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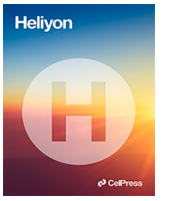

Research article

Plant growth-promoting rhizobacteria (PGPR) improve the growth and quality of several crops

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are known to have the effect of promoting plant growth. In this paper, three PGPR strains were selected from the previous work, which had plant growth-promoting activities such as phosphate solubilization, nitrogen fixation, phosphorus mobilization, etc. These strains named FJS-3(*Burkholderia pyromania*), FJS-7(*Pseudomonas rhodesiae*), and FJS-16(*Pseudomonas baetica*), respectively, were prepared into solid biological agents. Three widely planted commercial crops (tea plant, tobacco, and chili pepper) were selected for PGPR growth promotion verification. The results showed that the new shoots of tea seedlings under PGPR treatment were much more than the control. We also used tobacco, another important crop in Guizhou, to test the growth-promoting effect of individual bacteria, and the results showed that each of them could promote the growth of tobacco plants, and FJS-3(*Burkholderia pyrrocinia*) had the best effect. In addition, we carried out experiments on tobacco and pepper using multi-strain PGPR, the tobacco plants' height, fresh, and root weight increased by 30.15 %, 37.36 %, and 54.5 %, respectively, and the pepper plants' increased by 30.10 %, 56.38 % and 43.18 %, respectively, which both showed significantly better effects than that of a single strain. To further test the field performance, field trials were carried out in a mature Longjing43 tea plantation in Guizhou. There were four treatments: no fertilization (T_1), combined application of PGPR biological agent and compound fertilizer (T_2), only application of PGPR (T_3), and only application of compound fertilizer (T_4). In terms of yield, grouped with or without PGPR, there was a 15.38 % (T_2:T_4) and 92.31 % (T_3:T_1) increase between them, respectively. The tea's yield and tea flavor substances such as tea polyphenols, caffeine, and theanine were detected, and the T_2 showed the most significant positive effect on both sides. Especially, an important indicator of Matcha green tea is the color, chlorophyll content was then tested, and PGPR application increased it and improved the appearance. All these results demonstrated that the PGPR we screened could significantly promote plant growth and quality improvement, and had good application potential in crop planting, which could contribute to environmental protection and economic growth.

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1. Introduction

To resolve the series of agricultural production problems caused by the long-term application of chemical fertilizers, the low-carbon, purely natural, non-polluting, non-toxic, and harmless Plant Growth-Promoting Rhizobacteria (PGPR) biostimulant (or microbial fertilizer, microbial agents) have emerged in recent years, and recognized as a component of "fresh" green revolution [1].

Plant Growth-Promoting Rhizobacteria (PGPR) are soil bacteria that inhabit the rhizosphere of plants. These bacteria possess unique abilities such as phosphorus solubilization, nitrogen fixation, and potassium solubilization. They can convert insoluble forms of phosphorus and potassium in the soil into easily absorbable forms by plants through acidolysis, chelation, and exchange reactions. Furthermore, nitrogen-fixing microorganisms can hydrogenate N_2 in the air into NH_3, which enhances the availability of nutrients in the soil [2]. The mechanism of PGPR to promote plant growth is categorized into direct growth promotion, indirect growth promotion, soil nutrient cycling, regulation of plant growth, and biological control.

PGPR secretes a wide range of anti-fungal low molecular weight secondary metabolites, antibiotics, HCN, hydrolases, and cellulases to help plants resist stresses [2]. However, PGPR's most lauded function is to promote plant growth. Some studies showed that the application of PGPR can improve the growth environment of plants, such as regulating soil flora, delaying soil acidification, increasing soil nutrient conversion rate, etc., to promote the absorption of nutrients by plants to achieve the effect of promoting growth. Similarly, some PGPR strains also secrete plant hormones such as "Indole acetic acid (IAA)" to stimulate plant growth [3,4,5] It has been reported that the application of PGPR to crops improved their yield and quality [6–10]. For example, Fu [4] applied a combination of compound fertilizer and PGPR biostimulant in soybean planting, and the yield was 13.02 % higher than that of conventional fertilization. Aydinoglu [11] bioirrigated corn with *B. pumilus*, *B. licheniformis*, and *B. coagulans*, either alone or in combination, resulting in a 36 % increase in root fresh weight and a 39 % increase in aboveground fresh weight. Likewise, the application of PGPR biostimulant in other plants such as strawberries and cucumbers also have a good yield increase effect. Ipek [12] used bacterial suspensions of *Alcaligenes* sp. 637Ca, *Staphylococcus* sp. MFDCa-1 and MFDCa-2, A*grobacterium* sp. A18, *Panthenium* sp. FF1, and *Bacillus* sp. M3 to immerse strawberries before transplantation, respectively, to study the growth promotion effect of PGPR on strawberries under high calcium soil conditions. The results showed that all strains could significantly promote the growth and root development of strawberry plants, increase fruit yield, number of fruits, and fruit weight, and increase the content of N, P, K, and other nutrients in plant tissues. In the study of Mi et al. [13], the use of bacterial microbial fertilizer improved the quality of cucumber and increased the abundance of volatile substances in cucumber.

The tea plant, tobacco, and pepper are important commercial crops in Guizhou Province, China. Their yield and quality are closely related to the interests of a large number of farmers. The application of chemical fertilizers and pesticides are the most commonly used measures to increase production. However, it is not only a big input cost of planting but also leads to a series of problems such as environmental pollution and soil compaction, these negative effects have gradually seriously affected production and are not conducive to sustainable development, which has become an urgent problem to be solved.

In our previous research, we isolated three strains of PGPR, FJS-3 (*Burkholderia pyromania*), FJS-7 (*Pseudomonas rhodesiae*) and FJS-16 (*Pseudomonas baetica*). In this study, the effects of these three PGPR strains on the agronomic traits of tea (*Camellia sinensis*), tobacco (*Nicotiana tabacum*), and pepper (*Capsicum annuum*) were investigated in the greenhouse experiments. Several studies have developed PGPR for its application at field levels; however, it has not succeeded [14]. To better promote field application, we conducted field trials in Guizhou tea plantations, This commercial crop tea plant in there mainly used to produce matcha, which is a powdered type of green tea (*Camellia sinensis*), so it pays great attention to the color of the tea, as well as the low bitterness and astringency, and high freshness (umami flavor), so the contents of high chlorophyll, relatively low bitter and astringent substances caffeine and polyphenols, and high theanine are the main quality goals. Because it is not obtained through complex tea processing technology like oolong or black tea, it just steaming-drying-grinding, and its quality mainly depends on the quality of fresh tea leaves, so the cultivation and management of tea plants are particularly important, and fertilization is one of the most important steps to determine yield and quality. Delightfully, we have achieved good results in both indoor and field trials.

2. Materials and methods

2.1. Production of solid biological agent

The strains used in this experiment were isolated from the rhizosphere soil of the ancient tea plant in Fanjingshan Nature Reserve (Tongren City, Guizhou Province, China). The strains used in the experiment were FJS-3 *Burkholderia pyromania* (GDMCC No.62432), FJS-7 *Pseudomonas rhodesiae*, and FJS-16 *Pseudomonas baetica* (GDMCC No.62433).

Preparation of single-strain biostimulant: Take 100 μL of single-strain glycerol bacteria and add 250 mL LB liquid medium (LB medium composition: yeast extract 5 g, tryptone 10 g, sodium chloride 10 g per liter), cultured at 30 °C and 220 rpm for 24 h, centrifuge 4000r/min for 10 min, The bacterial cells were suspended in sterilized deionized water and the OD_{600} value was adjusted to 1.2. The bacterial resuspension is evenly mixed with equal volume sterilized peat and placed in a sterile plastic bag. After being sealed and fixed for 1 day, it was cultured in a constant temperature incubator at 30 °C for 7 days. Remove the bag and store it at room temperature for later use.

Multi-strain biostimulant preparation: According to the single-strain bacterial culture method, the three cultured bacterial cultures were thoroughly mixed according to 1:1:1, centrifuged, suspended, OD adjusted to 1.2 with sterile deionized water, mixed with sterilized peat, and placed in sterile plastic bags. After being sealed and fixed for 1 day, it was cultured in a constant temperature

incubator at 30 °C for 7 days. Remove the bag and store it at room temperature for later use.

2.2. Pot growth promotion test of PGPR agent

In the pot experiment, 2-year-old Fuding Dabai tea plants (*Camellia sinensis)*, K326 tobacco (*Nicotiana tabacum)* seeds, and Chaotian pepper (*Capsicum annum)* seeds were used as plant materials.

2.2.1. Camellia sinensis

Uniformly trimmed Fuding Dabai tea seedlings were transplanted into 500 g sterilized soil experimental pots, with three plants in each pot. Two treatment groups were established, one as the blank control, and the other with the application of a PGPR agent. Each treatment had three pots, and there were three replicates for each treatment. The PGPR consortia treatment group was applied with a solid agent of 1 g/pot every 15 days, as mentioned in 2.1. The control group was treated with 1 g/pot of sterilized peat soil. The first treatment was the starting time. After 30 days, the growth status of Fuding Dabai tea seedlings was observed, and the new shoot rate was measured. Tea seedlings were planted outdoors without additional irrigation, except for the first irrigation. During the planting period, there was plenty of rain, and the average temperature was 9–16 °C.

2.2.2. Nicotiana tabacum

Tobacco seeds were sterilized and placed in floating seedling trays for incubation. After germination, the test materials with similar growth status were selected for transplanting. The application method and dosage of PGPR fertilizer or sterilized peat soil for tea plants were the same. After 30 days, the growth status of tobacco was observed and their fresh weight and plant height were measured, and statistics from 15 plants of each species. Tobacco planting was divided into two parts. One part was used to test the growth promotion effect of single-strain, and four treatment groups were set up: Control, FJS-3, FJS-7, and FJS-16. The other part is to verify the growth promotion effect of multi-strain, and two treatment groups are set up: control and bacterial fertilizer. All experiments were performed in a greenhouse. The greenhouse conditions are 16 h/8 h day and night, and the temperature is 22 °C.

2.2.3. Capsicum annum

The peppers are planted in the greenhouse, the greenhouse conditions are 16 h/8 h, 22 °C. After disinfection, the pepper was planted and incubated in a floating seedling tray and then transplanted when the pepper developed to the fourth true leaf. The peppers are planted in the greenhouse, the greenhouse conditions are 16 h/8 h, 22 °C. After disinfection, the pepper was planted and incubated in a floating seedling tray and then transplanted when the pepper developed to the fourth true leaf. Peppers with the same growth condition were selected for the experiment Peppers with the same growth condition were selected for the experiment, and two treatment groups, control and multi-strain, were set up. Similarly, every 15 days, administer 1 g of multi-PGPR fertilizer or sterilized peat to each pot of pepper. After 30 days, the growth status of the pepper was observed and their fresh weight and plant height were measured, and statistics from 15 plants each species. Two treatment groups with similar growth conditions were selected for the test, and blank control and PGPR multi-strain groups were set up. Start with the transplanting time and make the first treatment.

Similarly, every 15 days, administer 1 g of multi-PGPR fertilizer or sterilized peat to each pot of pepper. After 30 days, the growth status of the pepper was observed and their fresh weight and plant height were measured, and statistics from 15 plants of each group.

2.3. Field experiment

The experimental area is located in the tea planting area of Guicha Company in Jiangkou County, Tongren City, Guizhou Province, China (E108° 47′, 27° 41′ north latitude). The average annual temperature is 16.2 °C, the average annual sunshine time is 1257.3 h, the frost-free period is 288 days, the average annual precipitation is 1369.6 mm, and the average annual relative humidity is 81 %. The tested tea variety is Longjing 43, and the field planting design is double-row strip planting, with a large row spacing of 150 cm, a small row spacing of 40 cm, and a cluster spacing of 20 cm.

The randomized block design was used in the experiment, with a total experiment area of 1334 m^2. The experiment site was divided into three replicate areas, and each area was set up with four treatment groups viz T_1 was the control without fertilization. T_2 application of compound fertilizer and PGPR biological agent mixed; T_3 was applied with PGPR, and T_4 was applied with compound fertilizer. The compound fertilizer used in the trial was provided by the WENGFU GROUP.

Among them, the application standard of compound fertilizer is compound fertilizer (total nutrient ($N + P_2O5 + K_2O$) $\geq$ 38 %, organic matter $\geq$60 %, nitrate nitrogen content $\geq$10 %) 160 kg/acres, and the application standard of biological bacteria agent is 2 g/m^2 (the total number of living bacteria is about 1.30×10^8 cfu/g). The fertilizers were applied to the T_2 and T_4 areas in November 2021, and an additional 40 kg/acre of nitrogenous fertilizer was applied to each group in March 2022. In addition to the fertilization method, the tea garden management is the same as the conventional management method, and no additional irrigation is carried out for natural rainwater.

2.4. Measurement of yield and related indexes

In April 2022, one bud and three leaves of tea in each plot were picked and weighed to calculate the yield. A 1 m^2 wooden frame was used to calculate the new shoot rate of tea, and each treatment was repeated 5 times. 100 buds were randomly selected from tea plants in the same area and weighed with an electronic balance with a precision of 0.001 g. 5 replicates were performed for each

treatment.

2.5. Determination of major chemical components in tea leaves

To prepare the tea juice, 0.15 g of dried tea leaf powder was mixed with 20 mL of freshly boiled distilled water in a boiling water bath. The mixture was shaken manually and boiled for 45 min. Afterward, the tea juice was filtered through double-layer filter paper and adjusted to a volume of 50 mL with distilled water. The solution was then cooled to room temperature for further biochemical analysis. Determination of total polyphenolic content (TPC) was conducted using spectrophotometry following the national standard GB/T 8313–2018. The determination of free amino acids (AA) was performed according to the national standards GB/T 8314–2013, respectively.

The freeze-dried tea powder (0.1 g) was combined with a 1.5 % magnesium oxide solution (30 mL) for caffeine analysis [15], and then immersed in ultrapure water (w/v) at 100 °C for 30 min. Subsequently, the resulting extract was subjected to double-filtration using a 0.22 μm Millipore membrane, followed by collection of 1 mL of the solution. The filtrate was then injected into a Select HSS C18 SB column (4.6 × 250 mm, 5 μm) with an injection volume of 10 μL, at a flow rate of 0.9 mL/min and a column temperature maintained at 35 ± 1 °C. The mobile phase consisted of 100 % methanol (A) and 100 % ultrapure water (B), with a 30 % A/70 % B isocratic gradient. Caffeine was detected at 280 nm.

For the determination of chlorophyll content, take 0.2 g of fresh tea leaves and divide them into three parts. Place each part in a mortar and add a small amount of quartz sand, calcium carbonate powder, and 2–3 mL of 95 % ethanol. Grind the mixture until it forms a homogenized pulp. Then, add 10 mL of ethanol and continue grinding until the tissue turns white. Allow it to stand for 3–5 min. Take one piece of filter paper and place it in a funnel. Moisten the filter paper with ethanol and pour the extract into the funnel using a glass rod to filter it into a 25 mL brown volumetric bottle. Rinse the mortar, pestle, and residue several times with a small amount of ethanol, ensuring that all remnants are transferred into the funnel as well. Use an eyedropper to absorb any remaining ethanol from the residue on the filter paper while washing away chloroplast pigments into the volumetric bottle until there is no green color left on both the filter paper and residue. Finally, fill up to 25 mL with ethanol to obtain accurate measurements. Shake well before pouring out this chloroplast pigment extract into a colorimetric cup with a light diameter measuring approximately 1 cm. The absorbance should be determined at wavelengths of both 665 nm and 649 nm using blank samples containing only pure ethanols as references.

2.6. Sensory evaluation

The sensory evaluation was performed to evaluate the green tea quality processed by the fresh tea leaves after tea after application of PGPR. The taste features, including bitterness, sweetness, astringency, umami, and thickness of the green tea were estimated by a group of professionally trained panelists (3 females and 3 males, aged 22–55 years) from Guizhou University. These five taste characteristics were rated on a 10-point scale (0, zero intensity; 5, moderate intensity; 10 strong intensity). The detailed procedures were described previously with a slight modification [16]. Briefly, a 3-green tea sample was infused in 150 mL of boiled distilled water for 4

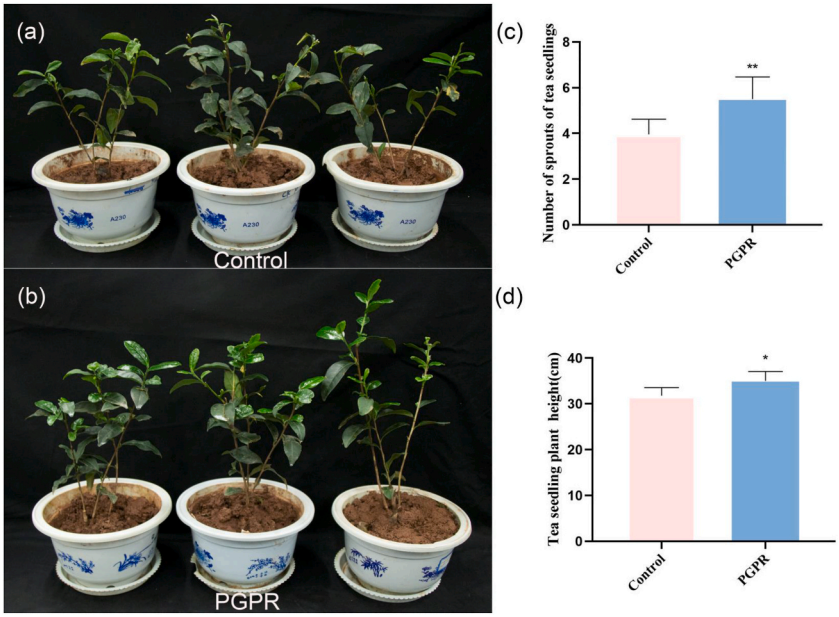

Fig. 1. The growth-promoting effect of PGPR on tea plants. (a) 1 g of peat soil per basin was applied as control (b)In the PGPR treatment group, 1 g microbial fertilizer was applied to each pot. (*c*–d) Plant height and germination number of tea seedlings in control group and PGPR treatment group. Different symbols indicate significant differences among groups in student's t-tests.

min in a specialized tea cup and then poured immediately into a tea bowl for sensory evaluation by the panel. All the green tea samples were evaluated blindly and repeated in triplicates on different days. The average scores of the five taste characteristics of the green tea samples were visualized using a radar chart.

The color difference of tea soup was determined according to Dong [17] take 3 g tea sample, add boiling distilled water 150 mL, and brew for 5 min, filter with filter paper while hot, cool the filtrate to room temperature, determine with a spectrophotometer, and repeat 3 times. L, a, b color difference system was used to determine, in which L value represented the degree; a represents the degree of red-green, "+" represents the degree of red, "-" represents the degree of green; b represents the degree of yellow blue, "+" represents the degree of yellow, "-" represents the degree of blue.

2.7. Statistical analysis

Means and SE values were calculated using Microsoft Excel 2013. Duncan's multiple comparison was used to calculate the significance among samples. The two-tailed student's *t*-test was used to calculate the significance between samples. All statistical analysis was performed using SPSS software Version 22.0(Colin and Paul, 2012), and GraphPad Prism version 8.0 (GraphPad Software Inc., San Diego, CA, USA; https://www.graphpad.com).

3. Result

3.1. The growth-promoting effect of PGPR on plants

Since the strains were isolated from the rhizosphere soil of ancient tea plants in Guizhou, we first verified the growth-promoting effect of tea plants. The growth status of Fudingdabai tea seedlings after 30 days of PGPR treatment is shown in Fig. 1a, b. The growth

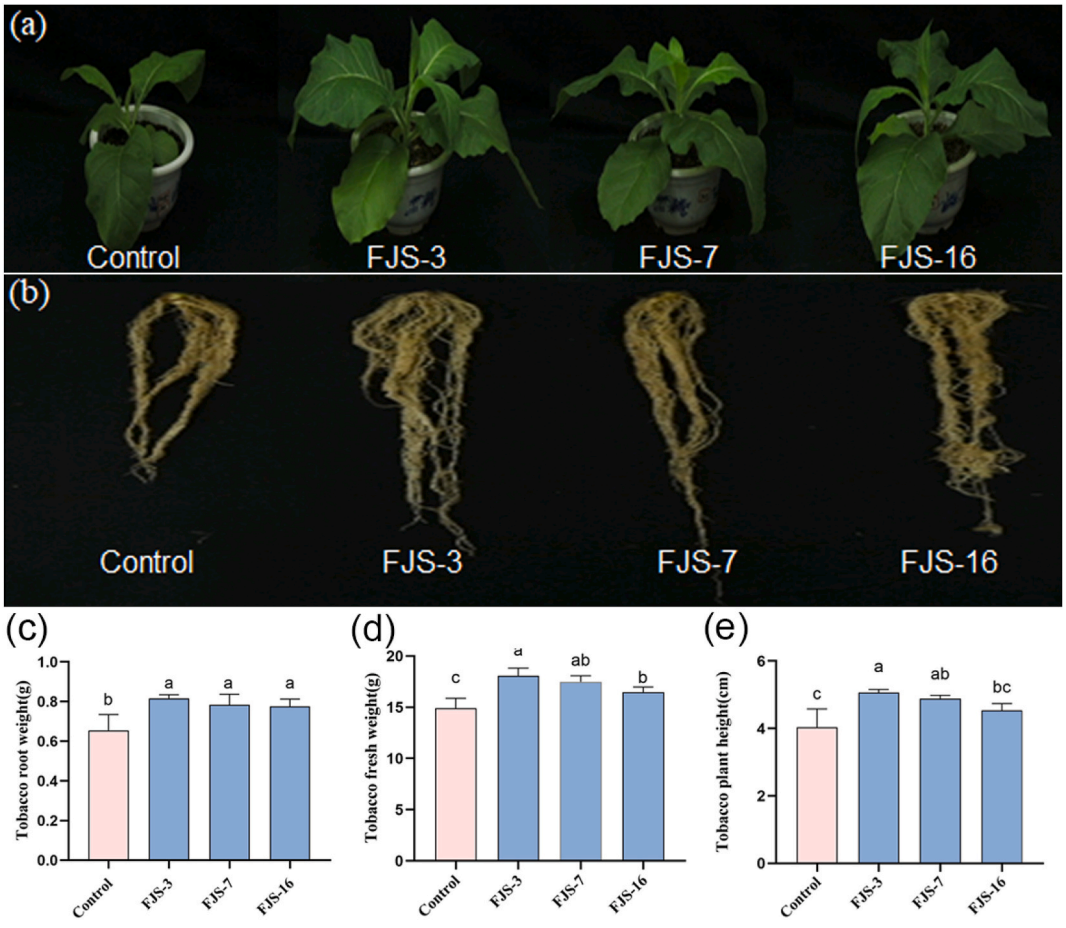

Fig. 2. Growth promoting effects of single PGPR strains on tobacco. (a–b) Tobacco growth status and the development status of tobacco roots. Tobacco with or without PGPR application were grown in basin for laboratory treatment. (*c*–e) Agronomic traits of tobacco in experimental group and control group were determined, including plant height, root weight and fresh weight. (*c*–e) Different letters indicate significant differences among different treatments in Duncan's multiple comparisons test.

of tea seedlings after the PGPR microbial agent applied was significantly better, and the new shoots rate and plant height were 41.03 % and 12.17 % higher than that in control, respectively (Fig. 1c and d).

At the same time, to test its broad spectrum and strain characteristics, we tested the effect of different strains of tobacco on plant growth promotion. The result shows that the plant height, root weight, and fresh weight of tobacco treated by PGPR were increased (Fig. 2). Different strains had different growth promotion efficiency on tobacco. The strain with the best growth promotion effect was FJS-3 (*Burkholderia pyrocinia*). Compared with the control, the plant height, root weight, and fresh weight of tobacco treated with FJS-3 increased by 25.56 %, 24.77 %, and 21.21 % respectively (Fig. 2 a-e). FJS-7 (*Pseudomonas rhodesiae*) and FJS-16 (*Pseudomonas baetica*) also promoted the growth of tobacco. Compared with the control, the plant height increased by 21.06 %, and 16.4 %, root weight increased by 19.87 % and 19.30 %, and fresh weight also increased by 17.25 % and 16.47 %, respectively. These indicated that these PGPR screened by us could be applied to the tobacco. Then, to test the growth promotion effect of multi-strain PGPR, we conducted repeated experiments on tobacco plants, and the three strains were mixed to form a multi-strain fertilizer to do the treatment. The growth status of tobacco after 30 days of treatment is shown in Fig. S1. The plant height, fresh weight, and root weight of tobacco in the PGPR treatment group increased by 30.15 %, 37.36 %, and 54.5 %, respectively, which is a much more significant growth promotion effect than that in single strain PGPR.

Similarly, we conducted further experiments on another important cash crop in Guizhou, chili pepper (Fig. 3). The results showed that compared with the control, plant height, root weight, and fresh weight of PGPR-applied plants were significantly increased by 30.10 %, 56.38 %, and 43.18 %, respectively (Fig. 3 a-e). It can be concluded that the multi-strain has a stronger effect on promoting plant growth.

3.2. Field experiment

3.2.1. can effectively increase tea yield

To test the effectiveness of the application of our PGPR bio-fertilizer at field levels, we experimented with Tongren tea plantation. Four treatment groups were set up, the details can be known in the methods section. The yield and quality components of each treatment were measured. The result showed that the tea bud weight and germination density in the treatment group (T_4) treated with compound fertilizer increased by 18.46 % and 20.18 %, respectively (Fig. 4 a,b), compared with the control group (T_1). However, the treatment group (T_2) that applied compound fertilizer and PGPR bacteria together had a more significant growth trend, and the two indexes increased by 31.01 % and 38.63 % respectively, treated with only PGPR (T_3) increased by 21.61 % and 17.01, respectively (Fig. 4). Compared to T_1, although the germination density is lower than that of T_4, the weight of 100 buds is higher, which also indicates that the quality of fresh leaves is better. The quality of tea will be better, which has also been verified in the follow-up

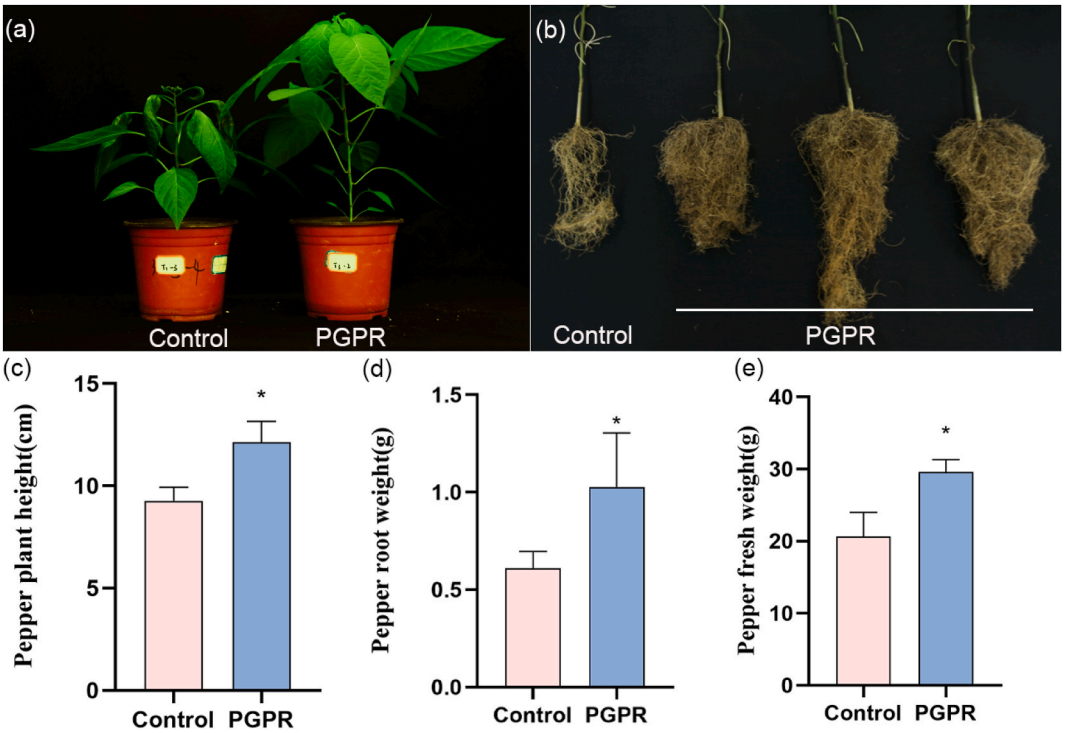

Fig. 3. The growth-promoting effect of PGPR on Pepper. (a, b) Growth performance (a) and roots development status(b) under Control or PGPR (c–e) Plant height, root weight and fresh weight of pepper in control group and PGPR treatment group. (c–e) Different letters indicate significant differences among different treatments student's *t*-test.

experiments (Figs. 5 and 6).

The yield of fresh tea leaves, $T_2 > T_4 > T_3 > T_1$ (Fig. 4 c). Compared with the T_1, the yield of fresh leaves in T_4 (compound fertilizer) increased by 95.05 %, while the yield increase in T_2 (compound fertilizer + PGPR) was as high as 125.06 %, and the yield increase in T_3 (PGPR)was 87.55 %, this is very close to T_4, and based on the aforementioned 100-bud weight, it can be seen that the application of PGPR has a very good potential to reduce the use of chemical fertilizers and improve the quality of tea.

3.2.2. improves tea quality

For the quality of tea, theanine is an important taste factor in tea quality, mainly reflected in umami flavor (freshness). The content of theanine in the four treatments: $T_2 > T_3 > T_4 > T_1$ (Fig. 5b), which is consistent with the sensory evaluation results (Fig. 6). Compared with the treatment group without fertilization (T_1), theanine in T_2 increased by 56.94 %, T_3 increased by 35.90 %, and T_4 increased by 16.67 %. Compared with no fertilization (T_1), the tea polyphenols of different fertilizer combinations were significantly decreased by 12.42 %, 8.62 %, and 4.75 %, respectively (Fig. 5a). Phenol-ammonia ratio refers to the ratio of tea polyphenols to theanine, which can effectively reflect the taste quality of green tea, the lower the ratio is, the fresher the taste of tea soup is. As shown in Fig. 5c, the phenol-ammonia ratio was $T_2 < T_3 < T_4 < T_1$, demonstrating that the application of PGPR could improve the umami flavor of green tea. For caffeine content, compared with T_1, T_2, T_3 and T_4 decreased by 9.25 %, 8.74 % and 7.71 %, respectively.

Chlorophyll is one of the important indexes to evaluate Matcha or Tencha (the raw ingredient for matcha), it is closely related to the evaluation criteria of appearance, and soup color in tea evaluation. In the determination of chlorophyll content in each treatment group (Fig. 5e), the chlorophyll content of T_2 was significantly higher than that of other treatments. T_2 treatment obtained the evaluation of 'greener' and 'tender green' in the appearance, soup color, and leaf bottom evaluation of sensory evaluation, and the scores were the highest, which was consistent with the trend of chlorophyll content. This result (Table S1) is also consistent with the results we obtained in the CIELAB system. The tea △a value of the T_2 treatment is the smallest among the four treatments, indicating that the T_2 color is the greenest. Tongren City, Guizhou Province is known as the ' capital of Chinese Matcha '. Color and taste are important indicators for judging the quality of Matcha. The tea variety Longjing 43 selected in this paper is suitable for matcha. In this study, the chlorophyll and amino acids of tea leaves were improved by the mixed application of PGPR and compound fertilizer. To a certain extent, it can be explained that PGPR biological agent has a certain positive effect on the quality formation of matcha.

In summary, the application of PGPR in tea plantations not only improves the yield of fresh tea leaves but also improves the quality of tea.

3.3. Tea sensory evaluations

The sensory evaluation results of tea with different treatments are shown in Fig. 6. Among the four treatments, T_2 had the highest scores in appearance, soup color, and aroma, and the taste of it was sweet and mellow (Fig. 6a and b). The scores were consistent with the results of theanine content and phenol ammonia ratio in the physical and chemical composition test results (Fig. 5), the comprehensive score ranking was $T_2 > T_3 > T_4 > T_1$, the quality of tea is closely related to soil and fertilizer management, PGPR bio-fertilization can promote the improvement of tea flavor quality.

4. Discussion

We isolated several PGPR strains from the root soil of an ancient tea plant in Guizhou, and three strains were identified and screened for subsequent growth-promoting test verification in our previous work. The three strains belong to *Pseudomonas* and *Burkholderia*, respectively. *Pseudomonas* and *Burkholderia* are two common genera in PGPR, *Burkholderia pyromania* is often reported to have nitrogen fixation, ACC deaminase activity, etc. [18]; *Pseudomonas rhodesiae* and P*seudomonas baetica* also have phosphate strains with these properties can increase crop biomass and yield by accelerating nutrient uptake [18,19].

We first conducted experiments on tea plants, and the results showed that the selected strains could significantly promote the growth of tea plants, and the growth state and new shoots rate were significantly improved (Fig. 1). Liu et al. inoculated tea plants with

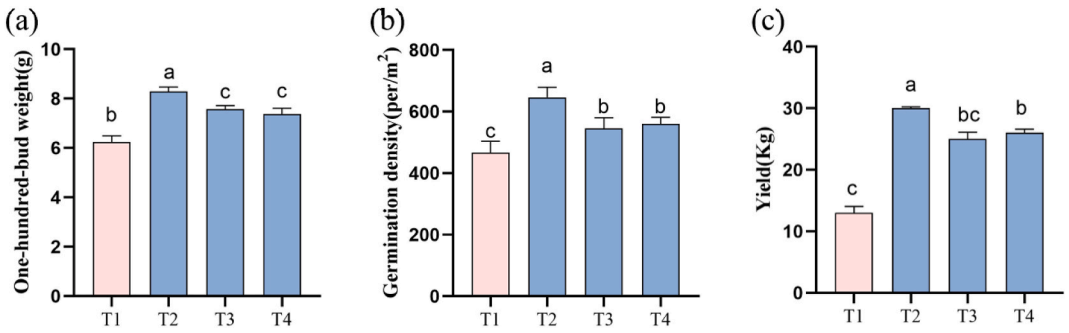

Fig. 4. Tea yield under different treatments. (a–c) The yield indexes were hundred-gain, germination density and fresh tea leaf yield. Different letters in front of the same compound and in bar graphs denote significant differences (one-way ANOVA test; $P < 0.05$).

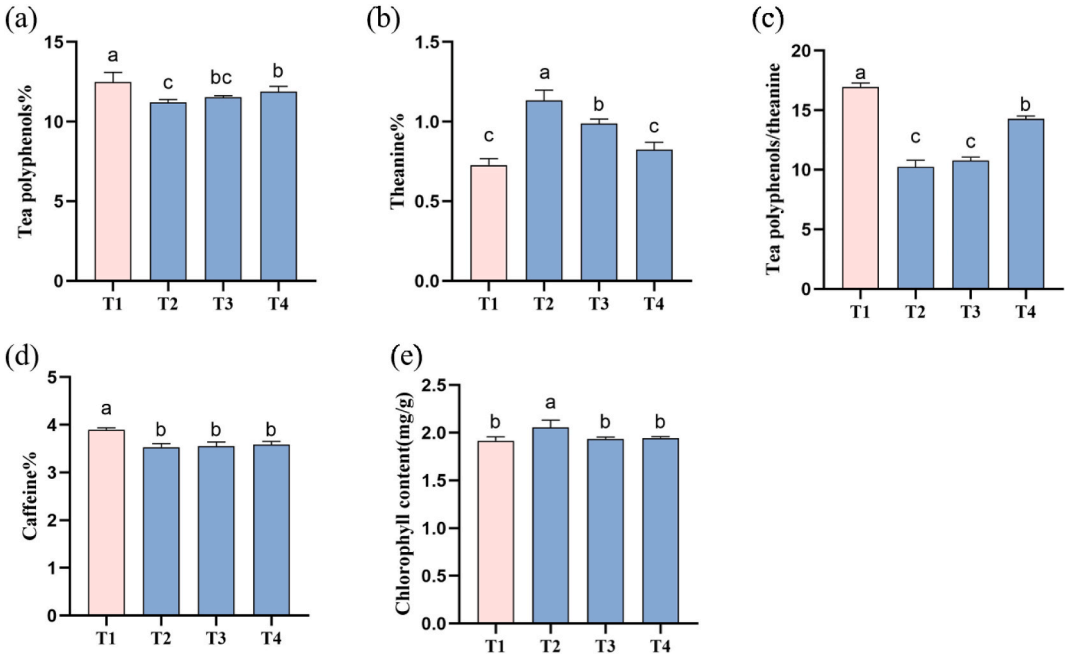

Fig. 5. Main chemical components of tea under different treatments. (a–e) The contents of tea polyphenols, amino acids, ratio of tea polyphenols to total amino acids, caffeine and chlorophyll after different fertilizer combinations were applied to tea plants. Different letters in front of the same compound and in bar graphs denote significant differences (one-way ANOVA test; $P < 0.05$).

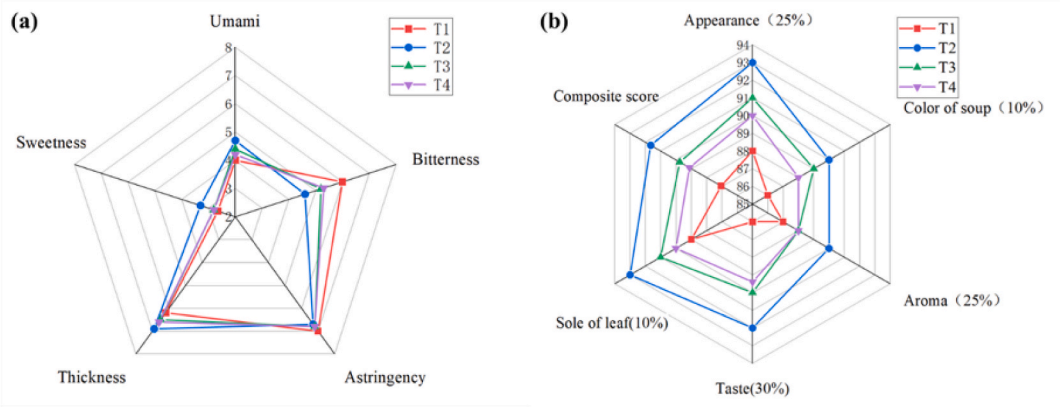

Fig. 6. The results of tea sensory evaluation. (a)Tea 5 kinds of sensory evaluation radar map. (b) According to GB/T 18,797–2012, green tea appearance, taste, soup color, sole of leaf, and aroma in proportion to the assigned radar map. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Streptococcus marcescens JW-CZ2 in a greenhouse, and the stem diameter and dry weight of tea plants were significantly increased by 11.37 % and 29.34 % compared with the control group on 180 days [20]. Similarly, Dhar et al. isolated *Streptococcus marcescens* ETR17 from tea plantations in West Bengal and Assam, which also showed a promoting effect on tea plants. On the 45th day after inoculation, the shoot length and root length increased by about 20 % and 32 %, respectively, compared with the control [21]. In this paper, the cultivation of tea trees was carried out outdoors, and the temperature and treatment time were low during the planting period, and the treatment time was still considerable, indicating that the combination of microbial fertilizers has good potential. We also used tobacco, another important cash crop in Guizhou, to test the growth-promoting effect of individual bacteria, and the results showed that each of them could promote the growth of tobacco plants, and FJS-3(*Burkholderia pyrrocinia*) had the best effect (Fig. 2). At the same time, we also carried out experiments on tobacco (Fig. S1) and peppers (Fig. 3), using multi-strain. The results showed that it not only has a significant growth-promoting effect. Compared with previous tobacco experiments, it can be seen that the effect is significantly better than that of single bacteria, which is in common with previous studies in wheat [22]. The results obtained by Karlidag et al. in treating apples with PGPR are also applicable to this study, the results showed that the growth promotion effect of composite strain on apples

was significantly higher than that of single strain [23]. As in previous studies, He et al. applied *Streptomyces* sp. TOR3209 significantly stimulated tobacco growth and root development [24]; Olasupon et al. treated peppers with different *bacilli* and increased biomass by 27–36 % [25]. In this paper, the PGPR biostimulant we used to show a good stimulant effect on both tobacco and pepper. The experiments of multi-crop showed that the stimulant effect of PGPR had a broad spectrum and could cope with various environments. In addition, it should be pointed out that since the yield of peppers is mainly focused on fruits, chili pepper fruits, but because the experimenters are not good at growing it, the chili peppers in the greenhouse have been vegetatively grown and have not produced ripe fruits, so the yield has not been counted. According to the analysis of the experimental results in the field of tea plantations in the later stage (Fig. 4), it may be implied that the strains we selected from the roots of tea plants may be more suitable for leaf crops and can significantly promote the absorption of N, P, and K, which are more important for reproductive growth, flowering, and fruiting, may need to be matched with strains with stronger phosphorus and potassium desolation effects. It has been reported that P and K have the effect of promoting plant branching, flowering, and fruiting [26], and PGPR with phosphorus mobilization and potassium mobilization characteristics can enhance the utilization efficiency of phosphorus and potassium in plants [1,14].

We will continue to verify this in future research and also provide a valuable reference for the production, promotion, and application of bacterial fertilizer.

After laboratory tests and good results, we conducted field trials to be truly applied to production. The results showed that the multi-strain fertilizer made from the selected strains could effectively improve the yield and quality of tea (Figs. 4–6). In terms of yield, T_3 (only PGPR) is very close to T_4 (only chemical compound fertilizer), and adding PGPR based on chemical fertilization (T_2) significantly increased the yield compared to T_4. Similar results appear in quality determinations. This may be related to the fact that PGPR can promote plant nutrient absorption, most of the applied fertilizers are not effectively used by plants or are lost or left in the soil to cause water eutrophication and soil compaction, acidification [21], and the application of PGPR can effectively improve it, so while promoting plant growth, reduce the chemical fertilizer application amount and the negative effects of excessing, to achieve the purpose of ecological development.

Studies have shown that the application of PGPR can improve soil biological activity and promote soil nutrient absorption. PGPR can recruit beneficial microorganisms after planting, improve crop disease resistance, and long-term application can effectively improve soil physical and chemical components. The utilization of phosphate solubilizing rhizobacteria (PSB) in agriculture has been found to enhance crop productivity and sustainability, particularly in arid regions, as highlighted by Maldonado's study [27]. The biological bacterial fertilizers are cheap enough to effectively reduce the cost of fertilization and improve the economic benefits obtained by growers, thus it has a good application prospect.

As we mentioned earlier, quality inspection of matcha, in addition to the conventional test of tea quality biochemical components, we also pay great attention to the content of chlorophyll, and surprisingly, it not only promotes the improvement of theanine, which is an umami flavor substance, reduces the content of caffeine and tea polyphenols, but also significantly increases the content of chlorophyll, which may be related to the fact that it can significantly promote the absorption of N [28], microbial agents can improve plant stem biomass and chlorophyll content [29]. In the production process of matcha, there is a step of shading, which can make the tea greener [30], but this means, the caffeine content will also rise, and the tea will become bitter. Theanine is one of the important C/N metabolites, and the application of exogenous nitrogen can effectively increase the content of theanine [31,32]. Tea polyphenols, caffeine, antioxidants, refreshing and cardiovascular protection, and other health effects [33–36] are also important tea flavor substances, mainly reflected as ' astringent ' and ' bitter [37–39]. Cao et al. inoculation of microbial fertilizers in acidic soils significantly increased the content of substances such as total amino acids, total proteins, and total sugars under greenhouse conditions [40]. Wu et al. also came to the consistent conclusion that tea quality was improved after the application of microbial fertilizers [41]. However, with our PGPR bio-fertilizer, apart from the chlorophyll content is increased, the caffeine content is also reduced (Fig. 5d), in addition, a low phenolic ammonia ratio is beneficial to the quality of green tea, so all the results are perfectly matched to the needs of the product (Figs. 5 and 6), so it has great promotion value.

There are many other reports of the field application of PGPR, but the effect is not as expected [42], Xiao et al. isolated three arsenic-resistant PGPR strains (S6, S7, and S10) to verify the role of PGPR in promoting rice growth. The results showed that all could promote rice growth. The increase was 10.50–51.30 % under greenhouse conditions and 4.83–9.16 % under paddy field conditions [43]. Our PGPR increased production by 15.38 % and achieved good results (our trial was conducted in 2021–2022, and to verify our results, we repeated the trial in 2022–2023 and the results were consistent), there may be due to species differences, while it could also be that their PGPR contains only a single strain, and the test proves that the effect of a single strain is far inferior to the composite strains, and in the complex environment of the field, the effect of this single strain may not be well reflected, therefore, the multi-strain fertilizer may have more field application effect and promotion value.

In summary, the biological agent prepared by our three PGPR strains can significantly promote the growth of plants and has a significant positive effect on the yield and quality of tea in the field, indicating that the biological agent has field application potential and promotion value.

5. data availability

Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.

Please select why. Please note that this statement will be available alongside your article upon publication. As follow-up to "Data

Availability.

All data has been included in article/supp. Material/referenced in article.

CRediT authorship contribution statement

Tongrui Zhang: Writing – original draft, Formal analysis. **Qinhao Jian:** Validation. **Xinzhuan Yao:** Validation. **Li Guan:** Validation. **Linlin Li:** Validation. **Fei Liu:** Funding acquisition. **Can Zhang:** Funding acquisition. **Dan Li:** Funding acquisition. **Hu Tang:** Conceptualization. **Litang Lu:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31553.

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