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ScienceDirect

Journal of Environmental Sciences 21(2009) 1135–1141

JOURNAL OF
ENVIRONMENTAL
SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Enhanced adaptability of *Sesbania rostrata* to Pb/Zn tailings via stem nodulation

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Received 28 September 2008; revised 07 November 2008; accepted 29 December 2008

Abstract

Sesbania rostrata is wellknown for its stem nodulation, but the roles of stem nodulation in root nodulation and adaptation of *S. rostrata* to Pb/Zn-enriched tailings environment has been poorly understood. We investigated the effects of inoculating (with stem nodule treatment) and non-inoculating (without stem nodule treatment) *Azorhizobium caulinodans* on the growth, root nodulation, and N fixation of *S. rostrata* grown on three different types of soil substrata: Pb/Zn tailings, garden soil amended tailings, and garden soil. The results showed that plant height, stem basal diameter, biomass, chlorophyll content, nitrogen content and N-accumulation per plant were 2.3%–4.9%, 2.2%–7.7%, 27.8%–72.2%, 17.1%–23.5%, 12.3%–34.2%, and 43.1%–131.2%, respectively, higher in treatments with stem nodule than those without stem nodule for the same soil substrate. With respect to soil substrata, all measurements had consistently higher values in tailings than in amended tailings and garden soil, indicating that the poorer the soil condition, the greater the contribution of stem nodule. In contrast, the number and fresh weight of root nodules on plants without stem nodule were 6.9–11.6 times and 5.8–29.0 times higher than those with stem nodule, respectively, especially with respect to the plants grew on Pb/Zn tailings. In general, stem nodulation favored plant growth and nitrogen fixation of *S. rostrata*, but suppressed root nodulation. With the ability of stem and root nodulation, *S. rostrata* can be used as a pioneer plant species for remediation of Pb/Zn tailings.

Key words: *Sesbania rostrata*; stem nodulation; root nodulation; Pb/Zn tailings; remediation

DOI: 10.1016/S1001-0742(08)62393-1

Introduction

Mining activities often generate large amounts of waste rocks and tailings that are eventually deposited on the fields. Waste rocks and tailings are physically unstable and easy to become sources of pollution, therefore causing environmental degradations and losses of biodiversity, amenity, and economic wealth (Freitas *et al*., 2004). Severe environmental problems caused by waste rocks and tailings have attracted worldwide attention. In China, restoring vegetation on waste rocks and mine tailings has become one of the important strategies for sustainable development at both local and regional scales (Gao *et al*., 1998). However, several limiting factors such as acidity, nutrient deficiency, toxic heavy metal ions, and poor physical structure, inhibit plants from establishing and growing on tailings (Pichtel and Salt, 1998). Therefore, amendment of physical and chemical properties of toxic metal-mined soils and choice of appropriate plant species are critically important in remediation of mine tailings (Bradshaw, 1987). Plant species that are tolerant to heavy metal toxicity and nutrient deficiency have better chance to establish and grow on mine tailings (Archer *et al*., 1988).

The genera of *Sesbania* (Papillionoideae), consists of about 500 species around the world, is a unique group of legumes native to the tropics and subtropics. They inhibit a wide range of ecologically diversified habitats from lowland, wetland to semi-arid ecosystems and from seacoasts to fresh-water swamps (Sharma *et al*., 2005). Many *Sesbania* species are fast growing annuals, able to grow in heavy metal contaminated soils, withstand water-logging, and tolerate soil salinity. They are also excellent green manure crops for rice and wheat (Evans and Rotar, 1987; Sharma *et al*., 2005). *Sesbania rostrata*, also called rostrate Sesbania, is an annual tropical legume shrub that originated in West Africa. It forms a symbiosis with *Azorhizobium caulinodans* and is renowned for its stem nodulation. Unlike root nodules, stem nodules of *S. rostrata* contain functioning chloroplasts in the nodule cortex and are therefore capable of carbon fixation. *S. rostrata* is a fast-growing species and probably the most rapid N_2-fixing plant known (Dreyfus and Dommergues, 1981; Dreyfus *et al*., 1988; Pareek *et al*., 1990; Somasegaran and Hoben, 1994). Furthermore, *S. rostrata* also has good saline and heavy metals tolerance to extreme environment such as Pb/Zn tailings (Evans and Rotar, 1987; Yang *et al*., 1997, 2004; Ye *et al*., 2001). For these reasons, *S. rostrata* had been used to reclaim Sn or Pb/Zn mine tailings in

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several places (Radziah and Shamsuddin, 1990; Yang *et al*., 1997, 2003; Jian *et al*., 2003), and has been somewhat studied for heavy metals tolerance mechanism (Chen *et al*., 2005; Zheng *et al*., 2005) and molecular aspects for understanding the N_2-fixing mechanisms of stem nodules (Suzuki *et al*., 2007, 2008).

Because the large contribution of stem and root nodulation in nitrogen fixation, *S. rostrata* could perhaps compensate for the lack of soil nitrogen on Pb/Zn tailings (Dreyfus *et al*., 1984). Since stem nodules do not contact directly with the extreme soil environment, they are expected to provide more nitrogen for plant growth than root nodules (Yang *et al*., 1997; Chan *et al*., 2003). Most microorganisms are sensitive to the toxicity of heavy metals, including *S. rostrata-A. caulinodans* symbiosis (Wu, 1989; McGrath, 1994). However, whether *S. rostrata* - *A. caulinodans* symbiosis could play important role in plant growth in heavy metal polluted environment has not been well understood. Furthermore, how stem nodulation influence root nodulation remains unknown.

The objective of this study was to investigate the influence of stem nodulation on root nodulation, plant growth, and N fixation by comparing *S. rostrata* with and without inoculation of *A. caulinodans* on different soil substrata: Pb/Zn tailings, garden soil amended Pb/Zn tailings, and non-polluted garden soil.

1 Materials and methods

1.1 Materials and methods

Three types of soil substrata were used to plant *S. rostrata* seedlings: Pb/Zn mine tailings, garden soil, and garden soil amended Pb/Zn mine tailings. The Pb/Zn mine tailings were excavated from a tailing dam at the Lechang Lead and Zinc Mine Factory located in Lechang County, Guangdong Province, China. The garden soil was obtained from the Bamboo Garden of Sun Yat-sen University, Guangzhou, China. All soil substrata were air-dried and sieved through a 2-mm mesh and mixed well. The pH value and electric conductivity were determined using pH/electric conductivity meter (CyberScan PC10, Eutech, USA) (solid:distilled water = 1:2). Total organic matter was determined using a CHNS/O analyzer (PE 2400, Perkin-Elmer, USA); total N was determined by the indophenol-blue method; total P was determined by the molybdenum-blue method; total K, Pb, Zn, Cu and Cd (digested with concentrated HNO_3 and concentrated $HClO_4$ at 5:1) and diethylenetriaminepentaacetic acid (DTPA)-extractable forms of them were determined by means of flame atomic absorption spectrometry (AAS, Perkin Elmer analyzer 100, Germany) (Page *et al*., 1982). All the measured chemical properties of tailings and garden soils are presented in Table 1. The tailings had much lower pH value, organic matter content and total N, P, K content, but much higher electric conductivity (EC) and concentrations of heavy metals (Pb, Zn, Cu and Cd) than the garden soil.

Table 1 Chemical and physical properties of tailing and garden soil used in this study (mean ± SD)

Property	Pb/Zn tailings	Garden soil
pH	4.51 ± 0.31	5.54 ± 0.17
Electronic conductivity (ms/cm)	1.95 ± 0.35	0.18 ± 0.02
Organic matter (g/kg)	5.5 ± 1.5	15.0 ± 2.3
Total N (g/kg)	0.69 ± 0.18	1.94 ± 0.19
Total P (mg/kg)	901.2 ± 198.0	2376.7 ± 145.4
Total K (mg/kg)	1501.3 ± 248.9	1916.1 ± 112.3
Total Pb (mg/kg)	2425.3 ± 481.0	198.1 ± 14.2
DTPA extractable Pb (mg/kg)	231.4 ± 35.6	13.21 ± 1.03
Total Zn (mg/kg)	2310.4 ± 453.7	266.8 ± 19.4
DTPA extractable Zn (mg/kg)	40.4 ± 15.1	14.4 ± 1.11
Total Cu (mg/kg)	140.2 ± 14.1	15.8 ± 1.09
DTPA extractable Cu (mg/kg)	0.71 ± 0.09	0.36 ± 0.03
Total Cd (mg/kg)	4.15 ± 0.59	ND
DTPA extractable Cd (mg/kg)	0.71 ± 0.08	ND

ND: not detected.

1.2 Experimental design

To test the effects of soil substrate, *S. rostrata* seedlings were planted in plastic pots filled with the three types of soil substrata: 100% tailings (T), 100% garden soil (S), and the half-half mixture of the two (M). Seedlings were also treated by inoculating (with stem nodule) (I) and non-inoculating *A. caulinodans* (without stem nodule) (N) to evaluate the effects of the stem nodulation. A full combination of the three soil types and two stem nodule manipulations received 6 types of treatments (TI, TN, MI, MN, SI, and SN) in total. Four replicates were conducted for each treatment. In total, 24 experimental pots were used to plant *S. rostrata* seedlings and were arranged in a randomized block design.

1.3 Seed germination and *A. caulinodans* inoculation

The seeds of *S. rostrata* were immersed in the 90°C water for 2 min and then 20 of them were sown in one plastic pot (13 cm high, 14 cm of top diameter, and 12 cm of bottom diameter) filled with 1.25 kg soil substrate, and watered daily. After the first seed germinated, the numbers of germinated seeds were recorded daily for each pot for 10 d. At day 20, 35, and 60, seedlings were thinned out to 5, 2, and 1 plant per pot gradually. At day 30, seedlings in 12 pots were inoculated with the mixed strains of *A. caulinodans* (i.e., with stem nodule treatment) by painting the bacteria mixed with water to the leaves and stem, which were placed apart from the other 12 pots of seedlings without inoculation (i.e., without stem nodule treatment). Although without inoculation, some seedlings still formed few small stem nodules, which maybe result from the presence of indigenous nodule-inducing bacteria in the soil. We removed those stem nodules carefully from the plants once they appeared and sterilized with ethanol to limit the damage to the host plant.

1.4 Growth measurement, tissue sampling and chemical analysis

All plants were allowed to grow for 100 d. Plant height, stem nodule location height, and number of stem nodules in each plant were recorded every 10 d. After 100 d of growth, all plants were harvested, separated into root and shoot parts, and washed with tap water to remove any other attached particles and then rinsed with deionized water. Plant height, basal diameter of stem, shoot and root biomass, height of stem nodule locate, number, diameter and fresh weight of stem and root nodules per plant were measured thereafter. Leaf chlorophyll *a* and *b* contents (extracted by acetone) were determined according to the molybdenum-blue method (Parkinson and Allen, 1975). N contents in plant roots, stems and leaves were determined according to the indophenol-blue method (digested with H_2SO_4 + H_2O_2) (Page *et al.*, 1982).

1.5 Statistical analysis

The data were analyzed using a SPSS statistical package by one-way analysis of variance (ANOVA) to compare the means of different treatments. Where significant F values were obtained, differences between treatments were tested using LSD test at the significance level of 0.05.

2 Results

2.1 Seed germination and plant growth

The seeds of *S. rostrata* started to germinate at day 3 after sowing. At day 8, the germination percentages in T, M and S were (59.6 ± 13.1)% (mean ± SD, the same as follows), (60.1 ± 9.2)% and (64.0 ± 10.5)%, respectively. Although the germination rate in garden soil was slightly higher than those in amended tailings and tailing, there is no statistically significant difference among three soil types.

Effects of the different treatments on plant growth are shown in Fig. 1. In the first 30 d, there was no significant difference in plant height among the six treatments (Fig. 1a). After 30 d growth, the plant heights in the garden soil and the amended tailings were higher than that in tailings. In the first 70 d, plant height showed almost no difference between the two stem nodule treatments. But after day 70, plant heights with stem nodules were slightly higher than that without stem nodules. After 100 d growth, plant heights reached 110–160 cm and were higher with stem nodule than those without stem nodule. Plant heights were also significantly different among different soil substrata, and were higher in the garden soil (S) than in the amended tailings (M) and in the tailings (T).

The basal diameter of stem (Fig. 1b) varied from 0.9 to 1.6 cm after 100 d growing, being larger for the treatments with stem nodule (TI, MI, SI) than those without stem nodule (TN, MN, SN). As for the soil types, the basal diameters of stem in garden soils were significantly larger than those in amended tailings and tailings.

The biomass (Fig. 1c) and leaf chlorophyll contents (Fig. 1d) in plants with stem nodule were significantly higher than in those without stem nodule, and significantly higher in the garden soil than in amended tailings and tailings. Averaged over all treatments, total plant biomass varied from 10 to 32 g dry matter per plant, most of which (84.4% ± 0.83%) was shoot biomass (Fig. 1c). The shoot/root ratios averaged 5.4 ± 0.37 for all treatments, did not respond markedly to stem nodulation treatments. The

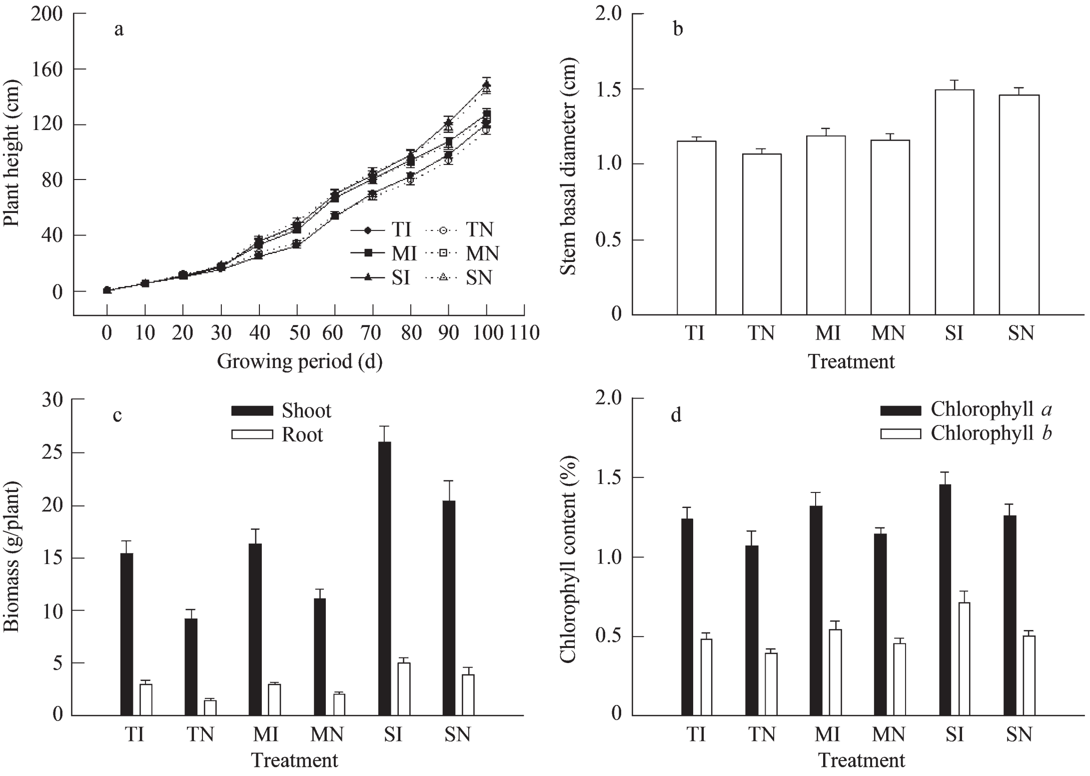

Fig. 1 Effects of stem nodulation on (a) plant height, (b) stem basal diameter, (c) biomass, and (d) chlorophylls content of *Sesbania rostrata* growing on Pb/Zn tailings (T), garden soil (S), and garden soil amended tailings (M). TI, SI and MI represent plants with stem nodules (I) and TN, SN and MN represent those without stem nodules (N). Error bars represent standard errors (n = 4).

total chlorophyll contents in leaves varied from 1.0% to 2.5%, and the content of chlorophyll *a* was much higher than that of chlorophyll *b* (Fig. 1d).

The relative increments of plant height, stem basal diameter, biomass and chlorophyll content of *S. rostrata* with stem nodule to those without stem nodule for the same soil substrate were 2.3%–4.9%, 2.2%–7.7%, 27.8%–72.2%, 17.1%–23.5%, respectively, and were in the order of: T (4.9%–72.2%) > M (2.6%–46.8%) > S (2.2%–27.8%), exhibiting the importance of stem nodules for plant growth and biomass accumulation.

2.2 Nodulation

Effects of different treatments on stem and root nodulations are shown in Fig. 2. Stem nodules started to appear at the basal of stem at day 9 after the mixed strains of *A. caulinodans* was inoculated (i.e., 39 d after germination). Twenty days after inoculation, stem nodules locations and their number increased rapidly over time (Figs. 2a and 2b). After 100 d growing, stem nodules formed at the height of about 70–120 cm on the stem, and the number of stem nodules increased in the order of tailings < amended tailings < garden soil with significant differences among each other.

At the end of the experiment, the number, diameter, and fresh weight of stem nodules were significantly greater than those of root nodules, and were also greater in garden soil than in amended tailings and tailings (Figs. 2b, 2c, and 2d). Interestingly, the root nodules on plants without stem nodule were significantly more than those on plants with stem nodule, so were the fresh weight and diameter of root nodules (Fig. 2d), indicating that root nodulation was suppressed by stem nodulation, especially with 100% tailing. The diameter of stem nodule descended in the order of TI > MI > SI (Fig. 2d), while the order for the diameters of root nodule was just a reverse of that.

2.3 Nitrogen accumulation

Effects of different treatments on nitrogen contents in plant tissues are shown in Fig. 3. Leaf nitrogen content varied from 2.3% to 4.0%, which was higher than stem (0.5%–1.1%) and root (1.1%–1.6%) nitrogen contents (Fig. 3a). N contents of leaves, stems, and roots for all plants with stem nodule were significantly higher than those without stem nodule, and the relative increments were 4.2%–27.4%, 12.5%–45.3% and 25.0%–28.9%, respectively, indicating that the stem nodules expended obviously nitrogen source for the plants with *A. caulonodans* inoculated. Total N accumulation, reflecting the absolute amount of N accumulated in plant body, followed the same trend as N contents, being significantly higher in plants with stem nodule than without stem nodule, and higher in the garden soil than in the amended tailings and tailings (Fig. 3b). The increments were in order of T (34.2%) > M (32.5%) > S (12.3%), exhibiting the importance of stem nodules for obtaining nitrogen.

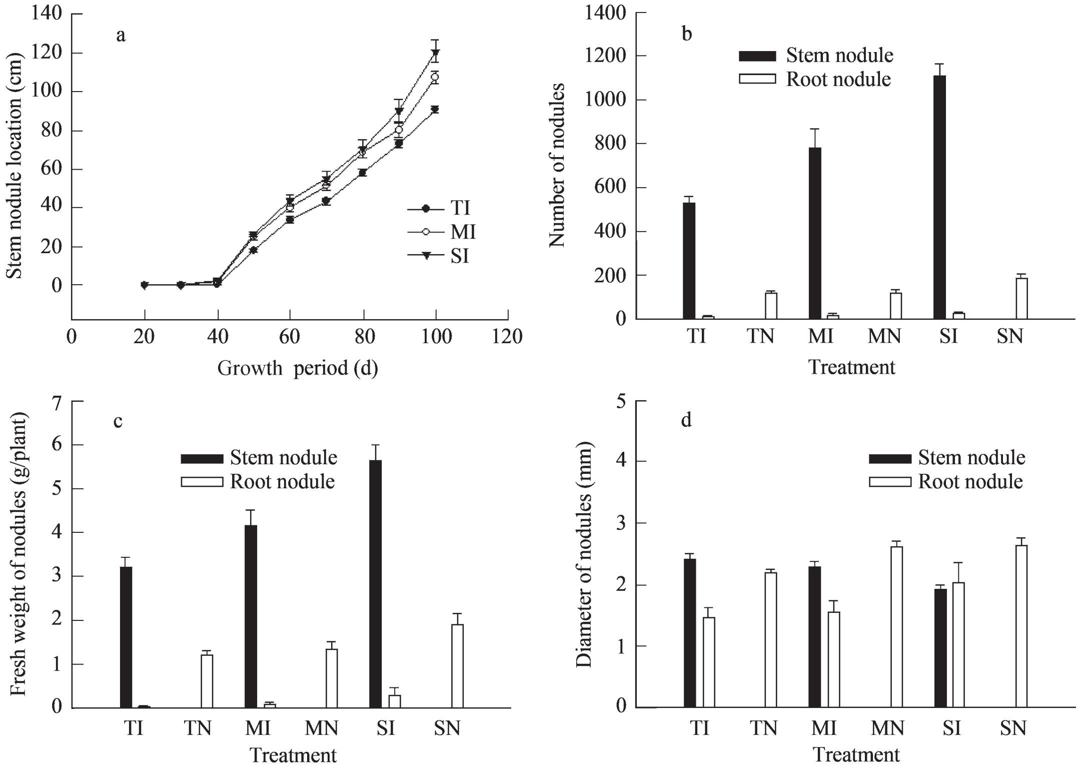

Fig. 2 Effects of different treatments on (a) the height of stem nodule location, (b) the number, (c) the fresh weight, and (d) the diameter of stem and root nodules. The meanings of treatment symbols (i.e., TI, SI, MI, TN, SN, and MN) are the same as shown in Fig. 1. Error bars represent standard errors ($n = 4$).

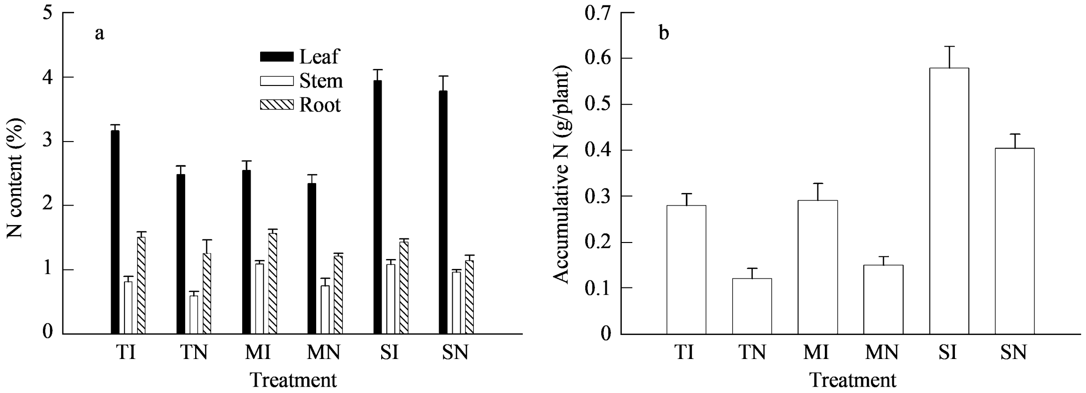

Fig. 3 Effects of stem nodulation on (a) the nitrogen contents of plant tissues and (b) N accumulations per plant. The meanings of treatment symbols (i.e., TI, TN, MI, MN, SI, and SN), are the same as shown in Fig. 1. Error bars represent standard errors ($n = 4$).

3 Discussion

Among several limiting factors that inhibit plant establishing and growing on mine tailings (e.g., acidity, nutrient deficiency, heavy metal toxicity, and poor physical soil structure), heavy metal toxicity and nutrient deficiency are the two most frequently faced obstacles for bioremediation of mining tailings (Bradshaw, 1987). The Pb/Zn tailings used in this study had most of these limitation features: low pH, high EC, high Pb and Cd contents, low organic matter and nutrient contents, and poor physical structure. According to the National Environmental Quality Standard for Soils of China (NEQSSC, GB 15618-1995), the contents of Pb and Cd in our experimental tailings largely exceeded the third grade (500 mg/kg for Pb and 1 mg/kg for Cd), which was considered to be the critical grade and above that plants can hardly survive (Xu and Liu, 1996). The results in this study showed that the condition of Pb/Zn tailings negatively affected the growth and nitrogen fixation of *S. rostrata*. On the Pb/Zn tailing dam at Lechang, where the experimental tailings was excavated, no plant species was found naturally growing even though the disposal had been stopped for ten years. In the present study, however, *S. rostrata* succeeded to germinate, grow, form nodules, and fix nitrogen on the experimental Pb/Zn tailings without amendments, indicating that the species possesses the capability to tolerate the acidification, nutrients deficiencies, toxicity of multiple heavy metals, and poor physical structure of the Pb/Zn mine tailings.

The two treatments with and without stem nodule in this experiment clearly exhibited the role of stem nodulation in the adaptation of *S. rostrata* to Pb/Zn tailings. The result showed that plant height, stem basal diameter, biomass, chlorophyll contents, nitrogen contents and N accumulation per plant of *S. rostrata* in treatments with stem nodule were 2.3%–4.9%, 2.2%–7.7%, 27.8%–72.2%, 17.1%–23.5%, 12.3%–34.2% and 43.1%–131.2% higher than those in treatments without stem nodule grown in the same soil substrate, respectively. It indicated that stem nodulation improved the growth and nitrogen fixation of *S. rostrata*, and the improvements for all the variables were consistently in the order of tailings > amended tailings > garden soil, indicating that the poorer the soil condition, the greater the contribution of stem nodules. This could be explained by that *S. rostrata* grown on Pb/Zn mine tailings depended greater on the nitrogen fixed by stem nodule than grown on amended tailings and garden soil. Moreover, it seemed that the stem nodulation may at least partly substitute the effect of the tailings amendment, because the biomass, chlorophyll content, nitrogen content and N accumulation per plant of *S. rostrata* grown on 100% tailings with stem nodules were even much higher than those of grown on 50% garden soil amended tailings without stem nodule.

We found that the plant height and stem basal diameter of *S. rostrata* in the treatments with stem nodule were higher than those without stem nodule grown in the same soil substrate, but no statistically significant difference were obtained (Figs. 1a and 1b). It is attributed to the short growing period of *S. rostrata*, which is deduced from the fact that the difference in plant heights between with and without stem nodule treatments was gradually enlarged after 70 d growth. The same phenomenon was also observed in stem basal diameter. Because plant height and radial growth constitute biomass, the overlap of the two factors resulted in the significant difference in plant biomass between with and without stem nodule treatments, indicating stem nodulation obviously improved the growth of *S. rostrata*.

Although heavy metals (e.g., Pb and Zn) were proved to be toxic to *A. caulinodans* and tend to reduce the nodulation rate of *S. rostrata*, especially at low pH (Ibekwe *et al*., 1995), our results showed that *S. rostrata*-*A. caulinodans* symbiosis still functioned and played an important role in nitrogen fixation and thus alleviated N limitation to plant growth in the experimental mine tailings. In addition, stem nodule contains functioning chloroplasts, which increase plant chlorophyll contents and photosynthetic ability and further benefit to plant growth. Ladha *et al.* (1989) found that *S. rostrata*-*A. caulinodans* symbiosis as green manure for the rice is advantageous because it adds to the soil not only organic C and N but also a large population of azorhizobia.

Interestingly, root nodules grew much worse on plants with stem nodule compared with those without stem nodule, especially with respect to the tailings. There may be competition between stem and root nodulation in *S. rostrata*. Stem nodules are more competitive since they do not direct contact with the harsh soil environment and receive more photosynthate (Yang *et al*., 1997). Tomekpe *et al*. (1996) found that root nodulation of *S. rostrata* inoculated by *A. caulinodans* could suppress stem nodulation in some degree. The interactions between stem and root nodulation of *S. rostrata* need to be further investigated.

4 Conclusions

Stem nodulation of *S. rostrata* favored plant growth and nitrogen fixation, but suppressed root nodulation. As *S. rostrata* is a fast-growing annual plant species with abilities to produce great biomass, and accumulate a large amount of nitrogen in a short period. When they die back each year, plant litter may add considerable amounts of organic matter into soil and improves soil chemical and physical conditions, which may facilitate the establishment and growth of other successive plant species on tailings. Therefore, *S. rostrata*, with the aid of its stem nodulation, could serve as an appropriate pioneer species in remediation of Pb/Zn mine tailings.

Acknowledgments

This work was supported by the Guangdong Sci-Tech Planning Project (No. 2005B33302012, 2008B020300011). The authors would like to thank Dr. Dafeng Hui at Duke University and Dr. Qinfeng Guo at Northern Prairie Wildlife Research Center of USGS for their suggestions and comments to the early versions of the manuscript, and the staff at the Lechang Lead/Zinc Mine Factory for supplying the tailings materials.

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