ROOT INITIATION AND PROLIFERATION OF BLACK PEPPER (Piper nigrum L.) STEM CUTTINGS BY INDOLE-3-ACETIC ACID PRODUCING INDIGENOUS BACILLUS SPP.

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ABSTRACT

Synthetic indole-3-acetic acid (IAA) stimulates the formation and initiation of main, lateral and adventitious roots and used widely in commercial vegetative propagation. Several indigenous plant growth promoting rhizobacteria (PGPR) are proficient in enhancing growth and health of root in vegetative propagation through secretion of IAA and potential to substitute synthetic IAA. Three indigenous Bacillus isolates were used to estimate the IAA production and evaluate their efficiency on root initiation and proliferation in black pepper stem cuttings under nursery condition. Bacillus megaterium UPMLH3 secreted the highest IAA level of 40.07 µg/mL, followed by Bacillus cereus UPMLH24 (22.51 µg/mL) and Bacillus cereus UPMLH1 (10.77 µg/mL) after 96 hours of incubation. Root initiation and proliferation experiments were assigned to six treatments (uninoculated control, 1,000 ppm indole-3-butyric acid (IBA), 2,000 ppm IBA, B. cereus UPMLH1, B. megaterium UPMLH3 and B. cereus UPMLH24) for a 45-day observation. The Bacillus isolates had significantly improved the survival and rooting rate of black pepper cuttings by 15% and 22% respectively over the uninoculated control. B. cereus UPMLH24, a moderate level of IAA producer, enhanced the number, total length, fresh and dry weights of the roots in black pepper cuttings. B. cereus UPMLH24 has demonstrated comparable result on total root number to the standard rooting practices using 1,000 ppm IBA. All the three Bacillus isolates had shown good survival rates on the roots and in the sand medium with the viable counts of 0.17-1.01 x 10⁹ cfu/g root (dry weight) and 1.53-2.67 x 10⁸ cfu/g sand respectively. Our findings suggest that the indigenous PGPR Bacillus cereus UPMLH24 has great potential to serve as biostimulant in improving the rooting performance of black pepper stem cuttings.

Key words: Plant-growth promoting rhizobacteria; Bacillus cereus; Bacillus megaterium; Biostimulant; Indole-3-butyric acid.

INTRODUCTION

Black pepper is notable and high demand spices in worldwide since 13th century. It has become an important economic plant to Vietnam, Brazil, Indonesia, India and Malaysia since 19th century. To furnish pepper berries according to the world demand, farmers are insistently using physical and chemical approaches to sustain the soil fertility and pepper production. Recently, organic and biological approaches are favourable in enhancing the growth and productivity of black pepper plants (Kandiannan et al., 1994; Diby et al., 2005; Thankamani et al., 2005). Auxin (IAA) is a notable exogenous hormone that is capable to manipulate morphology and physiology of plant roots. Even though auxin is naturally presence in plants, exogenous auxins are still act as effective inducer of adventitious roots in many woody species (Goldfarb et al., 1998). IAA synthesizing plant growth-promoting rhizobacteria (PGPR), including Azotobacter spp., Azospirillum spp., Bacillus spp. and Pseudomonas spp. are reported to directly promote rooting system and indirectly improve abiotic tolerance, nutrient uptake, biomass and yield of industrial plants (Diby et al., 2005; Sangeeth et al., 2008; Gholami et al., 2009; Saharan and
Nehra, 2011). Thus, the present study aimed to estimate the IAA production of three indigenous Bacillus isolates and to evaluate their efficiency on root initiation and proliferation of black pepper stem cuttings.

MATERIALS AND METHODS

Microorganisms used: Bacillus cereus UPMLH1, Bacillus megaterium UPMLH3 and Bacillus cereus UPMLH24 used in this study were previously isolated from soil and root samples of black pepper vine (Piper nigrum L.) of Kuching and Semengok Emas varieties under stress and low soil fertility condition and had been identified as IAA synthesizer (Zakry et al., 2010).

Estimation of IAA production by indigenous Bacillus spp.: Bacterial cultures were grown in nutrient broth (NB) at 28±2°C and shaken at 140 rpm. Fully grown bacterial cultures were harvested after 96 hours of incubation and IAA production was quantified with Salkowski reagent (50 ml, 35% of sulfuric acid, 1 ml 0.5 M FeCl₃) as described by Gordon and Weber (1951). Developed pink colour was read at 540 nm using spectrophotometer and compared with standard graph of IAA that was obtained in the range of 0-100 µg/ml.

Efficiency of IAA producing indigenous PGPR Bacillus spp. in root initiation and proliferation of black pepper stem cuttings: Three Bacillus isolates were evaluated for root initiation and proliferation of black pepper stem cuttings under nursery condition with 50% shading. Total treatments of six; including un-inoculated control, 1000 ppm indole-3-butyric acid (IBA), 2000 ppm IBA and PGPR inoculations (each isolate stood as a treatment); were arranged completely randomized design with 24 replicates (each replicate consisted of a cutting). Surface sterilization of five node black pepper stem cuttings were done with 70% ethanol and followed by 0.525% sodium hypochlorite (Clorox). Sterilized black pepper stem cuttings were dipped in overnight grown bacterial suspension (approximately 10⁸ cfu/ml) and air dried (Diby et al., 2005). Sterilized NB was used as a dipping treatment for uninoculated control. Three nodes from bottom of treated black pepper stem cuttings were planted in sterilized sand in black polyethylene bags. Plants were drenched twice a day. All plants were uprooted after 45 days of treatments. Data on total number, total and longest lengths, fresh and dry weights of roots were recorded.

Root colonization: Populations of rhizobacteria in sand and root surface were assessed after 45 days of treatment. Three plants per treatment were randomly selected with triplicated root samples. Treated plants were uprooted carefully and the lower 2 cm portion of root tips was used in bacterial population counts. Serial dilutions of bacterial suspension were spread on nutrient agar plates and the numbers of colony were recorded after 24 hours of incubation. Population was expressed in cfu/g root (dry weight) and cfu/g sand.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) using SAS 9.2 and means were compared by Duncan’s multiple range test (DMRT) at p ≤ 0.05.

RESULTS AND DISCUSSION

According to Pattern and Glick (1996), 80% of the PGPRs are by nature capable of synthesizing and exporting phytohormones (e.g. auxins, gibberellins, cytokikins, ethylene and abscisic acid). In present in vitro study, Bacillus megaterium UPMLH3 revealed the highest IAA level of 40.07 µg/mL, followed by Bacillus cereus UPMLH24 (22.51 µg/mL) and Bacillus cereus UPMLH1 (10.77 µg/mL) after 96 hours of incubation (Table 1). However, the actual association of IAA source rhizobacteria to the IAA pool in the rhizosphere depends on several factors, including population size of IAA synthesizing bacteria and amount of IAA produced by an individual cell in plant rhizosphere. The inoculation of Bacillus spp. efficiently improved survival (14%), rooting (21%) and sprouting (6%) rate of black pepper cuttings over uninoculated control (Table 2). B. cereus UPMLH24 has comparable rooting and sprouting rate with IBA 1000 ppm. Treatment with 2000 ppm IBA showed stunted responds on sprouting rate of black pepper stem cuttings over uninoculated control. Fresh stem cuttings or seeds needs a small amount (10⁻⁵ to 10⁻¹¹ M) of exogenous IAA to gain rapid root formation (Scott, 1972). Introduction of B. cereus UPMLH24 (a moderate IAA producer) to fresh black pepper stem cuttings efficiently enhanced total number (54%), total length (87%), longest length (25%), fresh (28%) and dry (112%) weights of roots over uninoculated control (Table 3). Inoculation of B. cereus UPMLH24 and commercial practices of 1000 ppm IBA showed similar effect on number of root production in black pepper cuttings at 11.99 and 131.17 respectively.
Meanwhile, IBA (2000 ppm) and B. megaterium UPMLH3 did not show significant difference as compared with the uninoculated control on total number; total length and dry weight of root. Even though at the beginning stage of root formation, plants require high concentration of endogenous IAA (Caboni et al., 1997), but if the optimum exogenous IAA level was exceeded, this will indirectly raise the ethylene biosynthesis (Riov and Yang, 1989) and inhibit root development and elongation (Edson et al., 1991).

Successful root colonization by PGPR depends on the rapid multiplication and adaptability of rhizobacteria in specific plant rhizosphere (Weller and Cook, 1983). The Bacillus isolates were well adopted and associated with the pepper plant rhizosphere ecosystem recording population density of $0.17-1.07 \times 10^9$ cfu/g root (dry weight) and $1.5-2.7 \times 10^8$ cfu/g sand respectively (Table 4). The Bacillus bacterial population in black pepper rhizosphere is correlated with the general increases of carbon and nitrogen nutrient content in root exudates (Raja et al., 2006).

Table 1: IAA production of indigenous Bacillus spp. after 96 hours of incubation

<table>
<thead>
<tr>
<th>Rhizobacteria</th>
<th>IAA production (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cereus UPMLH1</td>
<td>10.77 ± 1.01</td>
</tr>
<tr>
<td>B. megaterium UPMLH3</td>
<td>40.07 ± 1.98</td>
</tr>
<tr>
<td>B. cereus UPMLH24</td>
<td>22.51 ± 1.96</td>
</tr>
</tbody>
</table>

NOTE: means followed by different letters within each column are significantly different at p<0.01 level.

Table 2: Effects of Bacillus spp. on rooting and sprouting of pepper stem cuttings after 45 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival rate (%)</th>
<th>Rooted cutting (%)</th>
<th>Sprouted cutting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.50</td>
<td>79.17</td>
<td>75.00</td>
</tr>
<tr>
<td>2000 ppm IBA</td>
<td>83.33</td>
<td>83.33</td>
<td>25.00</td>
</tr>
<tr>
<td>1000 ppm IBA</td>
<td>100.00</td>
<td>87.50</td>
<td>79.17</td>
</tr>
<tr>
<td>B. cereus UPMLH1</td>
<td>91.67</td>
<td>79.17</td>
<td>62.50</td>
</tr>
<tr>
<td>B. megaterium UPMLH3</td>
<td>100.00</td>
<td>87.50</td>
<td>58.33</td>
</tr>
<tr>
<td>B. cereus UPMLH24</td>
<td>95.83</td>
<td>95.83</td>
<td>79.17</td>
</tr>
</tbody>
</table>

NOTE: Observation means of 12 plants; means followed by different letters within each column are significantly different at p<0.01 level.

Table 3: Effects of indigenous Bacillus spp. on root initiation and proliferation of pepper stem cuttings after 45 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total root (number/cutting)</th>
<th>Longest root length (cm/cutting)</th>
<th>Total root length (cm/cutting)</th>
<th>Root fresh weight (mg/cutting)</th>
<th>Root dry weight (mg/cutting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.33 ± 6.62</td>
<td>8.33 ± 0.47</td>
<td>150.32 ± 35.07</td>
<td>781.33 ± 45.88</td>
<td>154.25 ± 8.84</td>
</tr>
<tr>
<td>1000 ppm IBA</td>
<td>131.17 ± 37.80</td>
<td>18.88 ± 2.14</td>
<td>480.28 ± 74.30</td>
<td>3920.33 ± 29.35</td>
<td>873.00 ± 31.14</td>
</tr>
<tr>
<td>2000 ppm IBA</td>
<td>27.22 ± 2.35</td>
<td>11.63 ± 1.36</td>
<td>93.99 ± 8.65</td>
<td>963.33 ± 48.35</td>
<td>195.00 ± 3.70</td>
</tr>
<tr>
<td>B. cereus UPMLH1</td>
<td>83.70 ± 6.36</td>
<td>9.50 ± 0.32</td>
<td>166.87 ± 8.88</td>
<td>936.33 ± 31.39</td>
<td>237.59 ± 4.35</td>
</tr>
<tr>
<td>B. megaterium UPMLH3</td>
<td>27.22 ± 5.22</td>
<td>8.53 ± 0.45</td>
<td>113.89 ± 40.77</td>
<td>607.00 ± 35.84</td>
<td>135.25 ± 12.85</td>
</tr>
<tr>
<td>B. cereus UPMLH24</td>
<td>112.99 ± 1.36</td>
<td>11.06 ± 0.75</td>
<td>280.99 ± 31.72</td>
<td>1003.00 ± 14.73</td>
<td>327.50 ± 4.05</td>
</tr>
</tbody>
</table>

NOTE: Observation means of 12 plants; means followed by different letters within each column are significantly different at p<0.01 level.

Table 4: Population of indigenous Bacillus spp. recovered from pepper roots and sand after 45 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cfu/g root (dry weight)</th>
<th>cfu/g sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cereus UPMLH1</td>
<td>$10.1 \times 10^9$</td>
<td>$26.7 \times 10^8$</td>
</tr>
<tr>
<td>B. megaterium UPMLH3</td>
<td>$2.6 \times 10^9$</td>
<td>$16.7 \times 10^8$</td>
</tr>
<tr>
<td>B. cereus UPMLH24</td>
<td>$1.7 \times 10^9$</td>
<td>$15.3 \times 10^8$</td>
</tr>
</tbody>
</table>

NOTE: Observation means of 3 plants; means followed by different letters within each column are significantly different at 0.05 level.
CONCLUSION
 Apparently IAA producing PGPR could play important role as an alternative green technology for synthetic IAA or IBA in plant propagation. *B. cereus* UPMLH24 isolate has high potential to serve as biostimulant based on its ability in colonizing the roots and improving the rooting in black pepper plants. Further studies under field conditions should be conducted to verify the present findings as well as to evaluate the interaction between the PGPR and soil parameters in order to boost the growth and yield of black pepper plants.

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REFERENCES


