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Effects of Phosphate Solubilizing Bacteria on the Growth, Photosynthesis, and Nutrient Uptake of *Camellia oleifera* Abel.

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Abstract: Phosphorus (P) is a necessary nutrient for plant growth and plays an important role in plant metabolisms; however, the majority of P in soil is in insoluble forms. Phosphate solubilizing bacteria (PSB) can convert the insoluble phosphates into plant-available forms and may have the potential for use in sustainable agricultural practices. This study examined the effects of two native PSB, namely *Bacillus aryabhattai* (JX285) and *Pseudomonas auricularis* (HN038), and a mixture of both strains (1:1) on the growth of *Camellia oleifera* Abel. seedlings. The results showed a significant promotion of the growth of *C. oleifera* plants by three inoculation treatments. All the PSB inoculation treatments could improve the leaf nitrogen (N) and P content and had positive effects on the available N, P, and potassium (K) content of rhizosphere soil. A co-inoculation of the two native PSB strains caused a synergistic effect and achieved the best benefit. In conclusion, *B. aryabhattai* and *P. auricularis* could be used as biological agents instead of chemical fertilizers for agricultural production to reduce environmental pollution and increase the yield of tea oil.

Keywords: phosphate solubilizing bacteria; nutrition; oil tea

1. Introduction

Phosphorus (P) is an essential nutrient for plant growth and development. Although a large amount of P is present in soil, the majority of it is unavailable to plants [1]. In agro-forestry practices, it is a fact that many problems arise in the application of phosphate fertilizer [2]. On one hand, phosphate rock is a nonrenewable resource and may run out in 50–100 years due to anthropogenic exploitation [3]. On the other hand, about 70% of the phosphate fertilizer in soluble forms that is applied to the soil is rapidly combined with cations such as Ca^{2+}, Mg^{2+}, Al^{3+}, and Fe^{3+} and converted to insoluble forms [4]. Therefore, improving the absorption and use of P by crops is of great significance from both the ecological and economical perspective [5].

Phosphate-dissolving microorganisms are microbial resources closely associated with plant nutrition and account for 10% of all soil microorganisms [6,7]. Phosphate solubilizing bacteria (PSB)

are a group of these microorganisms that can transform insoluble P compounds into available forms by secreting organic acids, and they may be used as inoculants to enhance P availability for plants [8]. In addition, they can also promote plant growth via producing hormones, such as cytokinin and indole acetic acid [9,10]. Despite some PSB present in plant rhizospheres and in soil, the amount of P released by these microorganisms is usually insufficient to meet the demand of growing plants [11]. High-efficiency PSB have the potential for making a great contribution to the decrease of environmental pollution and promoting ecological balance by replacing chemical fertilizers [12]. Consequently, there is an urgent need to investigate the effects of selected high-efficiency PSB on plant nutrition and growth.

Camellia oleifera Abel. (Theaceae), a unique edible oil tree species to China, is one of the world's four famous woody oil plants [13]. Tea oil obtained from seeds has an unsaturated fatty acid content of up to 90%, much higher than vegetable oil, peanut oil, and soybean oil, and contains special physiologically active substances, such as camellia, which have extremely high nutritional values [14]. *Camellia oleifera* generally grows in the mountains and hills of subtropical regions in southern China and is also of great value in soil and water conservation and maintaining ecological balance [15]. However, the acid soil in southern China has a low available P content and limits the growth and productivity of *C. oleifera* [16]. In our previous study, we screened two high-efficiency PSB strains (JX285 and HN038) from the rhizosphere soil of *C. oleifera* [17,18]. The present study's aim was to determine the impacts of JX285 and HN038 on the growth, photosynthesis, and nutrient uptake of *C. oleifera*. The results may provide a theoretical basis for the development of microbial fertilizers for use in agroforestry.

2. Materials and Methods

2.1. Strains, Plant Material, and Growth Medium

The two PSB strains, JX285 (*Bacillus aryabhattai*) and HN38 (*Pseudomonas auricularis*), were isolated from the rhizosphere of *C. oleifera* in our previous experiments [17,18]. The PSB inoculum was prepared as follows: the two PSB strains were individually grown in liquid Luria-Bertani (LB) medium (containing tryptone 10 g/L, yeast extract 5 g/L, and NaCl 10 g/L) with shaking at 180 rpm for 24 h at 28 °C, and then the broth was centrifuged at 10,000 r/min for 10 min. The supernatant was discarded, and the pellet was re-suspended and washed with sterilized water three times. The final concentration of PSB inoculum was adjusted to 10^8 colony-forming units (CFU) mL^{-1}.

The seeds of *C. oleifera* were provided by the Jiangxi Academy of Forestry Research, China. The seeds were first surface sterilized with 0.3% potassium permanganate solution for 1 h and rinsed with sterilized water three times. They were then germinated on wet gauze at 30 °C. The germinated seeds were transplanted into 1 kg pots with sterilized sand. Finally, uniform seedlings were selected and transferred to a new pot containing 3 kg of growth medium. Each pot was planted with one seedling.

The growth medium was a mixture of krasnozem soil, sand, and peat soil (6:3:1, *v*/*v*). The soil used in the experiment was collected from the campus of Jiangxi Agricultural University (China) and was sieved after being air dried (1 mm). The growth medium was autoclaved at 0.11 MPa and 121 °C for 2 h. The basic physicochemical properties of the growth medium were as follows: pH 5.25 (soil:water—1:5), 43.71 mg/kg organic matter, 94.68 mg/kg available nitrogen (N), 2.80 mg/kg available P, and 15.80 mg/kg available potassium (K).

2.2. Experimental Design and Growth Condition

A pot experiment was conducted using a two-factor completely randomized block design containing five inoculation treatments and three P treatments [19]. The inoculation treatments were as follows: inoculated with *P. auricularis* HN038 (T1), inoculated with *B. aryabhattai* JX285 (T2), inoculated with the mixture of HN038 and JX285 (1:1) (T3), inoculated with LB medium (CK1), and inoculated with sterile water (CK2). The P treatments were as follows: no phosphate fertilizer (0 g/kg), (P1), added 3 g calcium superphosphate (1 g/kg) (P2), added 6 g calcium superphosphate (2 g/kg) (P3). Each treatment contained 5 replicates. Furthermore, in order to prevent the plant grow from stopping

due to a lack of nutrient requirements, urea and potassium nitrate were added to the soil to reach 300 mg/kg N and 200 mg/kg K, respectively. The pot was placed in a greenhouse with 12 h of light per day. After five months of growth, the growth and photosynthetic parameters were measured and the seedlings were harvested.

2.3. Plant Growth Measurement

Three healthy seedlings with consistent growth were selected for each treatment to measure their growth parameters. A measuring tape (Swordfish, China) was used to measure the plant height. The leaves, stems, and roots were harvested and dried at 75 °C to a constant weight and weighed to calculate the total biomass of each plant.

2.4. Measurement of Gas Exchange Parameters and Relative Chlorophyll Content

A LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) was used to determine the photosynthetic parameters. When the seedlings were growing vigorously (mid-September) and the temperature was relatively stable (09:00–11:00 am), healthy leaves in the upper part of the seedlings were chosen to determine the net photosynthetic rate (Pn) and the transpiration rate (Tr). The water use efficiency (WUE) was calculated as WUE = Pn/Tr. A chlorophyll meter (SPAD-502) was used to measure the relative chlorophyll content of the plants. A random measurement method was used between each treatment to eliminate the inevitable errors caused by different measurement times.

2.5. Measurement of Plant Nutrient and Soil Nutrient

The dry leaves were finely ground and homogenized to determine the N and P concentrations. The P content was assayed by the dry ash digestion method [20]. The N content was determined by the Kjeldahl digestion method [21].

Samples of rhizosphere soil were collected to test the soil's nutrient status. The N content was measured using the alkaline hydrolysis method [22]. The P content was determined by the molybdenum ruthenium anti-colorimetric method [23]. The K content was measured by atomic absorption spectrometry [24].

2.6. Data Analysis

Statistical tests were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A two-way ANOVA was performed to analysis the significance of P application, the PSB inoculation treatment and the interaction of P × PSB treatment. A one-way ANOVA was employed to examine the differences among the different inoculation treatments under each P level. Duncan's multiple range test was performed at $p = 0.05$ in case of significant impact by factor.

3. Results

3.1. Plant Height and Biomass

The two-way ANOVA results showed that plant height and biomass were significantly ($p \leq 0.01$) influenced by the interaction between P application and PSB inoculation (Table 1). The plant height and biomass were significantly ($p \leq 0.05$) improved by the single and mixed inoculation of two PSB strains compared to the non-inoculation control under each P level (Table 1). Co-inoculated plants showed a greater plant height and dry weight than those of plants inoculated with single PSB strains and non-inoculated plants. There was no significant difference in plant height and biomass between the CK1 and CK2 treatments. Moreover, the degree of the increase by PSB inoculation differed to some extent under different P levels. The maximum positive effects of the PSB inoculation on plant height and biomass were observed at the P3 level.

Table 1. Effect of PSB on plant height and biomass of *C. oleifera* seedlings.

P Application	PSB Inoculation	Plant Height (cm)	Biomass (g)
P1	T1	22.5 ± 0.9 b	10.13 ± 0.90 c
	T2	22.7 ± 1.9 b	12.00 ± 0.60 b
	T3	26.2 ± 1.2 a	14.27 ± 1.40 a
	CK1	15.7 ± 1.2 c	6.67 ± 0.61 d
	CK2	17.8 ± 0.3 c	7.00 ± 0.72 d
P2	T1	34.5 ± 1.0 c	23.40 ± 1.59 c
	T2	36.7 ± 1.8 b	24.40 ± 0.72 b
	T3	37.5 ± 1.0 a	26.97 ± 1.66 a
	CK1	16.5 ± 2.1 d	7.67 ± 1.01 d
	CK2	18.2 ± 0.9 d	7.97 ± 1.53 d
P3	T1	32.3 ± 1.5 a	15.73 ± 0.90 b
	T2	33.5 ± 0.9 a	16.47 ± 0.99 b
	T3	34.5 ± 0.8 a	19.13 ± 0.92 a
	CK1	16.9 ± 1.3 b	7.24 ± 0.47 c
	CK2	17.9 ± 2.1 b	7.50 ± 0.95 c
Two-way ANOVA results			
P		0.00 **	0.00 **
PSB		0.00 **	0.00 **
P × PSB		0.00 **	0.00 **

Data are means ± SD ($n = 3$). P: phosphorus; PSB: phosphate solubilizing bacteria; P1: no phosphate fertilizer (0 g/kg); P2: added 3 g calcium superphosphate (1 g/kg); P3: added 6 g calcium superphosphate (2 g/kg); T1: inoculated with *P. auricularis* HN038; T2: inoculated with *B. aryabhattai* JX285; T3: inoculated with the mixture of HN038 and JX285 (1:1); CK1: inoculated with LB medium; CK2: inoculated with sterile water. Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$). **, significant effect at $p \leq 0.01$.

3.2. Gas Exchange Parameters

The Pn was significantly ($p \leq 0.05$) influenced by P treatment and PSB treatment, while the Tr and WUE were significantly ($p \leq 0.05$) influenced by the interaction between P application and PSB inoculation (Table 2). At the same P application level, the Pn, Tr, and WUE were significantly ($p \leq 0.05$) enhanced by the single and mixed inoculation of PSB strains (Table 2). The Pn, Tr, and WUE of plants co-inoculated with *B. aryabhattai* and *P. auricularis* were higher than that of other treatments. The inoculation of single or mixed PSB strains promoted Pn and Tr more effectively at a high P level (P3 treatment), while the PSB's best improvement effect on WUE appeared at the intermediate level (P2 treatment).

Table 2. Effect of PSB on the photosynthetic characteristics of *C. oleifera* leaves.

P Application Levels	PSB Inoculation	Pn [μmol/(m^2·s)]	Tr [μmol/(m^2·s)]	WUE (μmol/mmol)	SPAD
P1	T1	3.41 ± 0.07 c	2.47 ± 0.07 a	1.38 ± 0.03 b	48.40 ± 1.64c
	T2	3.64 ± 0.05 b	2.59 ± 0.06 a	1.40 ± 0.03 b	51.37 ± 0.64 b
	T3	4.48 ± 0.20 a	2.69 ± 0.09 a	1.66 ± 0.07 a	64.83 ± 2.70 a
	CK1	2.46 ± 0.25 d	1.58 ± 0.09 b	1.74 ± 0.10 a	43.63 ± 2.21 d
	CK2	2.79 ± 0.43 d	1.73 ± 0.03 b	1.62 ± 0.23 a	44.83 ± 1.72 d
P2	T1	4.14 ± 0.35 c	2.20 ± 0.09 ab	1.88 ± 0.08 a	62.93 ± 2.40 b
	T2	4.73 ± 0.40 b	2.66 ± 0.07 a	1.78 ± 0.14 a	68.87 ± 1.66 a
	T3	5.69 ± 0.36 a	2.80 ± 0.04 a	2.03 ± 0.21 a	71.30 ± 1.64 a
	CK1	2.93 ± 0.45 d	1.87 ± 0.04 b	1.56 ± 0.21 b	47.83 ± 1.50 c
	CK2	3.07 ± 0.36 d	1.83 ± 0.04 b	1.68 ± 0.16 b	48.31 ± 1.14 c
P3	T1	3.76 ± 0.10 c	2.37 ± 0.03 ab	1.58 ± 0.02 a	52.73 ± 2.01 c
	T2	3.93 ± 0.29 b	2.42 ± 0.05 a	1.62 ± 0.10 a	57.87 ± 0.40 b
	T3	4.82 ± 0.20 a	2.87 ± 0.06 a	1.68 ± 0.04 a	66.17 ± 2.15 a
	CK1	2.89 ± 0.23 d	1.79 ± 0.07 b	1.61 ± 0.10 a	46.23 ± 1.00 d
	CK2	3.12 ± 0.16 d	1.90 ± 0.10 b	1.59 ± 0.02 a	47.36 ± 0.92 d
Two-way ANOVA results					
P		0.00 **	0.03 *	0.00 **	0.00 **
PSB		0.01 *	0.00 **	0.01 **	0.00 **
P × PSB		0.17 ^{NS}	0.00 **	0.17 ^{NS}	0.00 **

Data are means ± SD ($n = 3$). Pn: net photosynthetic rate, Tr: transpiration rate, WUE: water use efficiency, SPAD: relative chlorophyll content. Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$). *, significant effect at $0.01 < p \leq 0.05$; **, significant effect at $p \leq 0.01$; NS, no significant effect.

3.3. Chlorophyll Content

The chlorophyll content was significantly ($p \leq 0.01$) influenced by the interaction between P application and PSB inoculation (Table 2). The single and mixed inoculations of PSB strains could significantly ($p \leq 0.05$) increase the chlorophyll content of leaves regardless of the amount of P applied in the medium (Table 2). The co-inoculated plants had a higher chlorophyll content than that of either plants inoculated with single PSB strains or non-inoculated plants. The best promoted effect of the PSB strains on chlorophyll content was found at the intermediate P level. No significant difference in chlorophyll content was observed between the CK1 and CK2 treatments.

3.4. Nitrogen and Phosphorus Content in Leaves

The N content of the leaves was significantly ($p \leq 0.05$) influenced by P treatment and PSB treatment, while the P content of the leaves was significantly ($p \leq 0.01$) influenced by the interaction between P treatment and PSB treatment (Table 3). The trend of the changes in the N content of leaves was similar to that of the P content of leaves. The N and P content of the leaves increased to varying degrees by the single and mixed inoculations of PSB strains under different P application levels (Table 3). At the low (P1) and intermediate (P2) P levels, the N and P content of leaves were significantly increased by the PSB inoculation. The mixed inoculation of PSB strains increased the leaf N and P content more effectively than the single inoculation. However, there were no significant differences in the N and P content with the inoculation of PSB strains at a high P level (P3).

Table 3. Effect of PSB on the nitrogen content of *C. oleifera* leaves.

P Application Levels	PSB Inoculation	Nitrogen Content (mg/kg)	Phosphorus Content (g/kg)
P1	T1	1771.36 ± 86.22 b	0.50 ± 0.03 b
	T2	1924.55 ± 128.97 a	0.56 ± 0.02 a
	T3	1989.24 ± 100.17 a	0.55 ± 0.01 a
	CK1	1761.09 ± 102.14 b	0.44 ± 0.02 c
	CK2	1788.55 ± 85.44 b	0.46 ± 0.02 c
P2	T1	1849.29 ± 73.71 b	0.59 ± 0.03 b
	T2	2045.05 ± 50.33 a	0.61 ± 0.02 b
	T3	2122.31 ± 101.49 a	0.66 ± 0.01 a
	CK1	1791.32 ± 195.53 c	0.47 ± 0.03 c
	CK2	1886.69 ± 52.92 b	0.48 ± 0.01 c
P3	T1	1750.58 ± 105.83 b	0.50 ± 0.04 a
	T2	1766.99 ± 141.89 b	0.51 ± 0.05 a
	T3	1973.33 ± 95.04 a	0.59 ± 0.04 a
	CK1	1752.88 ± 92.92 b	0.49 ± 0.06 a
	CK2	1831.46 ± 83.27 b	0.51 ± 0.05 a
Two-way ANOVA results			
P		0.01 *	0.00 **
PSB		0.00 **	0.00 **
P × PSB		0.65 ^{NS}	0.01 **

Data are means ± SD ($n = 3$). Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$). *, significant effect at $0.01 < p \leq 0.05$; **, significant effect at $p \leq 0.01$; NS, no significant effect.

3.5. Nitrogen, Phosphorus, and Potassium Content of Rhizosphere Soil

The soil available N content was significantly ($p \leq 0.01$) influenced by P treatment and PSB treatment (Table 4). A significant increase in the available N content was found in the treatment of the inoculated PSB strains compared to the non-inoculated control ($p \leq 0.05$) (Figure 1). The maximum available N in the soil was found in the co-inoculated treatment under the intermediate P level. Moreover, no significant difference was observed in the available N content between the CK1 and CK2 treatments.

Table 4. Results of a two-way ANOVA of the effects of phosphate solubilizing bacteria (PSB) inoculation, phosphorus (P) application and their interactions on the soil parameters.

Index	P	PSB	P × PSB
Soil N content	0.00 **	0.00 **	0.06 ^{NS}
Soil P content	0.00 **	0.00 **	0.00 **
Soil K content	0.08 ^{NS}	0.00 **	0.00 **

**, significant effect at $p \leq 0.01$; NS, no significant effect.

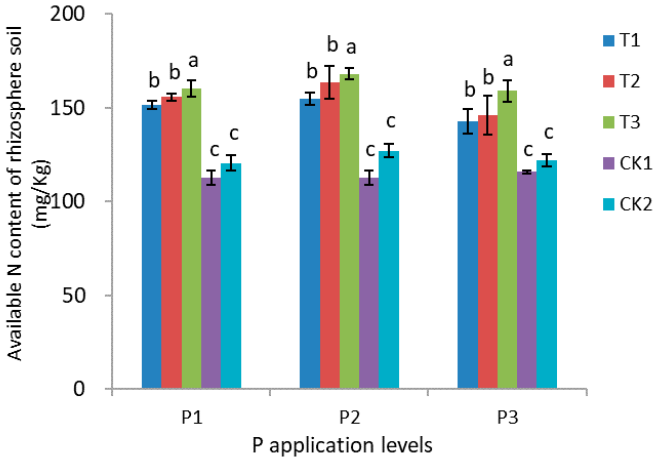

Figure 1. Effects of PSB on the available nitrogen content of *C. oleifera*. Note: Data are means ± SD ($n = 3$). P1: no phosphate fertilizer (0 g/kg); P2: added 3 g calcium superphosphate (1 g/kg); P3: added 6 g calcium superphosphate (2 g/kg); T1: inoculated with *P. auricularis* HN038; T2: inoculated with *B. aryabhattai* JX285; T3: inoculated with the mixture of HN038 and JX285 (1:1); CK1: inoculated with LB medium; CK2: inoculated with sterile water. Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$).

The results showed that the soil available P content was significantly ($p \leq 0.01$) influenced by the interaction between P treatment and PSB treatment (Table 4). Different inoculation treatments caused different degrees of increase in the available P content in the soil (Figure 2). Under the intermediate and high P levels, the inoculation of single and mixed PSB strains significantly increased the content of available P in the soil ($p \leq 0.05$). Conversely, there were no significant differences in the available P content among the inoculation treatments under a low P level ($p > 0.05$).

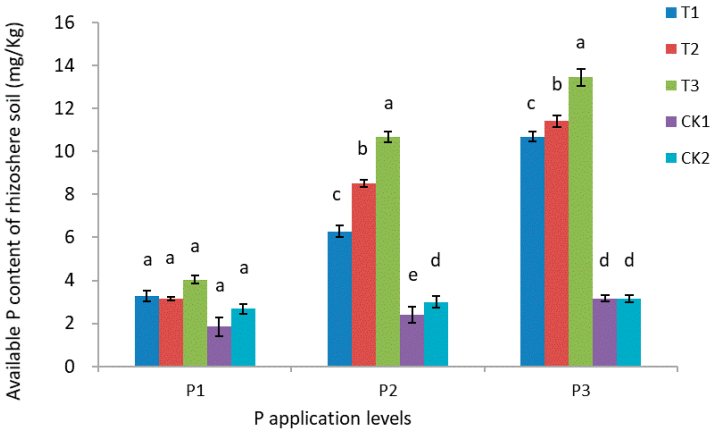

Figure 2. Effects of PSB on available phosphorus content of *C. oleifera*. Note: Data are means ± SD ($n = 3$). Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$).

The soil available K content was significantly ($p \leq 0.01$) influenced by the interaction between P treatment and PSB treatment and (Table 4). An inoculation of PSB strains increased the available K content in the soil (Figure 3). The co-inoculated treatment had a higher available K content than single and non-inoculation treatments under each P level.

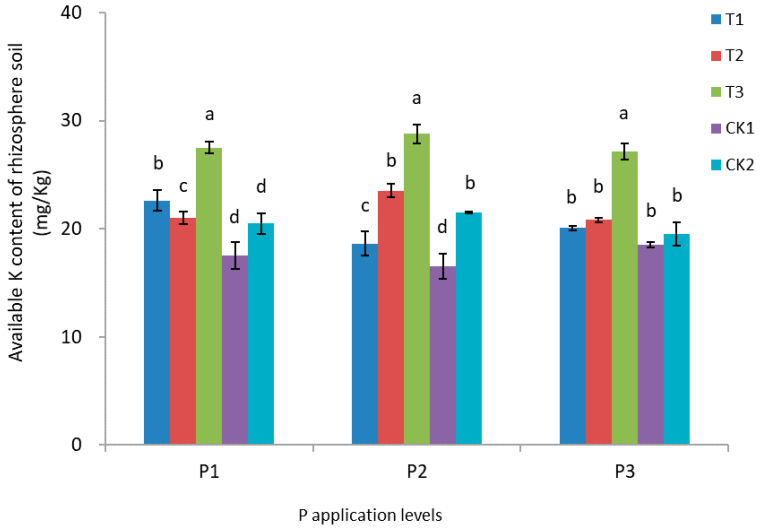

Figure 3. Effects of PSB on the available potassium content of *C. oleifera*. Note: Data are means ± SD ($n = 3$). Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$).

4. Discussion

Phosphorus deficiency in soil is an important limiting factor in global agro-forestry production [25,26]. Some microorganisms can increase the concentration of available P by secreting organic acids and various degrading enzymes (phytase, nuclease, phosphatase, etc.) to decompose insoluble phosphate in the soil [27,28]. However, these microorganisms release little P in their natural state [11]. Therefore, it is necessary to artificially inoculate some high-efficiency P solubilizing microorganisms to increase the amount of available P. In our previous experiments, we isolated native PSB strains from the rhizosphere soil of *C. oleifera* and screened two strains (JX285 and HN38) with a high phosphorus solubilizing ability [17,18]. The results of the present study showed that the single and mixed inoculations of JX285 and HN38 had positive effects on the growth, photosynthetic capacity, N and P content in the leaves of *C. oleifera*, and available N, P, and K content in the soil.

Plant growth is the most obvious characteristic for evaluating the effects of PSB [29]. In this study, the single and mixed inoculations of two native PSB strains increased the plant height and biomass of *C. oleifera*, supporting the findings reported by previous studies [10,30]. This might be due to the PSB strains of JX285 and HN38 dissolving the insoluble phosphate in the soil and enhancing the available P content by producing organic acid and extracellular phosphatases [27,28]. Another possibility might be related to the metabolism of PSB, producing a variety of plant hormones, acids, and vitamins [30].

Plants use the light energy absorbed by chlorophyll molecules to drive photosynthesis [31]. This study showed the beneficial effect of PSB inoculation on the chlorophyll content and photosynthetic capacity of *C. oleifera* that is consistent with a previous observation in rice [32]. The higher Tr and WUE found in plants inoculated with PSB strains indicated that PSB improved the water status of *C. oleifera* [33]. An enhancement of Tr suggested an increased water uptake capacity, providing additional water for transpiration and improving soluble nutrient uptake [34].

Phosphorus is an essential nutrient, being a component of vital molecules in plants, and is involved in many metabolic processes [28]. PSB may convert insoluble P compounds into soluble forms by the processes of chelation, acidification, and exchange reactions [30]. In the current study, the increased available P content in the rhizosphere soil by inoculating PSB strains was only found in the intermediate and high insoluble P levels, while no significant difference was observed between the

inoculated and non-inoculated plants under a low P level. This may result from the uptake of P by *C. oleifera* for growth under a low P level, as supported by the improved leaf P content and the growth performance of PSB under a low P level. Under a high P level, the available P released by PSB was sufficient for the plants' growth, which led to the PSB with no effect on the leaf P concentration. PSB not only solubilize and mineralize P from insoluble compounds but also release other nutrients [1,35]. In this study, the available N and K content of rhizosphere soil and the leaf N content were promoted by the PSB inoculation, demonstrating that PSB elevated the amounts of available N, P, and K in the soil and subsequently provided better nutrition for plant growth [36,37].

This study also found that the inoculation effect of mixed PSB strains was better than that of single strains. According to previous studies, there may be a synergistic effect between different microorganisms [38,39]. On one hand, the combination of different PSB strains may have a higher and more stable cell activity than a single strain [40]. On the other hand, the combination could better exploit the limited P sources in soil [30]. However, the mechanisms of a synergistic interaction remain to be explored.

5. Conclusions

In this study, we probed the effects of single and mixed inoculations of two native PSB strains, JX285 and HN38, on the growth of *C. oleifera* seedlings. The results showed the positive influence of JX285 and HN38 on plant growth, photosynthetic capacity, the N and P content of the leaves, and the available N, P, and K content of rhizosphere soil. The two PSB strains in the co-inoculation acted synergistically with each other and strengthened the beneficial effects on plant growth performance. The use of PSB as inoculants may provide an alternative to chemical fertilizers and promote sustainable agroforestry.

Author Contributions: L.Z. conceived the idea and established the direction; F.W. wrote the first draft of the manuscript; J.L. (Jianrong Li), Y.C., S.W., Y.Z., L.L. and X.S. carried out the experiment. S.W., J.L. (Junsheng Liang), and Y.C. analyzed the data. All authors contributed with suggestions and corrections, and approved the final manuscript.

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