[3] up to 20% and yield losses up to 54.3% were reported in India[4].

The development of sheath blight resistant varieties has not been particularly successful due to the lack of available resistant donors among cultivated rice varieties[5]. Chemical control has been reported to be a successful control measure of sheath blight disease[6]. However, the effective control of soil-borne *R. solani* using fungicide soil drenching is impractical, especially under flooded conditions, as it involves a high fungicide cost and creates environmental pollution concerns[7]. An alternative control of sheath blight using plant growth-promoting rhizobacteria (PGPR) and silicon (Si) fertilizer have gained attention in recent years.

Silicon is a bioactive element that helps to alleviate abiotic and biotic stresses. Recently, Si fertilizer has widely been used to enhance crop production and reduce the incidence of fungal disease such as rice sheath disease caused by *R. solani*[8]. However, due to the high cost of Si fertilizer, much effort has been placed on recent study into sustainable strategies to alleviate the use of silicon fertilizer. The use of silicon-solubilising rhizobacteria (SSR) has been introduced into rice cultivation systems to control rice plant disease, due to the poor solubilisation of soil silicon and the low distribution of SSR in soils for rice cultivation. The SSR are also considered to be PGPR, which exert beneficial effects on plant growth, yield and health in a range of crops including cereals, either via direct or indirect mechanisms[9]. The application of SSR was reported to be one of the important mechanisms for the biological suppression of rice phytopathogens through polymerisation of silicate from the soil for plant uptake[10].

Limited study has been conducted in the application of SSR to control rice sheath blight disease caused by *R. solani*. Therefore, the objective of the study was to screen and evaluate the phytobeneficial and plant growth-promoting properties of indigenous SSR against *R. solani* infection.

MATERIALS AND METHODS

Soil sampling and bacterial isolation: Rhizosphere soil samples were randomly collected from the rice rhizosphere at Besut, Terengganu, Malaysia. Ten grams of dried rhizosphere soil samples were transferred to 90 mL sterile distilled water in a 250 mL Erlenmeyer flask. One milliliter of soil dilutions (10^2 and 10^4) was transferred to magnesium trisilicate (0.25% w/v) medium and was incubated at $28\pm2°C$ for 24-36 h. After incubation, the bacterial isolates with a halo zone formed around the colony were selected as positive silicon-solubilising rhizobacteria (SSR)[11]. A total of 31 SSR were isolated and were designated SSR1-SSR31.

In vitro screening for phytobeneficial properties

Dual culture testing: A 5 mm diameter *R. solani* agar plug obtained from the periphery of a 7 days old colony was transferred to a Potato Dextrose Agar (PDA) plate and the respective SSR isolates were streaked approximately 2 cm apart. The plates were incubated at $28\pm2°C$ for 5 days. A petri dish containing *R. solani* alone was used as a control[12]. The inhibition of *R. solani* mycelial growth was recorded.

Volatile compound production: A 250 µL aliquot of SSR suspension (1×10^6 CFU mL^{-1}) was placed in a petri dish containing Nutrient Agar (NA). A 5 mm disk of a 7 days old pure culture of *R. solani* was placed at the center of another petri dish containing PDA. Both plates were placed face to face (without lids) and were sealed to prevent the loss of the volatile compounds formed. Plates with only *R. solani* were used as a control. The plates were incubated at $28\pm2°C$ for 5 days. The growth of *R. solani* was observed, measured and compared to that of the controls[13]. The results were expressed as the Percentage Inhibition of Radial Growth (PIRG) using the following equation:

$$\text{Inhibition in radial growth}(\%) = \frac{R1-R2}{R1}\times100\%$$

where, R1 is the radial growth in the control and R2 is the radial mycelial growth in the treatments.

Hydrogen cyanide production: The picrate assay described by Rakh *et al.*[14] was used for the qualitative estimation of HCN production. The SSR isolates were streaked onto tryptic soy agar supplemented with 4.4 g L^{-1} glycine. Then, 1.5 cm diameter pieces of sterilised filter paper were saturated with picric acid (5.0% w/v) and placed in the upper lids of the inoculated petri dishes. The plates were sealed with parafilm and incubated at $28\pm2°C$ for 48 h. Hydrogen cyanide production was determined by the colour change of the filter paper from yellow to reddish brown. The colour was eluted by placing the filter paper in a clean test tube containing 10 mL distilled water and the absorbance was measured at 625 nm using a spectrophotometer.

In vitro screening of plant growth-promotion properties

Indole-3-acetic acid (IAA) production: All the SSR isolates were screened for IAA production using spectrophotometric estimation[15]. The bacterial culture was grown in Nutrient Broth (NB) supplemented with L-tryptophan (5 μg mL^{-1}) and was incubated at 28±2°C for 5 days. The cultures were centrifuged at 3,000 rpm for 30 min and 2 mL of the supernatant was mixed with 2 drops of orthophosphoric acid and 4 mL Salkowski reagent (50 mL 35% perchloric acid added to 1 mL 0.5 M FeCl$_3$). The development of a red colour indicated IAA production. Colour changes were measured at 530 nm and the concentration of IAA (μg mL^{-1}) was determined against a standard curve of IAA.

Phosphate-solubilization: A phosphate (P) solubilisation test was conducted using National Botanical Research Institute Phosphate (NBRIP) medium containing precipitated tri-calcium phosphate[15]. The SSR isolates were cultured in NB for two days and 100 μL was spotted onto the surface of the NBRIP plates and incubated at 28±2°C for 5 days. The ability to solubilise phosphate was assessed by measuring the radius of the clearing zone formed around the bacterial colony.

Selection of potential SSR: The most potential SSR were selected based on the significance values from the inhibitory properties against *R. solani* and the plant growth-promotion tests conducted. Eight SSR (SSR2, SSR12, SSR13, SSR24, SSR25, SSR26, SSR27 and SSR31) were selected to further confirm their bio-efficacy against *R. solani* through screening for diffusible antibiotics and for the production of extracellular metabolites.

Confirmation of the inhibitory efficiency of the selected SSR

Diffusible antibiotic production testing: The PDA plate was covered with a sterile cellulose tube. The SSR was inoculated in the centre of the cellulose tube and was incubated at 28±2°C for 24 h. The cellulose tube with the growth of respective SSR was removed before placing a 5 mm colony of *R. solani* at the center on the plate and incubating at 28±2°C for 5 days. The control plate was inoculated with the cellulose tube without SSR. Three replicates were conducted for each SSR isolate. The diameter of the *R. solani* colony was measured[16].

Extracellular production testing: The extracellular production of selected SSR was determined using the method described by Tariq *et al.*[16]. Each of the SSR was incubated in

NB on a rotary shaker (100 rpm) at 28±2°C. After 6 days of incubation, the SSR suspensions were centrifuged at 6,000 rpm at 4°C for 12 min and the bacterial cells were discarded. The SSR supernatants were filtered through a membrane filter (0.20 μm) and were mixed with PDA (25% v/v). A 5 mm of 4 days old *R. solani* plug was placed at the center of the solidified PDA and was incubated at 28±2°C for 5 days. The control consisted of PDA mixed with only NB filtrate. The growth diameter of *R. solani* was measured and five SSR (SSR2, SSR13, SSR24, SSR25 and SSR26) were confirmed as the most potent inhibitors of *R. solani*, based on their significant values of diffusible antibiotic and extracellular metabolite production using the percentage growth inhibition rate according to the equation:

$$\text{Inhibition (\%)} = 1 - \left(\frac{\text{Treatment growth}}{\text{Control growth}} \right) \times 100\%$$

Inoculation of silicon-solubilising rhizobacteria: The greenhouse experiment was conducted at the School of Food Science and Technology of the Universiti of Malaysia Terengganu (UMT). The daily temperature ranged from 28-34°C. The rice variety MR219 (susceptibility to *R. solani*) was selected as commonly planted in Malaysia. Sterilised rice seeds were immersed in respective SSR inoculants (1×10^8 CFU mL^{-1}) for 45 min before sowing[13]. Rice seeds immersed in distilled water served as a control. Soil infected with *R. solani* was prepared 3 days before seed sowing by drenching 5 kg of soil per pot with 20 mL *R. solani* inoculant (1×10^8 CFU mL^{-1}) and control pots were drenched with distilled water. The soils treated with *R. solani* were covered with plastic film for 3 days to retain moisture. Rice seeds were sown and the seedlings were thinned at 7 days after sowing to allow only 10 seedlings per pot. A second inoculation of *R. solani* (1×10^8 CFU mL^{-1}) was conducted at the four-leaf seedling stage by spraying.

Evaluation of rice sheath blight disease development and plant growth-promoting traits: After 7 days of *R. solani* inoculation, the symptoms of sheath blight disease were assessed[13]. The disease severity was determined based on the scale in Table 1. The disease incidence and disease susceptibility index were evaluated using the following equation:

$$\text{Disease incidence (\%)} = \frac{\text{Total No. of infected plants}}{\text{Total No. of plants}} \times 100$$

Table 1: Rice sheath blight disease severity scale

Disease scales	Disease symptoms
0	No lesion
1	The appearance of water-soaked lesions
2	The appearance of necrosis on the leaf area
3	Less than 50% necrosis on the leaf area
4	More than 50% necrosis on the leaf area
5	Necrosis across the entire leaf section and leaf death

$$\text{Disease susceptibility index (DSI\%)} = \sum \frac{\text{No. of plant in the specific scale} \times \text{disease scale}}{\text{Total No. of plants}} \times 100$$

Rice plant growth-promoting traits were assessed 30 days after sowing. Plant height was measured between the root crown and the tallest shoot tip using a ruler.

Identification of the potential SSR isolates: The identification of the selected SSR isolates were identified using VITEK 2 system version 05.04. A sterilized swab was used to transfer the fresh (24 h after incubation) colony of a pure culture and the turbidity was adjusted and measured using a turbidity meter. Identification cards were inoculated with isolate suspensions using an integrated vacuum apparatus. The filled cassette is placed into a vacuum chamber station, sealed and inserted into the VITEK 2 reader-incubator (incubation temperature, $35.5 \pm 1°C$) and subjected to a kinetic fluorescence measurement every 15 min. The results were interpreted by the database automatically. Used card were automatically discarded into a waste container.

Experimental design and statistical analysis: All the *in vitro* experiments were conducted in a complete randomized design with five replicates and repeated twice. The greenhouse experiment was conducted in a randomized complete block design with five replicates. All data were subjected to analysis of variance and significance tests using the Duncan multiple range test at $p \leq 0.05$ with the Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Soil sampling and bacterial isolation: A total of 31 potential SSR were isolated from the healthy rice rhizosphere. These results indicated that all 31 SSR isolates capable to solubilized silicate by production of yellow hallo zone on media containing magnesium trisilicate (0.25% w/v). The solubilization of insoluble minerals such as silicates into soluble form was reported associated to the production of organic acids such as 2 keto-gluconic acid, alkalis and polysaccharides[17]. The scarce of soil microorganisms with capability to solubilizing silicon was also reported[18].

In vitro screening for phytobeneficial properties: Rice is a silicaceous plant; however, silicates in the soil exist in a polymerised form and are not available for plant uptake. The application of SSR is important to augment the availability of silica in the soil and to improve the productivity of rice production systems. In addition, induced systemic resistance by SSR was suggested to be one of the most important mechanisms for biological suppression of rice phytopathogens[10]. In dual culture testing, 4 isolates: SSR2, SSR24, SSR25 and SSR26, exhibited a significant PIRG value of more than 75% which indicated a strong inhibition to *R. solani* (Table 2, Fig. 1). However, other SSR isolates (27 isolates) were identified as poor inhibitors of *R. solani* with PIRG value ranged 0-38.89% (Table 2). All isolated SSR were able to produce volatile compounds except SSR31. The SSR that produced the greatest amount of volatile compounds in the suppression of *R. solani* was SSR12 (Fig. 1) with of a PIRG value of 84.55% (Table 2). The results of HCN production indicated that only four SSR (SSR24, SSR25, SSR26 and SSR27) showed a positive HCN production ranging from 0.069-0.017 of colour density measured at 625 nm (Table 2). Hydrogen cyanide is a volatile secondary metabolite that can inhibit the growth of various soil-borne pathogens[19], due to the inhibition of metal enzymes, especially cytochrome C oxidases in electron transport systems[20].

All the *in vitro* inhibitory tests conducted were effective to screen the suppression potential of SSR isolates against *R. solani*[21]. Under dual culture plate testing, all SSR isolates tested, varied in their ability to suppress *R. solani*. This ability to suppress the growth of *R. solani* might be associated with competition and the production of volatile compound by the potential SSR. Inactivation of the pathogen by the inhibition via the production of volatile antibiotic compounds is one mechanism of biological control[21]. In this study, it was observed that SSR with high PIRG values were always associated with high values of volatile compound production. This finding was supported by that of Bos *et al.*[22] who reported that volatile compound producers can reduce disease infection.

In vitro screening of plant growth-promotion properties: Six out of 31 SSR isolates tested produced IAA (Table 2). Isolate SSR31 produced the greatest amount of IAA (0.74 µg mL^{-1}) among the other SSR isolates. The ability to solubilise precipitated phosphate was demonstrated by 19 tested SSR isolates, with the P-solubilising index ranging from 14.90-33.33%. Isolate SSR13 showed a remarkably high P-solubilising index value (33.33%) followed by SSR14 (30.13%). The production of IAA among the SSR tested varied greatly ranging from 0.04-0.74 µg mL^{-1}. The variation in IAA
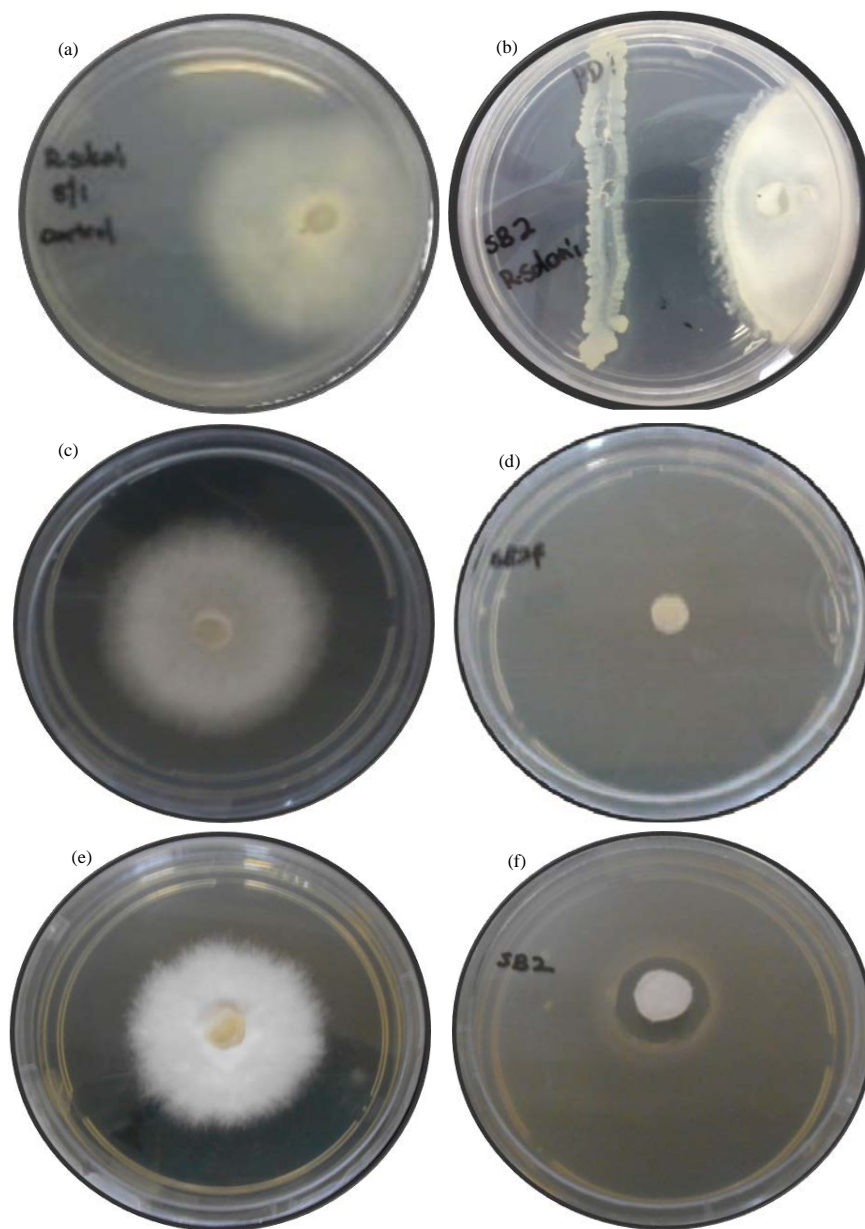
Fig. 1(a-f): Inhibition effects of SSR against *R. solani* using dual culture test (a-b) Served as control; diffusible antibiotics test, (c-d) Served as control; extracellular metabolites production and (e-f) Served as control; after 5 days of incubation at 28±2°C

production between bacteria depends on the species and strain, culture conditions, growth stages and substrate availability[23].

Confirmation of the inhibitory efficiency of the selected SSR: Eight SSR isolates (SSR2, SSR12, SSR13, SSR24, SSR25, SSR26, SSR27 and SSR31) were selected based on their inhibitory activities against *R. solani* and plant growth-promotion properties. The inhibitory efficacy against

R. solani of all selected SSR was further confirmed through the testing of diffusible antibiotics and extracellular metabolite production (Table 3). All selected SSR isolates produced diffusible antibiotic and extracellular metabolites. Isolates SSR24, SSR25 and SSR26 recorded significantly high values of diffusible antibiotic production with PIRG value of 78.90%, respectively. Additionally, isolates SSR2 and SSR13 exhibited the highest production rates of extracellular metabolites with PIRG value of 66.90%, respectively. Therefore,

Table 2: *In vitro* screening of phytobeneficial and plant growth-promoting properties of all silicon-solubilising rhizobacteria (SSR)

Isolates	Dual culture test (PIRG)	Volatile compound testing (%)	Hydrogen cyanide test OD (625 nm)	IAA production test ($\mu g\ mL^{-1}$)	Phosphate solubilising index (%)
SSR1	0.00[e]	4.88[c,d]	0[c]	0[c]	0[e]
SSR2	76.54[a]	52.03[b]	0[c]	0[c]	0[e]
SSR3	25.93[b,c]	20.33[b,c,d]	0[c]	0[c]	26.93[a,b,c,d]
SSR4	9.26[c,d,e]	26.02[b,c,d]	0[c]	0[c]	14.90[e]
SSR5	0.00[e]	6.50[c,d]	0[c]	0[c]	21.86[d]
SSR6	0.00[e]	19.51[b,c,d]	0[c]	0[c]	22.56[c,d]
SSR7	1.85[d,e]	23.58[b,c,d]	0[c]	0[c]	0[e]
SSR8	0.00[e]	13.82[c,d]	0[c]	0[c]	24.96[b,c,d]
SSR9	0.00[e]	30.08[b,c,d]	0[c]	0[c]	21.40[d]
SSR10	0.62[e]	12.20[c,d]	0[c]	0[c]	23.83[b,c,d]
SSR11	38.89[b]	52.03[b,c]	0[c]	0[c]	25.80[b,c,d]
SSR12	12.35[c,d,e]	84.55[a]	0[c]	0[c]	23.60[b,c,d]
SSR13	4.94[c,d,e]	26.02[b,c,d]	0[c]	0[c]	33.33[a]
SSR14	24.69[b,c,d]	17.07[b,c,d]	0[c]	0[c]	30.13[a,b]
SSR15	0.00[e]	22.76[b,c,d]	0[c]	0[c]	0[e]
SSR16	7.41[c,d,e]	16.26[b,c,d]	0[c]	0[c]	0[e]
SSR17	9.88[c,d,e]	29.27[c,d]	0[c]	0.04 [b,c]	21.76[d]
SSR18	4.32[c,d,e]	30.08[b,c]	0[c]	0.27[a,b,c]	0[e]
SSR19	1.23[e]	18.70[b]	0[c]	0.55[a,b]	0[e]
SSR20	9.88[c,d,e]	10.57[c,d]	0[c]	0[c]	24.33[b,c,d]
SSR21	10.49[c,d,e]	17.07[b,c,d]	0[c]	0 [c]	0[e]
SSR22	7.41[c,d,e]	23.58[b,c,d]	0[c]	0[c]	0[e]
SSR23	0.62[e]	16.26[b,c,d]	0[c]	0[c]	23.36[b,c,d]
SSR24	78.00[a]	38.21[b,c]	0.032[b]	0.13[b,c]	0[e]
SSR25	79.63[a]	37.40[b,c,d]	0.017[b,c]	0[c]	29.66[a,b,c]
SSR26	75.93[a]	13.01[c,d]	0.018[b,c]	0[c]	25.53[b,c,d]
SSR27	3.70[c,d,e]	13.82[c,d]	0.069[a]	0[c]	21.76[d]
SSR28	0.62[e]	13.01[c,d]	0[c]	0[c]	0[e]
SSR29	0.63[e]	18.70[b,c,d]	0[c]	0[c]	21.83[d]
SSR30	8.02[c,d,e]	36.59[b,c,d]	0[c]	0.27[a,b,c]	0[e]
SSR31	25.31[b,c]	0.00[d]	0[c]	0.74[a]	28.43[a,b,c,d]

Means within columns with the same letters were significantly different according to Duncan's test at p≤0.05, each value represents the mean of five replications with two repeated trials

Table 3: Production of diffusible antibiotics and extracellular metabolites of selected silicon-solubilising rhizobacteria (SSR) against *Rhizoctonia solani*

Treatments	Diffusible antibiotic production (PIRG)	Extracellular metabolite production (PIRG)
SSR2	32.32[b]	66.90[a]
SSR12	23.83[c]	3.07[c]
SSR13	29.90[bc]	66.90[a]
SSR24	78.90[a]	5.44[c]
SSR25	78.90[a]	45.63[ab]
SSR26	78.90[a]	52.72[ab]
SSR27	18.59[c]	42.55[b]
SSR31	9.70[c]	5.44[c]

Means followed by the same letter in the same column were not significantly different at p≤0.05, each value represents the mean of five replications with two repeated trials

Table 4: Severity of rice sheath blight as measured by the incidence of disease and susceptibility to disease of the plants

Treatments	Disease incidence (%)	Disease susceptibility index (%)
Control	56.67[a]	59.00[a]
SSR2	46.67[ab]	26.67[bc]
SSR13	33.33[bc]	12.00[bc]
SSR24	16.67[d]	6.00[c]
SSR25	36.67[ab]	16.00[bc]
SSR26	20.00[bc]	12.00[bc]

Means followed by the same letter in the same column were not significantly different at p≤0.05, each value represents the mean of five replications with two repeated trials

five potential SSR isolates (SSR2, SSR13, SSR24, SSR25 and SSR26) were selected for greenhouse evaluation as potential inhibitors against *R. solani*.

Evaluation of rice sheath blight disease development and plant growth-promoting traits: The development of rice sheath blight disease and plant growth-promoting traits were

evaluated under greenhouse conditions. A low degree of disease incidence and disease susceptibility index indicated that selected SSR isolates possessed the highest potential to control rice sheath blight disease under greenhouse conditions by inhibiting the growth of *R. solani* (Table 4). In control rice plants, the highest disease incidence (56.67%) was recorded together with a disease susceptibility index of 59.00%. However, isolate SSR24 demonstrated a significantly low degree of disease incidence (16.67%), followed by SSR26 (20%), SSR13 (33.33%), SSR25 (36.67%) and SSR2 (56.67%)

(Table 4). The production of non-volatile antibiotics and extracellular metabolites even at low concentrations plays an important role in the suppression of plant pathogens[24] including *R. solani*[25]. Moreover, the production of antibiotics by SSR was also related to induce systemic resistance in rice plants and contributed to disease suppression[24]. This was especially evident in greenhouse experiments, a significantly low disease susceptibility index was found in rice plants inoculated with the selected SSR isolates. The potential of SSR24 was associated with the significant degree of inhibition and the production of volatile and non-volatile antibiotics against *R. solani*. According to Labuschagne *et al.*[26], the production of cyanides destroys the cell wall of pathogens and is also associated with the inhibition of mycelial growth of pathogens in plants. The deposition of Si beneath the leaf cuticle to form a cuticle-Si double layer that mechanically impedes penetration of fungus and disrupt the infection process[27]. Furthermore, another defence mechanism reported by Vijayapriya and Muthukkaruppan[10] was related to the increase in silicon availability by introduced SSR under *in vitro* conditions. The SSR not involve directly in combating phyto-pathogenic fungi but indirectly through releasing of Si in soil helps to induce disease resistance in plant either by

acting as physical barrier or as a modulator of host resistance to pathogen[18]. Therefore, the application of SSR is important for the solubilisation of soil silicates via microbial metabolism[10].

Generally, uninoculated rice plants with *R. solani* were taller than those infected with *R. solani* in plants treated with the selected SSR (Fig. 2). Rice sheath blight disease is commonly found in young plants[28], where it destroys the plant tissues and affects the absorption of water and nutrient uptake of plants but the disease does not affect mature plants. No significant difference in plant height was recorded between SSR-treated plants and the controls in *R. solani* inoculated plants. However, healthy rice plants (without inoculation with *R. solani*) showed a significant difference in plant height when treated with SSR2, SSR13, SSR25 and SSR26 compared to the control (Fig. 2). The tallest rice plants were those treated with SSR13. The difference in plant height between rice plants treated with SSR and control in plants inoculated or uninoculated with *R. solani* was associated to the phosphate-solubilising capability.

Under greenhouse conditions, rice plants inoculated with SSR were significantly taller without *R. solani* inoculation compared to the controls, which was associated with the
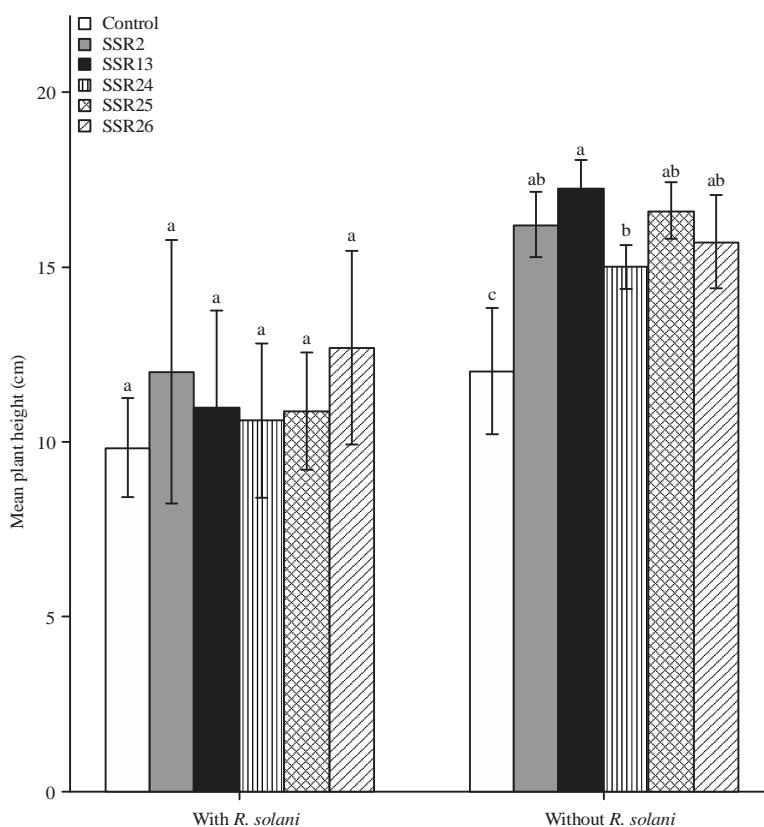

Fig. 2: Height of rice plants treated with respective SSR with *R. solani* and without *R. solani* inoculation at 30 days after sowing

solubilisation of phosphate or IAA production. Moreover, the plant growth promotion observed in plants treated with SSR2 might be related to plant health. However, the production of HCN was also reported to inhibit the growth of seedlings[19]. The HCN is a toxic gas that possible to cause phytotoxic and lead to growth reduction through inhibiting of CO_2 and nitrate assimilation, disruption of reduction of oxygen in the cytochrome respiratory chain and electron transport in photosynthesis[29]. This agrees with our findings, where no significant increase in plant dry biomass was observed in rice plants inoculated with SSR24, SSR25 and SSR26.

The isolates that showed the greatest potential for rice sheath blight disease management caused by *R. solani* of rice variety MR219 were SSR13 and SSR24 as the most potent plant growth promoter.

Identification of the potential SSR isolates: Isolates SSR13 and SSR24 were identified as *Serratia marcescens* and *Pseudomonas aeruginosa*, respectively. The *S. marcescens* and *P. aeruginasa* were reported as plant growth promoting rhizobacteria that improved rice plant growth and plant health against *R. solani* infection[30].

CONCLUSION

The application of *P. aeruginasa* (SSR24) and *S. marcescens* (SSR13) improved rice plant growth and plant health against *R. solani* infection. Therefore, the selected of *S. marcescens* (SSR13) and *P. aeruginasa* (SSR24) demonstrate the potential to be developed as a consortium of biological control agents to control rice sheath blight disease caused by *R. solani*, as well as to improve rice plant growth. The application of eco-friendly measure is useful for rice sheath blight disease management through biological control agents to reduce chemical usage and toward sustainability in rice production system.

SIGNIFICANT STATEMENTS

• A total of 31 silicon-solubilizing rhizobacteria were isolated from the healthy rice rhizosphere
• Isolate SSR13 demonstrated a remarkably high of P-solubilising index (33.33%) and the highest plant growth in treatment without *R. solani* infection
• Isolate SSR24 showed a significantly low of disease susceptibility index (6%), compared to the control (59%)

• The isolates *Serratia marcescens* (SSR13) and *Pseudomonas aeruginosa* (SSR24) shown the most potential isolates to be developed as biocontrol agents in rice sheath blight disease management and plant growth promoter

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