Effect of co-inoculation with *Bradyrhizobium japonicum* and *Pseudomonas putida* on root morph-architecture traits, nodulation and growth of soybean in response to phosphorus supply under hydroponic conditions

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Abstract


Plant growth promoting *Rhizobacteria* with plant root inoculation plays important role in plant growth and yield. In our research, the root morph-architecture traits, nodulation and growth on soybean by *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* TSAU 1 strains effects were studied in high (250 μmol) and low (50 μmol) phosphorus nutrient solutions under hydroponic conditions. Soybean plants were grown for 45 days at 29°C day temperature and 20°C night temperature in phosphorus supply hydroponic conditions. Results revealed that the combination of *B. japonicum* and *P. putida* inoculation soybean significantly raised root morph-architecture traits, nodulation, and growth on soybean compared to control and single inoculation with *B. japonicum* USDA 110 alone under both nutrient solutions. There was a significant and positive correlation between root architecture and nodule number of soybean coinoculated with *B. japonicum* USDA 110 and *P. putida* TSAU 1 under high phosphorus (250 μM) nutrient condition. Our results indicate that the higher efficiency of the combination with *B. japonicum* USDA 110 and *P. putida* TSAU 1 symbiotic system is related to the ability to increase root morph-architecture traits, nodulation, and growth under low and high phosphorus nutrient solutions.

Keywords: Rhizobacteria; root morph-architecture; nodulation; soybean growth; phosphorus

Introduction

Phosphorus (P) is the most important microelement for plant development and yield. It also plays a major role in controlling enzymes activity and molecular structure. The nucleic acids, proteins and lipid molecules in a cell of the plant contain P (Qin et al., 2012; Niste et al., 2014). Phosphorus participates in many metabolic processes, energy generation, synthesis of nucleic acids, photosynthesis, respiration, glycolysis, membrane synthesis and stability, carbohydrate metabolism, nitrogen (N) fixation and activation of enzymes (Vance et al., 2003). P is taken up by the plant
in increased root growth, enhanced lateral root formation, increased length and number of root hairs (Liao et al., 2001; Lynch and Brown, 2001; Chaudhary et al., 2008). Also, P rise the nodulation, plant development, root growth and architecture (Gentili and Huss-Danell, 2003; Vance et al., 2003; Kuang et al., 2005).

P deficit is one of the major abiotic factors. The decline of P has a negative effect on the development of plant leaf areas and the process of photosynthesis in the leaf area (Chaudhary et al., 2008). P deficiency limits root weight, shoot weight, root length, shoot growth, total phosphorus and number of nodule, as a result decreasing nitrogen fixation of leguminous plants (Liao et al., 2004; Jebara et al., 2005; Beebe et al., 2006; Palermo et al., 2012; Kontopoulou et al., 2015; Sulieman and Tran, 2015).

Soybean (Glycine max L. Merr.) crop is the main source of oil and protein in the world (Guo et al., 2011; Qin et al., 2011). The role of P is, therefore, the most important for soybean growth and development. P is needed protein and oil yield for soybean grain. Several studies reported the negative effects of P deficiency root morphology, length of root, length of shoot, weight of root, weight of shoot, nodule weight, weight of biomass, nodule number, total plant nitrogen and phosphorus in soybean under hydroponic condition (Miao et al., 2007; Win et al., 2010; Mukhtar, 2015).

P plays major role in the legume-rhizobia symbiosis. Many studies have suggested that the P supply of a nutrient significantly affects the nodulation and growth in legumes, such as common bean (Liao et al., 2001; Liao et al., 2004; Jebara et al., 2005), soybean (Miao et al., 2007; Ao et al., 2010; Mukhtar, 2015; Jabborova and Davranov, 2015) and Medicago truncatula (Sulieman et al., 2013).

The aims of this study were to investigate the role of P supply in nodulation and root morph-architecture traits in soybean plants, and to investigate the interaction between Bradyrhizobium japonicum and Pseudomonas putida strains in plant tissues. In the greenhouse experiment, plants were grown at both sufficient and limiting P concentrations to examine interactions among strains.

**Materials and Methods**

Soybean (Glycine max L.) genotype YC03-3 was used in this study provided by the Root Biology Centre, South China Agricultural University. The P. putida TSAU 1 was grown on King’s B agar (KB) and B. japonicum USDA 110 on yeast extract mannitol (YEM) agar at 28°C. Pseudomonas was grown on King’s B medium, which contained (g L−1) peptone, 20.0; glycerol, 10.0 mL; K2HPO4, 1.5; MgSO4, 1.5; agar, 15.0; pH 7.2; YEMA medium contained (g L−1) mannitol, 10.0; yeast extract, 0.3; dipotassium phosphate (K2HPO4), 0.25; magnesium sulphate (MgSO4), 0.2; agar, 15.0; pH 7.0.

The surface of grain was sterilized in 10% NaOCl and germinated on paper tissue towels soaked in 0.5 mM CaSO4 for 5 days in a dark room at 25°C (Liao et al., 2001). Bradyrhizobium japonicum strain USDA 110 uninoculated to the seedlings which were 10⁶ CFU ml⁻¹. Co-inoculation the cell suspensions of both strains were mixed in a ratio 1:1 and vortexed. The seedlings were transferred into a 2 L pot under hydroponic conditions. Two concentrations of the nutrient solution (modified solution) were used as treatments, responding to HP (high phosphorus concentration; 250 μM) and LP (low phosphorus concentration; 50 μM). Soybean were grown in a greenhouse at 29°C during days and at 20°C during night and the nutrient solutions were renewed twice a week. The pH of the solution was adjusted to 6.5-7.0 daily. Three replicates were also used for each treatment.

Soybean was harvested 45 days after planting. At harvest, each plant was separated into shoots and roots and the shoot height of each plant was measured using a ruler. After taking the fresh root weight of each plant, the roots were then scanned using Win-RHIZO LA1600 (Regent Instruments, Quebec, Canada) to measure the surface of the root, the area of the root, the diameter of the root, the volume of root and total root length. Shoots were oven-dried to constant weight at 75°C for 48 hours and the shoot dry weights were recorded using an electronic balance. The roots of each plant were then oven-dried to constant weight at 75°C for 48 hours and the root dry weights were recorded using the same electronic balance.

Experimental data were analysed with the StatView Software using ANOVA. The significance of the effect of treatment was determined by the magnitude of the F value (P < 0.05 < 0.001).

**Results and Discussion**

The growth of soybeans was increased by high P (250 μM) when soybeans were inoculated and not inoculated, under all treatments. High P (250 μM) increased the length of the shoot by 32% compared to control at low P condition. Co-inoculation with B. japonicum USDA 110 and P. putida TSAU 1 significantly increased the length of the shoot by 19% compared to control of high P (250 μM) condition (Fig. 1a). P deficiency induced a significant decrease in shoot
Fig. 1. The effect of the P supply in the solution on shoot length per plant (a), number of nodules per plant (b), root weight per plant (c), shoot weight per plant (d), nodule dry weight per plant (e) and total plant weight (f) of inoculated soybean plants, grown at 50 and 250 μM for 45 days
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In low P (50 μM) both single *B. japonicum* USDA 110 and co-inoculation *B. japonicum* USDA 110 with *P. putida* TSAU 1 improved the length of the shoot on average by 15-32.2% (Fig. 1a).

The root dry weight, shoot dry weight and total dry weight of soybean inoculated with single *B. japonicum* USDA 110 plants significantly increased by 71%, 74% and 78%, compared to control of high P (250 μM) nutrient condition (Fig. 1 c, d, f). The highest root dry weight, shoot dry weight and total dry weight of soybean was increased from coinoculated soybean tissues grown in high P (250 μM) nutrient condition.

The combination of *B. japonicum* USDA 110 and *P. putida* TSAU 1 significantly increased the root dry weight, shoot dry weight and total dry weight of soybean by 103%, 153%, and 155% compared to control of high P (250 μM) condition. Co-inoculation of soybean with *B. japonicum* USDA 110 and *P. putida* TSAU 1 strains significantly increased the root dry weight 18%, shoot dry weight 45% and total dry weight 43% of plants compared to *B. japonicum* USDA 110 alone in 250 μM P condition (Fig. 1 c, d, f).

The root dry weight, shoot dry weight and total dry weight of control plants sharply decreased by 16%, 47%, and 40%.

**Fig. 2a, b.** The root system of soybean grown in Hoagland solution supplemented with HP (250 μM) and LP (50 μM) supply under hydroponic condition.
Table 1. P supply in the solution on root morphological traits of soybeans by inoculation with B. japonicum USDA 110 and co-inoculation with P. putida TSAU 1

<table>
<thead>
<tr>
<th>Nutrient solution</th>
<th>Treatment</th>
<th>Total root length, cm</th>
<th>Root surface area, cm²</th>
<th>Projected area, cm²</th>
<th>Root average diameter, mm</th>
<th>Root volume, cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP 250 μM</td>
<td>Control</td>
<td>3362.4 ± 1.69</td>
<td>352.5 ± 1.64</td>
<td>109.1 ± 0.90</td>
<td>0.30 ± 0.05</td>
<td>3.35 ± 0.98</td>
</tr>
<tr>
<td>USDA110 USDA</td>
<td>5183.7 ± 2.42***</td>
<td>570.5 ± 1.27***</td>
<td>163.2 ± 2.20***</td>
<td>0.32 ± 0.03</td>
<td>4.18 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>+ TSAU 1</td>
<td>6855.5 ± 1.10***</td>
<td>1037.2 ± 1.56***</td>
<td>292.5 ± 2.78***</td>
<td>0.44 ± 0.04**</td>
<td>11.3 ± 1.38***</td>
<td></td>
</tr>
<tr>
<td>LP 50 μM</td>
<td>Control</td>
<td>1499.6 ± 1.22</td>
<td>123.5 ± 1.33</td>
<td>85.67 ± 1.21</td>
<td>0.27 ± 0.06</td>
<td>2.80 ± 0.03</td>
</tr>
<tr>
<td>USDA110 USDA</td>
<td>3253.3 ± 1.92**</td>
<td>426.0 ± 1.35**</td>
<td>138.8 ± 1.73*</td>
<td>0.29 ± 0.08</td>
<td>4.28 ± 0.02*</td>
<td></td>
</tr>
<tr>
<td>+ TSAU 1</td>
<td>4231.4 ± 1.02**</td>
<td>510.4 ± 1.06**</td>
<td>175.0 ± 1.96**</td>
<td>0.38 ± 0.06**</td>
<td>5.59 ± 0.07**</td>
<td></td>
</tr>
</tbody>
</table>

During 45-days soybean plant was grown under hydroponic conditions in high phosphorus 250 μM and low phosphorus 50 μM nutrient solutions.

44% respectively in low P (50 μM) nutrient condition (Fig. 1c, d, f). Inoculation of soybean alone B. japonicum USDA 110 strain significantly increased the root dry weight 59%, shoot dry weight 103% and total dry weight 107% of plants compared to control in 50 μM P condition (Fig. 1c, d, f).

The combination of B. japonicum USDA 110 and P. putida TSAU 1 strains increased the root dry weight, shoot dry weight and total dry weight of soybeans by 122-270%, compared to inoculated control and inoculation with the B. japonicum USDA 110 alone by 39-81% in low P (50 μM).

The high P (250 μM) supply significantly increased both the number of nodules (Fig. 1b) and the nodule dry weight per plant (Fig. 1e) when soybeans were inoculated both single B. japonicum USDA 110 and co-inoculation with B. japonicum USDA 110 with P. putida TSAU 1. The combination of B. japonicum USDA 110 and P. putida TSAU 1 inoculated soybean significantly increased the number of nodules 13% and the nodule dry weight 114% per plant compared to single inoculation with B. japonicum USDA 110 alone in 250 μM P condition (Fig. 1b, c). P deficiency decreased both the number of nodules (Fig. 1b, e) and the nodule dry weight per plant when soybeans were inoculated with single B. japonicum USDA 110 alone. The LP (50 μM) significantly increased a number of nodules 82% and the nodule dry weight 62% per plant when soybeans were coinoculated B. japonicum USDA 110 with P. putida TSAU 1 compared to single-inoculated B. japonicum USDA 110 alone (Fig. 1b, e).

According to the results, in the HP (250 μM) nutrient solutions the length of root on soybean was increased by 54%, the surface of root to 62% and the area of root to 49% and the volume of root to 24% when inoculation B. japonicum USDA 110 strain compared to control (Fig. 2a, b, Table 1). Consequently, it was observed that at coinoculation of B. japonicum USDA 110 with P. putida TSAU 1 strains, the length of root of soybean increased to 104%, the surface of root to 194%, the area of root to 168% and the diameter of the root to 47% for the soybean plants grown in the HP nutrient solutions (250 μM).

P deficiency induced a significant decrease in root morph-architecture growth in the control. The root length, surface area, projected area, root volume and root average diameter of control plants sharply decreased by 55%, 65%, 22%, 17% and 10% respectively in LP (50 μM) nutrient condition (Table 1). Inoculation single B. japonicum USDA 110 strain significantly increased the root length 116%, surface area 244%, projected area 62% and root volume 52% compared to control in LP (50 μM) condition. The combination of B. japonicum USDA 110 and P. putida TSAU 1 increased the root length, projected area, root volume and average diameter of roots by 182%, 104%, 97% and 40% compared to uninoculated control and inoculation with the symbiont alone by 30%, 20%, 30% and 41% in LP (50 μM) solution. Significantly positive correlations were recorded for root architecture and nodule number of soybean coinoculated with B. japonicum USDA 110 and P. putida TSAU 1 in hydroponic culture with high phosphorus supply (Table 2).

Table 2. Correlation of root length (RL), surface area (SA), project area (PA), root volume (V), root diameter (AD) and nodule number (NN) of soybean co-inoculated with Bradyrhizobium japonicum USDA 110 and P. putida TSAU 1 in hydroponic culture with high phosphorus supply (HP 250 μM)

<table>
<thead>
<tr>
<th></th>
<th>RL</th>
<th>SA</th>
<th>PA</th>
<th>V</th>
<th>AD</th>
<th>NN</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SA</td>
<td>0.67**</td>
<td>1</td>
<td>0.69***</td>
<td>0.94***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>0.98***</td>
<td>0.69***</td>
<td>1</td>
<td>0.97***</td>
<td>0.96***</td>
<td>1</td>
</tr>
<tr>
<td>V</td>
<td>0.86***</td>
<td>0.92***</td>
<td>0.94***</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>0.92***</td>
<td>0.79***</td>
<td>0.97***</td>
<td>0.96***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NN</td>
<td>-0.23*</td>
<td>0.48**</td>
<td>-0.17*</td>
<td>0.24*</td>
<td>0.02</td>
<td>1</td>
</tr>
</tbody>
</table>

Significance levels for positive correlations: *P < 0.05 (significant), **P < 0.01 (highly significant), ***P < 0.001 (extremely significant)
ent solution. Root length values were in an extremely significant positive correlation with root project area \((r = 0.98)\), root volume \((r = 0.86)\) and root average diameter \((r = 0.92)\). Root surface area values were in an extremely significant positive correlation with root project area \((r = 0.69)\), root volume \((r = 0.92)\) and root average diameter \((r = 0.79)\). Root project area values were in an extremely significant positive correlation with root volume \((r = 0.94)\) and root average diameter \((r = 0.97)\).

Significantly positive correlations were recorded for root architecture and nodule number of soybean coinoculated with \(B. japonicum\) USDA 110 and \(P. putida\) TSAU 1 in hydroponic culture with low phosphorus supply (Table 3). Nodule number area values were in a significant positive correlation with root surface area \((r = 0.85)\) and root average diameter \((r = 0.23)\) coinoculated with \(B. japonicum\) USDA 110 and \(P. putida\) TSAU 1 under LP (50 μM) nutrient solution. Root surface area values were in an extremely significant positive correlation with root nodule number \((r = 0.85)\). Also, root volume values were in an extremely significant positive correlation with root average diameter \((r = 0.88)\). Significantly, positive correlations were recorded for root architecture and nodule number of soybean coinfected with \(B. japonicum\) USDA 110 and \(P. putida\) TSAU 1 under LP (50 μM) nutrient solution. Root length values were in a highly significant positive correlation with root surface area \((r = 0.44)\) and root project area \((r = 0.40)\).

**Table 3. Correlation of root length (RL), surface area (SA), project area (PA), root volume (V), root diameter (AD) and nodule number (NN) of soybean coinoculated with \(B. japonicum\) USDA 110 and \(P. putida\) TSAU 1 in hydroponic culture with low phosphorus supply (LP 50μM)**

<table>
<thead>
<tr>
<th></th>
<th>RL</th>
<th>SA</th>
<th>PA</th>
<th>V</th>
<th>AD</th>
<th>NN</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>0.44**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>0.40**</td>
<td>0.06</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>-0.58**</td>
<td>-0.32</td>
<td>-0.85**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>-0.17*</td>
<td>0.00</td>
<td>-0.78***</td>
<td>0.88***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NN</td>
<td>0.00</td>
<td>0.85***</td>
<td>-0.12*</td>
<td>0.03</td>
<td>0.23*</td>
<td>1</td>
</tr>
</tbody>
</table>

Significance levels for positive correlations: **P < 0.05 (significant), P < 0.01 (highly significant), ***P < 0.001 (extremely significant)**

**Results and Discussion**

This study demonstrated that P supply in the solution had an important role in root morph-architecture traits, nodulation, and growth of soybean. High P (250 μM) supply increased length of shoot, dry weight of root, dry weight of shoot and dry weight of total plant (Fig. 1a, c, d, f). Similar results have been obtained in common bean, in which root length, root surface, average diameter, total root dry weight, total shoot dry weight and dry weight of total plant were increased in HP solution (Liao et al., 2004; Win et al., 2010; Ochigbo and Bello, 2014). Many studies reported the high P positive effects on root length of leguminous plants (Liao et al., 2001, 2004; Chaudhary et al., 2008).

In this study, the highest nodule number and nodule dry weight were observed on soybean roots grown under high P solution (Fig. 1b, e). Miao et al. (2007) reported an increased nodule weight per nodule and the nodule biomass in soybean grown in high P solution. Several studies have demonstrated that the P supply in the solution had a specific stimulation on nodulation, nodule growth, root growth and architecture (Reddell et al., 1997; Vadez and Drevon, 2001; Tang et al., 2001; Vance et al., 2003; Kuang et al., 2005; Chaudhary et al., 2008; Qin et al., 2012).

In other studies, the low P in the solution had a negative effect on nodulation and the nodule function (Robson et al., 1981; Miao et al., 2007; Sulieman et al., 2013). Our study showed that high P supply in the solution significantly increased the total root length, root surface area and root project area compared to low P supply (Table 1). Ao et al. (2010) reported that significantly increased root length, root surface area and root volume of soybean grown in high P solution.

Our study demonstrated that P supply in the solution at single inoculation \(B. japonicum\) USDA 110 strain significantly raised the dry weight of root, the dry weight of shoot, the dry weight of total plants (Fig. 1b, e), the length of total root, the surface of root and the project area of root compared to control (Table 1). Plant growth promoting \(Rhizobacteria\) on plant nutrition may result from effects on plant nutrient uptake and plant growth (Egamberdieva et al., 2016; Egamberdieva et al., 2017). Sulieman et al. (2013) conducted an experiment on the effect of inoculation with \(S. meliloti\) 2011 strain which increased the dry weight of root, the dry weight of shoot, number of the nodule and the dry weight of nodule of \(M. truncatula\) than \(M. truncatula\) under P-deficiency conditions. It has also been reported that plant height, root and shoot biomass, root surface area and root project area of soybean increased when seeds inoculated with \(Rhizobium\) under high P condition (Mukhtar, 2015).

The present study illustrated that the length of total root, the surface of root and the project area of root on soybean coinoculated with \(B. japonicum\) USDA 110 and \(P. putida\) TSAU 1 strains significantly increased compared to control and \(B. japonicum\) USDA 110 alone in both nutrient solutions. Co-inoculated soybeans grown in both nutrient solutions had
the highest number of nodules, the dry weight of root, the dry weight of shoot, the dry weight of nodule and the dry weight of total plant (Fig. 1 b, c, d, e, f). Similarly, combined inoculation of *Rhizobium* and plant growth-promoting bacteria in bean and chickpea has been reported to enhance nodulation, plant growth, and nutrient uptake (Sindhu and Dadarwal, 2001; Goel et al., 2002; Stajkovic et al., 2011). Jabborova and Davranov (2015) reported increased growth of soybean by co-inoculating the seedling with *B. japonicum* USDA 110 and *P. putida* TSAU 1 under low P (50 μM) nutrient solution. Further at the field level, could be an effective alternative to chemical fertilizer in order to promote soybean growth when coinoculation of *B. japonicum* USDA 110 and *P. putida* TSAU 1 under low P defence conditions.

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**References**


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