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Co-inoculation of plant growth-promoting rhizobacteria enhance phytoremediation efficiency of hybrid *Pennisetum* in Cu contaminated soils*

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Abstract: Plant growth-promoting rhizobacteria (PGPR) can promote the efficiency of phytoremediation, but most studies have concerned on single PGPR strain and knowledge of the effects of co-inoculating multiple strains is still lacking. This study explored whether, and if so how, consortia of PGPR with complementary traits can increase phytoremediation efficiency. Hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*) seeds were inoculated with 1−3 Cu tolerant PGPR strains with N_2-fixing or 1-aminocyclopropane-1-carboxylate deaminase-producing ability, and the treatments' effects on seed germination, seedling growth and Cu uptake were assessed in a range of conditions. Our results showed that the PGPR strains promoted both seed germination and seedling growth, and the effects were stronger under the high-Cu conditions. Hybrid *Pennisetum* is quite tolerant to Cu and its root-to-shoot translocation factor of Cu is lower than 0. 1. PGPR inoculation further decreased the translocation factor. Co-inoculation of multiple strains enhanced both the stimulation effects on plant growth and the inhibition effects on translocation factor. Our results suggest that co-inoculation of 2−3 PGPR strains with complementary plant growth promoting traits can enhance the phytoremediation efficiency of hybrid *Pennisetum*, and hybrid *Pennisetum* is an excellent candidate plant for phytostabilization of Cu mine tailings.

Key words: heavy metals; mine tailings; microbe-assisted phytoremediation; co-inoculation; hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*)

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联合接种植物生长益生菌提高杂交象草在铜污染土壤修复中的效率

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摘要：植物生长益生菌（PGPR，plant growth-promoting rhizobacteria）能提高植物修复的效率，虽然有研究显示联合接种可进一步提高植物修复效率，但目前人们仍大多关注于单菌株的促生作用，对于联合接种的作用和机理的研究仍有待进一步深入。我们将 1 ~ 3 种具有固氮能力或产 1-氨基环丙烷-1-羧酸脱氨酶能力的耐铜 PGPR 菌株接种于杂交象草（*Pennisetum americanum* × *P. purpureum*）的种子上，研究在不同铜含量及不同土壤条件下其对杂交象草种子萌发、幼苗生长及铜吸收的影响，探讨联合接种具有互补促生功能的菌株能否进一步提高以及如何提高植物修复的效率。结果显示，植物生长益生菌能有效促进种子萌发及幼苗生长，且在高铜的条件下促生效果更好。杂交象草对铜耐受性强，其植物体对铜元素由根向茎迁移的系数低于 0. 1，接种 PGPR 进一步降低迁移系数。联合接种进一步增强了 PGPR 对植物地下部分生长的促进作用和对迁移效率的抑制作用。我们的研究显示联合接种 2 ~ 3 种具有互补植物生长促进性状的 PGPR 菌株可提高杂交象草在植物修复中的效率，而杂交象草可作为铜尾矿植物修复的候选物种，具有较高的潜在应用价值。

关键词：重金属；尾矿；微生物辅助植物修复；联合接种；杂交象草（*Pennisetum americanum*×*P. purpureum*）

Mining activities have resulted in large amounts of metalliferous mine tailings around the world. The tailings' high contents of heavy metals（HM）and associated contaminants may spread in dust and/or leach into nearby watercourses, seriously polluting environments[1-2]. Phytoremediation（involving use of plants for metal reclamation）has received increased interest as it offers a cost effective and environmentally friendly technology for remediation of HM contaminated soils[3-4]. Phytostabilization is one category of phytoremediation, which refers to reduction of levels of available metal ions in soil through absorption and precipitation in the roots and root zones of suitable plant species. Rapidly growing plants with low root-to-shoot HM translocation factors（TFs）are most suitable for phytostabilization, as they minimize quantities of HMs that enter food chains via animals that eat aerial parts of plants[5].

Slow growth of plants on tailings soils due to the toxic effects of HM and extreme nutrient deficiencies poses a general challenge for phytoremediation. Thus, plant growth-promoting rhizobacteria（PGPR）are often introduced in the phytoremediation of HM tailings due to their ability to enhance plants' HM tolerance and growth[6-7]. PGPR promote plants' growth through numerous mechanisms, *inter alia*, synthesizing phytohormones, fixing nitrogen, increasing the availability of nutrients, and producing 1-aminocyclopropane-1-carboxylate（ACC, the immediate precursor of ethylene in plants）deaminase[8-10]. They can also produce antibiotic and antifungal metabolites, and/or induce systemic resistance in plants, which can protect them from pathogens[11], and stimulate growth of plants in HM-contaminated soils by decreasing the metals' toxicity to the plants[12]. PGPR mixtures with complementary traits generally enhance plant growth more effectively than single strains or single physiological classes of PGPR[8,13-14]. However, this is not always the case, and contradictory results are often found[15-16]. Hence, further assessments are needed to formulate optimal PGPR consortia for phytoremediation.

Copper（Cu）is involved in numerous physiological processes and essential for plant growth[17], however, it is strongly toxic to plants once the concentration surpasses a taxon-related threshold[18]. The high Cu and extremely low nitrogen contents are regarded as the factors that most strongly hinder plants' growth on Cu mine tailings[15]. Thus, in the study presented here, we inoculated several Cu-tolerant PGPR strains with the ability of N_2-fixation or ACC deaminase production, singly or in various combinations, and examined their effects on seed germination and seedling growth of a fast growing high-quality

forage species, hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*), in a range of conditions. We addressed the following specific questions. First, does co-inoculation of multiple strains of PGPR have stronger growth-promoting effects than inoculation with a single strain, and if so to what extent? Second, how do PGPR inoculations affect Cu accumulation and translocation in the plants? Third, is hybrid *Pennisetum* suitable for phytoremediation of HM tailings?

1 Materials and methods

We addressed our research questions with a germination experiment and a growth experiment. We had isolated 10 Cu-tolerant PGPR strains from the rhizosphere of plants growing on Yangshanchong Cu tailings (30º54′ N, 117º53′ E) discarded in 1990, in Tong Ling, Anhui Province, China[15]. Two N_2-fixing strains (*Stenotrophomonas* sp. and *Ochrobactrum* sp., designated FLN-B1 and FLN-B6 respectively) and two ACC-utilizing strains (*Microbacterium* sp. and *Klebsiella* sp., designated ACC-B1 and ACC-B2, respectively)[15] were used in the present study.

1.1 Germination experiment

The PGPR strains used in the germination experiment were FLN-B1, FLN-B6 and ACC-B2. They were inoculated singly or co-inoculated in all possible combinations, resulting in a total of eight treatments including a no inoculation (control) treatment (Control, FLN-B1, FLN-B6, ACC-B2, FLN-B1+FLN-B6, FLN-B1+ ACC-B2, FLN-B6+ACC-B2, FLN-B1+ FLN-B6+ACC-B2).

The seeds used in the germination experiment were hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*) seeds, supplied by Huafeng Grass Industry Technology Co., Ltd. (Zhengzhou, China). This species is widely used as animal fodder, with high yield, high quality and strong stress resistance. The seeds were surface-sterilized by soaking in 1.5% sodium hypochlorite solution for 10 min, then rinsed with sterile deionized water. Sets of seeds were then soaked in each of the PGPR suspensions prepared as described above for 2 h (or in sterilized water, for controls). Two sets of 24 plastic petri dishes (diameter 9 cm), were prepared by placing two layers of filter paper in them, then adding 10 mL of sterilized deionized water to each dish of one set (designated −Cu), and 10 mL of 0.4 mmol·L^{-1} $CuSO_4$ solution to each dish of the other set (designated +Cu). Sets of 20 seeds subjected to each of the eight treatments were placed in triplicate −Cu and +Cu petri dishes. Then the petri dishes were placed in an incubator in a climate chamber set at 25 ℃ and providing 12∶12 h light∶dark cycles with a photon flux density of 150 μmol·m^{-2}·s^{-1}. Ten mL of sterilized water or 0.4 mmol·L^{-1} $CuSO_4$ solution was added to each −Cu and +Cu petri dish, respectively, every 2 days to maintain the humidity. Two weeks later, the germination rate under each treatment was recorded. The shoot height and maximum root length of each seedling were measured and recorded (assigning values of 0 to seeds that did not germinate), and mean values in each pot were recorded.

1.2 Growth experiment

Tailings' high contents of heavy metals are easy to spread in dust and cause high content in soils nearby, including farmland, which might result in high heavy metals in food and potentially threaten human health. In order to test the efficiency of PGPR in both Cu contaminated soils and tailings, two kinds of soils (nutrient soils and tailings) were used in the growth experiment. The former was a general type of soil bought from Cuijun Co., Ltd. (Taiwan, China), consisting of a mixture of peat, sawdust, coconut shell powder, vermiculite, pearlite and organic manure, called General Nutrient Soil by the company. A batch of the soil was added with 1.36 g·L^{-1} $CuSO_4$ solution to a final concentration of 350 mg Cu·kg^{-1} soil (designated +Cu), and another batch of the soil was added with an equal volume of distilled water (designated −Cu). As Cu toxicity was the main stress factor in this part of the experiment, ACC-deaminase producing ability seemed to be a highly important plant growth promoting trait. The seeds sown in these pots were inoculated with two ACC-utilizing PGPR strains (ACC-B1 and ACC-B2) and one N_2-fixing PGPR strain (FLN-B1) both singly and in all combinations, resulting in a total of eight treat-

ments（Control，ACC-B1，ACC-B2，FLN-B1，ACC-B1+ACC-B2，ACC-B1+FLN-B1，ACC-B2+FLN-B1，ACC-B1+ACC-B2+FLN-B1）.

The tailings soils used in the growth experiment were collected from the 0－20 cm layer of Shuimuchong Cu mine tailings，in Tong Ling，Anhui Province，China（30°54′N，117°53′E）. The tailings have extremely low N，P，K and total organic carbon contents，but they are rich in Cu，S and Ca[19]. As the tailings sample was too infertile for the plants，it was mixed with the nutrient soil（5∶1）to enable their growth. To examine effects of indigenous microbes on the PGP efficiency of PGPR，a batch of this mixture was sterilized by autoclaving shortly before the experiment and another batch was not sterilized（designated sterilized and unsterilized tailings soils，respectively）. Both N deficiency and Cu toxicity are stress factors for plants grown on tailings soils. Thus，the seeds sown in these soils were inoculated with two N_2-fixing PGPR strains (FLN-B1 and FLN-B6) and one ACC-utilizing PGPR strain（ACC-B2）[15]，both singly and in all combinations，same as the germination experiment.

Seeds were surface-sterilized and inoculated with the selected single PGPR strains or mixtures（resulting in seven inoculation treatments and one control treatment in total），as in the germination experiment. Then 0. 7 kg portions of each of the four types of prepared soil（nutrient －Cu，nutrient +Cu，sterilized tailings and non-sterilized tailings）were placed in sets of 24 pots（diameter 13 cm，height 16 cm），corresponding to 3 replications for each of the 8 inoculation treatments. Sets of 15 seeds subjected to each inoculation treatment were sown in triplicate pots containing each of the growth substrates.

The pots were arranged in a randomized block in a wire house（Guangzhou，China），and kept at ambient light and temperature. The pots were watered with 200 mL water daily to maintain their moisture content. Two weeks after sowing，all but the five most average-looking individuals in each pot were removed to avoid overplanting and minimize variation caused by endogenous differences among the seeds. The experiment lasted for 60 d and then the plants were subjected to the analytical procedures described below. The temperatures during this period ranged from 20. 5 to 36. 2 ℃，respectively.

1. 3 Determination of growth and physiological indices

1. 3. 1 Chlorophyll *a* fluorescence The third fully developed leaf from the base of each individual plant was selected to determine chlorophyll *a* fluorescence *in vivo* using a Plant Efficiency Analyzer（PEA；Hansatech Ltd.，England）with an excitation light intensity of 3 000 $\mu mol \cdot m^{-2} \cdot s^{-1}$，after dark adaptation for at least 20 min. Photosynthesis Index（PI_{abs}）values were calculated according to Strasser B J and Strasser R J[20].

1. 3. 2 Proline and malondialdehyde (MDA) contents The 2^{nd}－4^{th} fully developed leaves from the base of each plant were used for determination. Proline was determined using the method described by Troll and Lindsley[21] and MDA was determined according to Wang[22].

1. 3. 3 Plant growth and Cu content The above-ground height and maximum root length of each plant were measured and mean values for plants in each pot were calculated. The fresh weights of the above- and below-ground part of plants in each pot were then determined（including leaves cut for physiological determinations）. The samples were then rinsed with tap water，washed three times with deionized water and oven-dried to constant weight at 65 ℃ to calculate their water content and dry weight. Finally，the Cu contents of the oven-dried above- and below-ground tissues of plants grown in tailings soils were determined by digestion with conc. HNO_3 and $HClO_4$ followed by analysis using an OPTIMA 2100 inductively coupled plasma optical emission spectrometer（ICP-OES system，Perkin-Elmer，Wellesley，MA，USA）.

1. 4 Data analysis

The efficiency of each plant's root-to-shoot translocation of the HM（Cu）was characterized by calculating a translocation factor（TF），using Equation 1[4]

$$TF = \frac{C_{shoot}}{C_{root}}, \quad (1)$$

where C_{shoot} and C_{root} are contents of the metal in shoots and roots，respectively.

Total biological accumulation（TBA）of Cu was calculated with Equation 2

$$TBA = C_{shoot} \times W_{shoot} + C_{root} \times W_{root}, \quad (2)$$

where W_{shoot} and W_{root} are the weights of shoots and roots respectively.

Two-way ANOVA was applied to determine effects of the PGPR and solution or substrate treatments（−Cu or +Cu，and unsterilized or sterilized），as well as their interactions on the analyzed parameters. For growth experiment，data from nutrient soil experiment and tailings soil experiment were analyzed separately. One-way ANOVA was applied to assess effects of the PGPR treatments on the analyzed parameters within each soil or solution treatment. The LSD post hoc test was used to identify significant difference among the treatments at the 5% probability level. ANOVA analysis was carried out using SPSS for Windows software version 20. 0.

To comprehensively analyze the responses of growth and physiological variables（shoot height，root length，aboveground biomass，belowground biomass，MDA and so on）to the explanatory variables（substrate treatments and inoculation treatments），redundancy analysis（RDA）was conducted using vegan package of R Studio 1. 4. 1717，with the data of nutrient soil pot experiment and tailings pot experiment being analyzed respectively. Prior to analysis，scale function was used to standard the data. The diagrams were drawn using ggplot2 package. RDA results show that the vector length of MDA，PI_{abs}，Cu contents of aboveground tissue，TF and TBA is very short，which indicate they have little impact on the model（Data not shown）. Thus，RDA not included these variables was conducted.

2 Results

2. 1 Germination

Both Cu addition and PGPR inoculation strongly affected the germination rate，shoot height and root length of hybrid *Pennisetum*，and there were significant interactions between Cu addition and inoculation for germination rate and root length（Table 1）. Germination rate of hybrid *Pennisetum* seeds exposed to the −Cu solution was not affected by PGPR treatment，but germination rate of those exposed to the +Cu solution was substantially stimulated，with no significant differences in effects of the seven PGPR treatments（Table 2）. PGPR inoculation significantly stimulated shoot and root lengths in the presence of both solutions. Under the −Cu conditions，the maximum increases in shoot and root length（by 32% and 34% higher than those of the control），were induced by FLN-B1+ACC-B2 and ACC-B2 inoculation，respectively，while corresponding maxima under the +Cu conditions were induced by FLN-B1 inoculation（up to 111% and 1 167%，respectively）.

Table 1 Effect of Cu addition（−Cu or +Cu）and PGPR inoculation on germination parameters of hybrid *Pennisetum*（*Pennisetum americanum* × *P. purpureum*）after 2 weeks incubation as shown by *F*- and *P*-values from two-way ANOVA[1)]

Treatment	df	Germination rate		Shoot height		Root length	
		F	*P*	*F*	*P*	*F*	*P*
Cu addition	1	113. 853	**0. 000**	408. 002	**0. 000**	1910. 491	**0. 000**
Inoculation	7	5. 817	**0. 000**	4. 738	**0. 001**	3. 610	**0. 006**
Cu addition × Inoculation	7	3. 657	**0. 005**	0. 456	0. 084	3. 527	**0. 006**

1）Values in bold are significant at *P*-values < 0. 05.

2. 2 Growth

Co-inoculation of two or three strains generally caused stronger increases in root length（Fig. 1）. For plants grown on −Cu and +Cu nutrient soils，the maximum increases in root length were 79% and 94%，and the corresponding maxima in height were 19% and 36%，respectively. The maximum increases in root length for plants grown on unsterilized and sterilized tailings soils were 32% and 30%，respectively. Effects of PGPR inoculation on the height of plants grown on sterilized tailings soils were not significant，while all PGPR treatments increased the height of plants grown on unsterilized tailings soils，by up to 43%.

Table 2　Effects of indicated PGPR on the germination parameters of *Pennisetum americanum*×*P. purpureum* after 2 weeks incubation in deionized water（−Cu）or 0. 4 mmol·L^{-1} $CuSO_4$ solution（+Cu）（means±SE）[1)]

PGPR treatment	Germination rate /%		Shoot height /cm		Root length /cm	
	−Cu	+Cu	−Cu	+Cu	−Cu	+Cu
CK	83±2	37±3a	4. 31±0. 23a	1. 49±0. 36a	6. 36±0. 44ab	0. 03±0. 01a
F1	78±3	67±7b	4. 70±0. 06ab	3. 15±0. 17c	5. 83±0. 10a	0. 38±0. 05c
F6	87±2	67±7b	5. 40±0. 52bc	2. 89±0. 27bc	7. 89±0. 98c	0. 38±0. 10c
ACC2	82±3	68±3b	5. 63±0. 31c	2. 27±0. 50ab	8. 55±0. 27d	0. 24±0. 03bc
F1+F6	87±2	63±2b	5. 68±0. 17c	2. 72±0. 17bc	7. 61±0. 53bcd	0. 18±0. 04bc
F1+ACC2	90±0	68±4b	5. 60±0. 27c	2. 45±0. 26bc	7. 13±0. 03abc	0. 13±0. 03ab
F6+ACC2	87±4	65±5b	5. 63±0. 21c	2. 75±0. 17bc	7. 33±0. 50bcd	0. 11±0. 01ab
F1+F6+ACC2	92±3	77±6b	5. 45±0. 12b	2. 48±0. 13bc	7. 54±0. 29bcd	0. 11±0. 01ab

1）F1，F6 and ACC2 refer to FLN-B1（*Stenotrophomonas* sp.），FLN-B6（*Ochrobactrum* sp.）and ACC-B2（*Klebsiella* sp.），respectively. Different letters in the same columns indicate significant differences between PGPR treatments（$P < 0.05$，one-way ANOVA，LSD test）.

PGPR induced significant increases in above- or below-ground biomass of plants grown on +Cu nutrient soils（up to 117% and 375%，respectively），but not those grown on −Cu nutrient soils（Fig. 1）. For plants grown on unsterilized and sterilized tailings soils，the maximum increases in aboveground biomass were 185% and 55%，respectively，while the maximum increases in belowground biomass were 192% and 134% respectively（Fig. 1）.

Contrary to our expectations，two-way ANOVA results showed that adding Cu to the nutrient soils significantly promoted the growth of aboveground parts of the plants，inducing significant increases in height and aboveground biomass（Fig. 1）.

2. 3　Physiological indices

For plants grown on nutrient soils，the single PGPR inoculations did not significantly affect PI_{abs} or the proline content，while co-inoculation of PGPR strains enhanced these indices，by up to 57% and 178% respectively. PGPR inoculation did not significantly affect the MDA content of plants grown on nutrition soils，but Cu addition increased the MDA content（Fig. 2）.

PGPR treatments increased PI_{abs} and inhibited the MDA content for plants grown on sterilized tailings，but did not have significant effects on these indices for plants grown on unsterilized tailings（Fig. 2）.

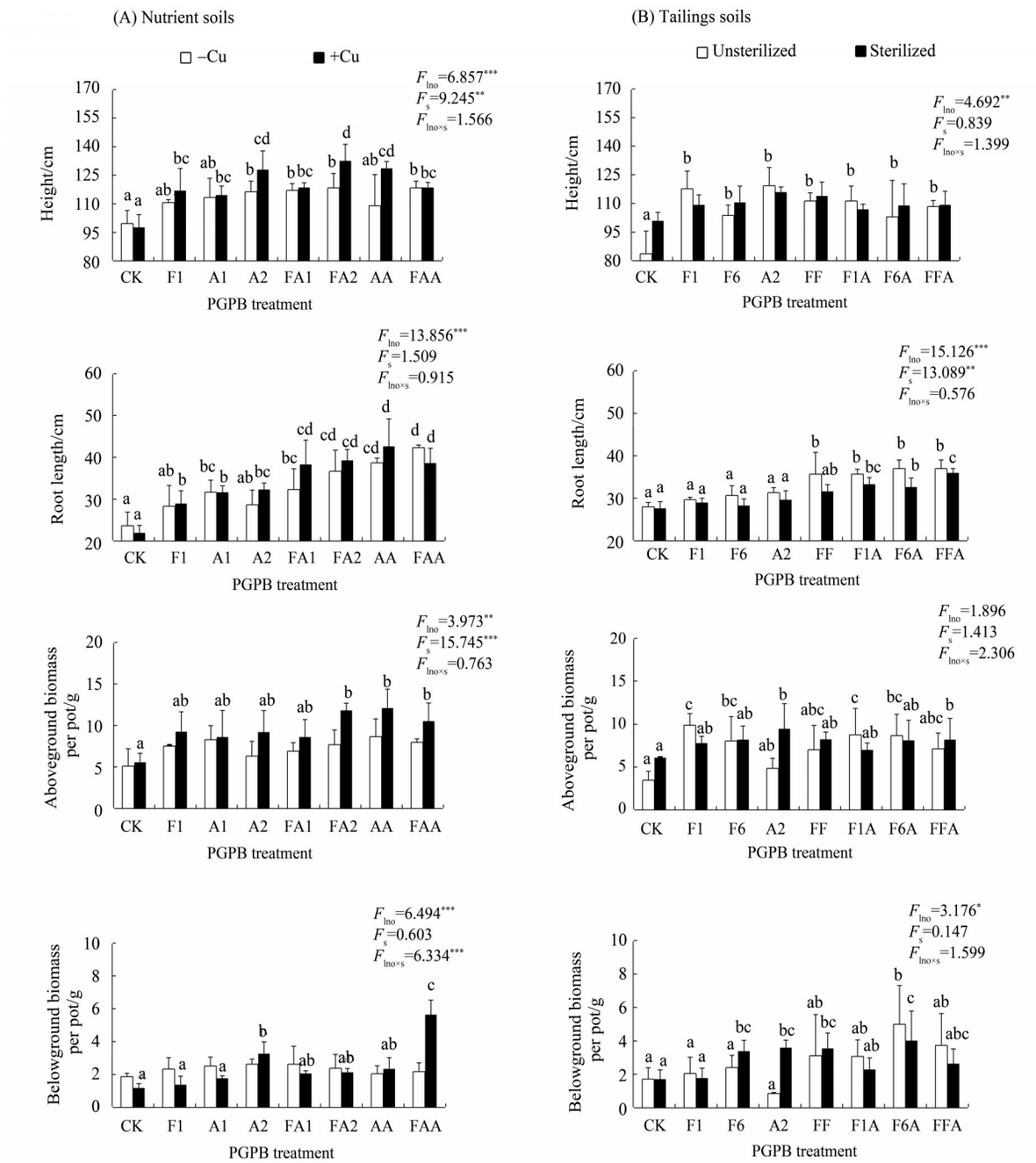
2. 4　Accumulation and translocation of Cu

The TF of Cu in the hybrid *Pennisetum* plants was lower than 0. 1. Both PGPR inoculation and soil sterilization significantly decreased the Cu contents of their aboveground tissue and TF，and co-inoculation generally had stronger effects（Fig. 3）. The maximum decreases in aboveground tissue Cu contents caused by PGPR treatments for plants grown on unsterilized and sterilized tailings were 25% and 51%，and the corresponding maxima in TF were 59% and 72%.

Moreover，although PGPR inoculation tended to increase the content of Cu in belowground tissue，and the TBA，the only significant effect（at $P < 0.05$）was an increase in the TBA of plants grown on sterilized tailings（Fig. 3）.

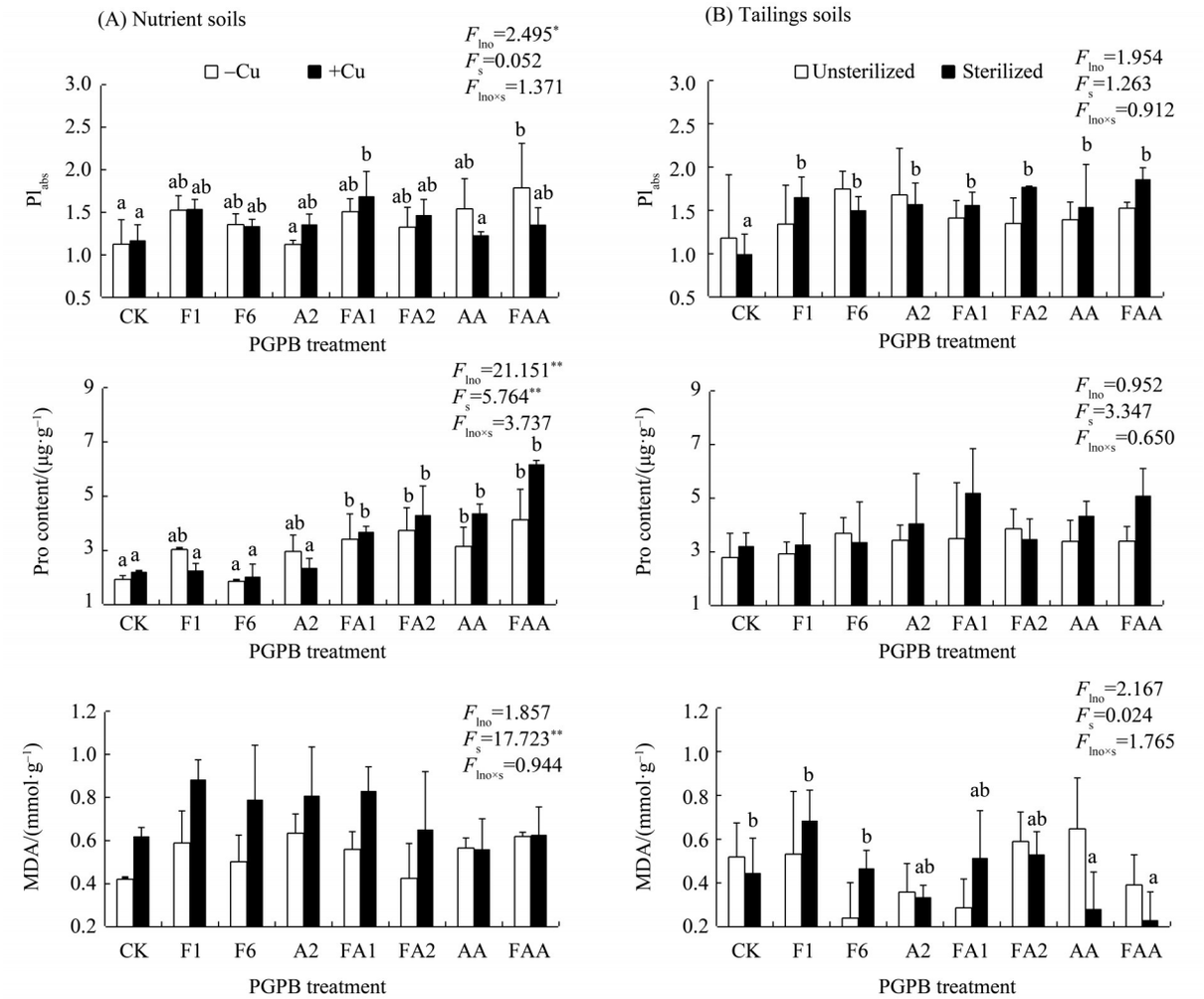
2. 5　Multivariate analysis of key factors driving growth，physiological indices and Cu translocation

As MDA，PI_{abs}，Cu contents of aboveground tissue，TF and TBA have little impact on the model，for clarity，biplot based on RDA not including these variables are shown（Fig. 4）. For nutrient soil experiment，all of the explanatory variables explained 52. 12% of the variance，with axis 1 explaining 87. 11% and axis 2 explaining another 11. 56%；for tailings soil experiment，all of the explanatory variables explained 34. 87% of the variance，with axis 1 explaining 82. 24% and axis 2 explaining another



In the nutrient soil panels, F1, A1, A2, FA1, FA2, AA and FAA refer to FLN-B1 (*Stenotrophomonas* sp.), ACC-B1 (*Microbacterium* sp.), ACC-B2 (*Klebsiella* sp.), FLN-B1+ACC-B1, FLN-B1+ACC-B2, ACC-B1+ACC-B2, FLN-B1+ACC-B1+ACC-B2 treatments, respectively. In the tailings soil panels, F1, F6, A2, FF, F1A, F6A, FFA refer to FLN-B1, FLN-B6 (*Ochrobactrum* sp.), ACC-B2, FLN-B1+FLN-B6, FLN-B1+ACC-B2, FLN-B6+ACC-B2, FLN-B1+FLN-B6+ACC-B2 treatments, respectively. *F*-values are indications from two-way ANOVA of effects of the PGPR and substrate treatments (with or without Cu addition for nutrient soils, designated +Cu and -Cu, respectively, and with or without sterilization for tailings soils, designated sterilized and unsterilized, respectively) on the indicated parameters (Ino, PGPR inoculation treatment; S, substrate treatment; Ino×S, interaction between bacterial and substrate treatment). For each substrate treatment, different letters over the bars indicate significant differences between-PGPR treatment ($P < 0.05$, one-way ANOVA, LSD post hoc tests). Where no letters exist, no significant differences were noted.

Fig. 1 Shoot height, root length, aboveground biomass and belowground biomass of hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*) inoculated with various PGPR combinations grown on nutrient soils (A) and tailings soils (B) (mean +1 SE, n=3)



Legend same as in Fig. 1.

Fig. 2　Photosynthesis index，proline and MDA contents（FW）of hybrid *Pennisetum*（*Pennisetum americanum* × *P. purpureum*）inoculated with various PGPR combinations grown on nutrient soils（A）and tailings soils（B）（mean +1 SE，n=3）

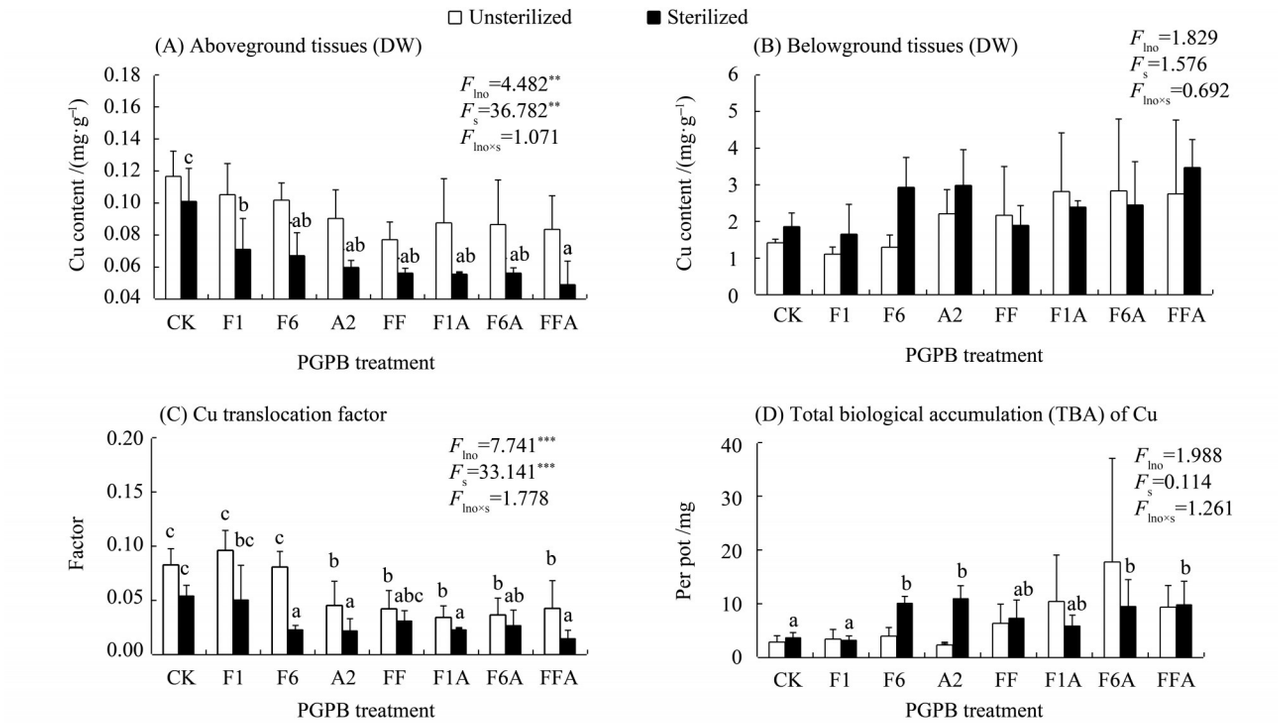
15. 45%.

The results show that for both nutrient soil pot experiment and tailings pot experiment，root length，belowground biomass and proline contents are generally negatively correlated with no inoculation（control）and single PGPR inoculation treatments，while positively correlated with co-inoculations. Shoot height and aboveground biomass are negatively correlated with control，and generally positively correlated or not correlated to PGPR inoculations（Fig. 4）. In addition，for plants grown on nutrient soils，Cu addition is positively correlated with shoot height and aboveground biomass. For plants grown on tailings，Cu content of belowground tissues is positively correlated with co-inoculations. Substrate sterilization is negatively correlated with root length，belowground biomass and Cu contents in belowground tissues. The results also show that root length，belowground biomass and proline content are strongly positively correlated. The responses of shoot height and root length are almost not correlated，indicating they responded quite differently to the treatments.

3　Discussion

3. 1　Seed germination

Seed germination is an extremely sensitive process that is affected by diverse environmental and developmental factors[23]. PGPR can affect seed germination and seedling growth via numerous mechanisms，*inter alia* phosphate solubilization and production of ACC deaminase and phytohor-



Legends same as in Fig. 1.

Fig. 3 Content, translocation and biological accumulation of Cu in hybrid pennisetum (*Pennisetum americanum* × *P. purpureum*) inoculated with various PGPR combinations grown on tailings soils (mean +1 SE, n=3)

mones[23-24]. The auxin production capacities of PGPR are reportedly related to their enhancement of seeds' germination rates[25]. However, we found that ACC-B2, which has higher IAA production capacity than the other PGPR strains[15], did not stimulate germination more strongly. As the PGPR used in our germination experiment have multiple PGP traits[15], we speculate that the germination-promoting effects we observed may have been overall effects of several activities.

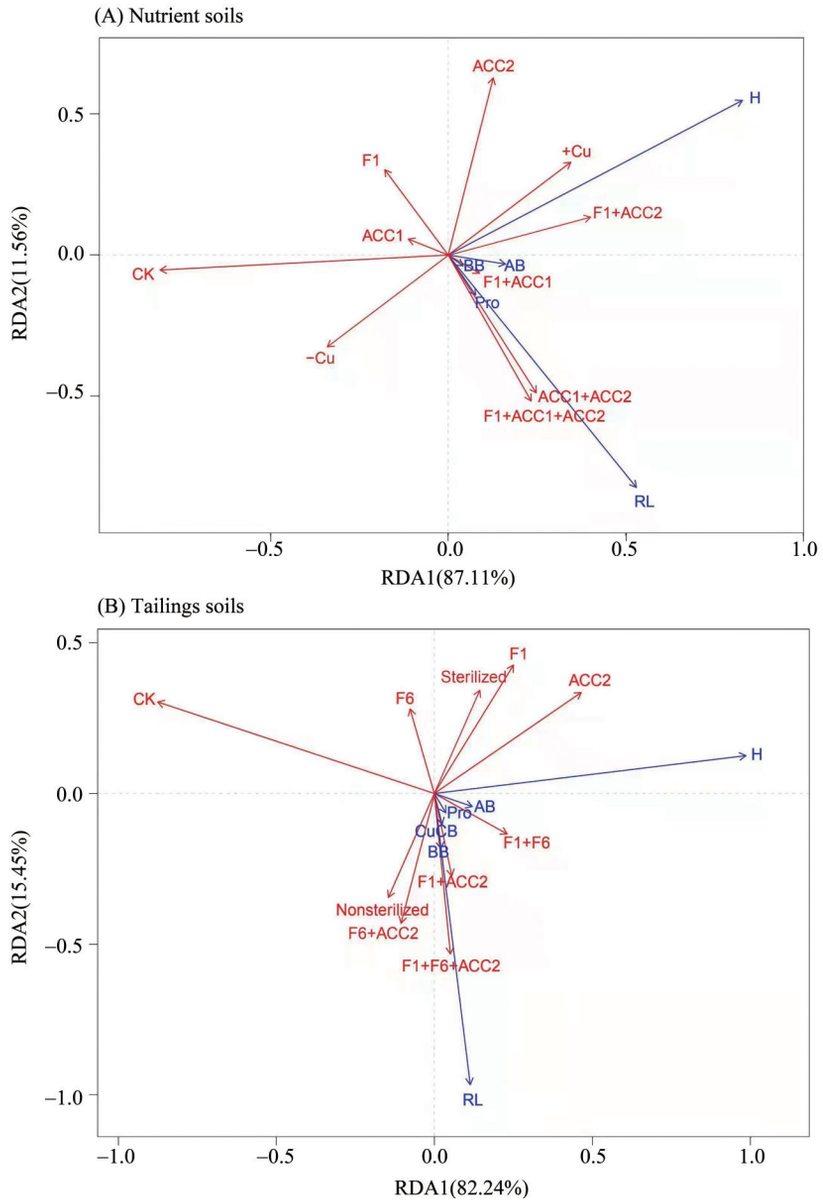
Our study also showed that PGPR had stronger germination-promoting effects in the presence of the +Cu solution, indicating that they had stronger PGP effect on plants under stress conditions, in accordance with previous reports, as reviewed by Glick[11].

3.2 Growth and physiology

Cu is quite toxic to plants, with EC50 values much lower than 300 mg Cu kg^{-1} soil for various crops[18]. We found that although adding 350 mg Cu kg^{-1} soil increased the content of MDA in hybrid *Pennisetum*, it did not significantly reduce the plant's growth or PI_{abs}, suggesting that the species is quite tolerant to Cu stress. The growth-promoting efficiency of PGPR reportedly depends not only on the strain, but also the plant species and environmental conditions[2]. Our results confirm that PGPR have stronger effects on plants under stress conditions[11]. In contrast, sterilization of tailings soils did not have consistent effects on the growth-promoting efficiency, although we previously found that soil sterilization greatly enhanced it[15]. This might have been because the tailings soils used in this study were from a recently discarded tailings with very sparse microbial populations[19], so competition from indigenous microbes and effects of sterilization were weak.

Co-inoculation of two or three PGPR strains promoted increases in root length, and to a less extant belowground biomass, of plants more effectively than inoculation with the strains singly, suggesting that the strains had synergistic effects on the plants' growth. On the other side, RDA results show that shoot height and aboveground biomass are generally not positively correlated with co-inoculations, indicating that co-inoculation are more effective on stimulating the growth of belowground part than aboveground part .

Although reductions in proline contents of plants



Cu accumulation and translocation indices as response variables (blue) and PGPR treatments and substrate treatments (−Cu/+Cu for nutrient soil pot experiment and sterilized/unsterilized for tailings pot experiment) as explanatory variables (red). Data were standardized prior to statistical analysis. H, AB, RL, BB, Pro, CuCB refer to shoot height, aboveground biomass, root length, belowground biomass, proline content and Cu content of belowground tissues. F1, F6, ACC1 and ACC2 refer to FLN-B1 (*Stenotrophomonas* sp.), FLN-B6 (*Ochrobactrum* sp.), ACC-B1 (*Microbacterium* sp.) and ACC-B2 (*Klebsiella* sp.) treatments respectively. Percentages along axis 1 and axis 2 indicate the proportion of explained total variation. The effectiveness of variations in explaining the response variables is represented by the direction and the length of the arrows.

Fig. 4 Biplot based on redundancy analysis using growth and physiological indices

exposed to HM following PGPR inoculation have been reported[26], we found that co-inoculation of PGPR strains increased proline accumulation in plants grown on the nutrient soils. However, PGPR inoculation neither increased MDA contents nor inhibited the growth or PI_{abs} of plants, and proline content show similar change pattern with root length and belowground biomass. As proline plays important roles in plants' stress resistance[27], we consider that the increase in proline contents of the plants following PGPR inoculation indicates an increase in stress resistance.

3.3 Copper uptake and translocation

PGPR inoculation can reportedly stimulate both increases in biomass and accumulation of HM in plants, and thus bioextraction of HM from soil[28-29]. However, effects of PGPR on HM accumulation in plants strongly vary as different organisms have different effects on heavy metal mobility[10,13,30]. We found that the effects of PGPR inoculation on Cu contents of roots are not significant, however, it significantly reduced Cu contents of aboveground tissues of plants grown on tailings soils due to reduction in the root-to-shoot translocation of Cu. Reductions in TF caused by inoculation of rhizobacteria have been widely reported[1,31] and may be one of the mechanisms that reduces HMs' toxicity to the plants. The reduction in Cu contents in aboveground parts of plants inoculated by PGPR will reduce amounts of Cu entering food chains via animals that eat aerial parts of the plants. On the other hand, PGPR inoculation, especially co-inoculations, caused an increase in biomass and Cu content of belowground tissues, which compensated for the lower accumulation of Cu in aboveground tissues and resulted in similar or higher metal removal from soils.

4 Conclusion

Hybrid *Pennisetum* is quite tolerant to Cu and can grow well on Cu contaminated soils and Cu tailings soils. PGPR inoculations enhanced the seed germination, especially under Cu stress conditions. The growth of hybrid *Pennisetum* grown on nutrient soils and tailings soils exhibited similar responses to PGPR inoculation in general, and co-inoculations stimulated the growth of belowground part more effectively than single strain inoculations. PGPR inoculations, especially co-inoculations, reduced the Cu content in the aboveground tissues of the plant due to reductions in translocation factor.

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