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Effects of Brassinolide on Photosynthetic Parameters of *Robinia pseudoacacia* Seedlings in Petroleum Polluted Soil

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ABSTRACT

Robinia pseudoacacia seedlings planted in petroleum polluted soil were treated with different concentrations of brassinolide (0, 0.1, 0.3 and 0.5 mg L^{-1}) by dipping the root before planting and spraying leaf during leaf expansion period. The gas exchange parameters and photosynthetic fluorescence characteristics of seedlings were determined to study the effects of brassinolide on photosynthesis of *R. pseudoacacia* seedlings in polluted conditions. The results indicated that the application of brassinolide significantly increased the leaf photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration, and it also significantly increased the PSII original light energy conversion efficiency, PSII noncyclic electron transport efficiency, photochemical quenching coefficient and the performance of the electron transfer rate of seedlings. In conclusion, brassinolide could effectively counteract the inhibitory effects of petroleum on the gas exchange and the carboxylation capacity of mesophyll cells of seedlings in 10, 15 and 20 g kg^{-1}, 0.3 mg L^{-1} was the favourable concentration to be used to counteract the adverse effects of petroleum pollution on *R. pseudoacacia*.

INTRODUCTION

Northern Shaanxi is an important petroleum producing region of China, which has made great contributions to the economic development of China. However, the spill of petroleum during the long-term exploitation has caused severe pollution of the soil, air and water environment and endangered the survival of plants and animals in this region (Zhang 2013). Lot of efforts have been made to remediate polluted soil in recent decades. In the existing approaches, phytoremediation has become one of the most preferred methods for its low cost and high absorbing capacity of petroleum pollutants (Ghazisaeedi et al. 2014, Merkl et al. 2005).

Robinia pseudoacacia is a species which is widely utilized in the afforestation in the Loess hilly region of Northern Shaanxi, and previous study has confirmed that this species also exhibit some ability of absorbing and transferring the petroleum pollutants (Zhang 2013). However, in the polluted regions, the growth and survival of *R. pseudoacacia* are still severely inhibited, which caused great limitations for the phytoremediation of petroleum pollution using this species (Zhang 2013). Hence, for speeding up the vegetation recovery of petroleum polluted regions and the remediation of the polluted soil, it is of great importance to improve the resistance of *R. pseudoacacia* to petroleum and to promote their growth.

Brassinolide, as a phytohormone, can adjust the physiological functions of plant and speed up the their development (Clouse 2015, Jiang et al. 2012, Wei & Li 2016). Especially in stressful conditions, such as drought, extreme temperature and saline-alkali or polluted soil, brassinolide can exhibit favourable abilities to improve the stress resistance of plants and promote their growth by improving the antioxidant ability, maintaining the cell water potential, and improving the photosynthetic efficiency (Ahammed et al. 2013, Ahammed et al. 2012a, Hayat et al. 2012, Hu et al. 2013, Jiang et al. 2013, Li et al. 2015, Wu et al. 2014). Our previous studies had demonstrated that brassinolide can significantly increase the activity of antioxidant enzymes and the content of antioxidants, thus counteract the stress of petroleum and promote the growth and biomass accumulation of *R. pseudoacacia* (Han et al. 2015a, 2015b). However, there were few literatures about the effects on brassinolide on the photosynthesis of *R. pseudoacacia* (which is directly associated with the growth) in petroleum polluted conditions. Hence, in this study, the brassinolide treated *R. pseudoacacia* seedlings were planted in petroleum polluted soil, and the effects of brassinolide on the gas exchange abilities and chlorophyll fluorescence characteristics were studied. The results might provide an effective approach to intensify the effects of phytoremediation.

MATERIALS AND METHODS

Studied area: The experiments were conducted in the nursery garden of the Northwest A&F University in Shaanxi, China. The climate here is classified as temperate continental monsoon climate, with an annual average sunshine duration of 2150 h, an annual average temperature of 12.9°C, an accumulated temperature ($\geq$10°C) of 4185°C, a frost-free period of 221 d and an annual average precipitation of 621.6 mm.

Materials processing: The *R. pseudoacacia* seedlings were purchased from the Huaziping nursery garden of the An'sai County, Northern Shaanxi, China. The soil used for experiments was collected from the surface layer (0-20 cm) of the waste grassland of the An'sai County. The organic matter content of the soil is 8.45 g kg^{-1}, the total N content 0.42 g kg^{-1}, the total P content 0.48 g kg^{-1} and the total K content 1.76 g kg^{-1}. The available N, P and K contents are 3.85 mg kg^{-1}, 5.52 mg kg^{-1} and 74.66 mg kg^{-1}, respectively, and the pH of soil is 8.2. The brassinolide is purchased from Xinchaoyang Institute of Biohormonal, Chengdu, China.

The collected soil was air dried and passed through a 4 mm sieve, and then 10.54 kg soil was placed into each plastic barrel (opening diameter is 31 cm, bottom diameter is 23 cm and the height is 27 cm). According to the investigation about the actual pollution degree of the studied region, crude oil purchased from the local oil well was added into soil with the concentrations of 10 g kg^{-1}, 15 g kg^{-1} and 20 g kg^{-1} (soil dry weight) respectively. The polluted soil samples were uniformly mixed without using any organic solvent. For each kind of soil, 20 barrels of soil was prepared.

The brassinolide emulsion was attenuated using 50-60°C distilled water and then re-attenuated to 0.1, 0.3 and 0.5 mg L^{-1} using distilled water (with these concentrations, brassinolide was the most favourable for the *R. pseudoacacia* seedlings according to the unpublished data). In early March, 60 similar *R. pseudoacacia* seedlings were chosen, 15 of them were treated (root dipping for 30 min) with distilled water, and each 15 of the remaining 45 seedlings were treated by a different brassinolide solution, respectively. Treated seedlings were then planted in the polluted soil media, each combination of soil pollution degree and brassinolide concentration was seen as a treatment, and each treatment has 5 replicates. After planting, sufficient water was added into soil to maintain the survival and well growth of seedlings. At the leaf-expansion period, the leaves of each seedling were sprayed using brassinolide solution (the concentration equalled to the concentration used for the root dipping), until the dropping water was observed. At the end of June, the seedlings were placed under a rainshed, and the soil moisture of each barrel was adjusted to 75% of the field water-holding capacity and maintained consistently.

Indices determination: At 9:00-11:00 of a sunny day in August, the net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and photosynthetic photon flux density (*PPFD*) were determined using a portable gas exchange system (Li-6400, Li-Cor Inc., Lincoln, NE, USA). When doing these, the parameters of the system were set as: gasflow- 500 μmol s^{-1}, light intensity-1000 μmol m^{-2} s^{-1} and temperature- 30°C.

A PAM-2000 portable chlorophyll fluorometer (Walz, Germany) was used to determine the initial fluorescence (F_0, determined after the dark adaptation), initial fluorescence under light (F_0'), maximal fluorescence (F_m, determined after the dark adaptation), maximal fluorescence under light (F_m'), steady-state fluorescence (F_s), and the variable fluorescence (F_v, $F_v = F_m - F_0$), PSII original light energy conversion efficiency (F_v/F_m), PSII noncyclic electron transport efficiency (Φ_{PSII}, $\Phi_{PSII} = (F_m' - F_s)/F_m'$) were calculated. In addition, the photochemical quenching coefficient (*qP*) and performance of the electron transfer rate (*ETR*) were calculated by the following equations (Bernard et al. 1989; Demmig-Adams & Adams III 1996):

$$qP=(F_m'-F_s)/(F_m'-F_0') \quad ...(1)$$

$$ETR=PPFD * \Phi_{PSII} * 0.85 * 0.5 \quad ...(2)$$

Data processing: The data were presented as average ± SE. SPSS 17.0 software was employed for the one-way analysis of variance, and Duncan's test was used for the *post hoc* analysis ($P< 0.05$). Origin 9.0 was used for drawing.

RESULTS

Effects of brassinolide on the gas exchange of seedlings: Brassinolide treatments significantly ($P< 0.05$) counteracted the inhibitory effects of petroleum pollution on the gas exchange abilities (P_n, T_r, G_s and C_i) of *R. pseudoacacia* seedlings (Fig. 1). In 10 g kg^{-1} polluted soil, 0.1 and 0.3 mg L^{-1} brassinolide treatments exhibited the most significant ($P< 0.05$) improving effects on the P_n and C_i of the seedlings, 0.3 mg L^{-1} brassinolide treatment exhibited the most significant ($P< 0.05$) improving effects on the T_r, while the improving effects of brassinolide on the G_s exhibited no significant difference among the 3 treatments. In 15 g kg^{-1} polluted soil, 0.1 and 0.3 mg L^{-1} brassinolide treatments exhibited the most significant ($P< 0.05$) improving effects on the C_i of the seedlings, while the improving effects of brassin-

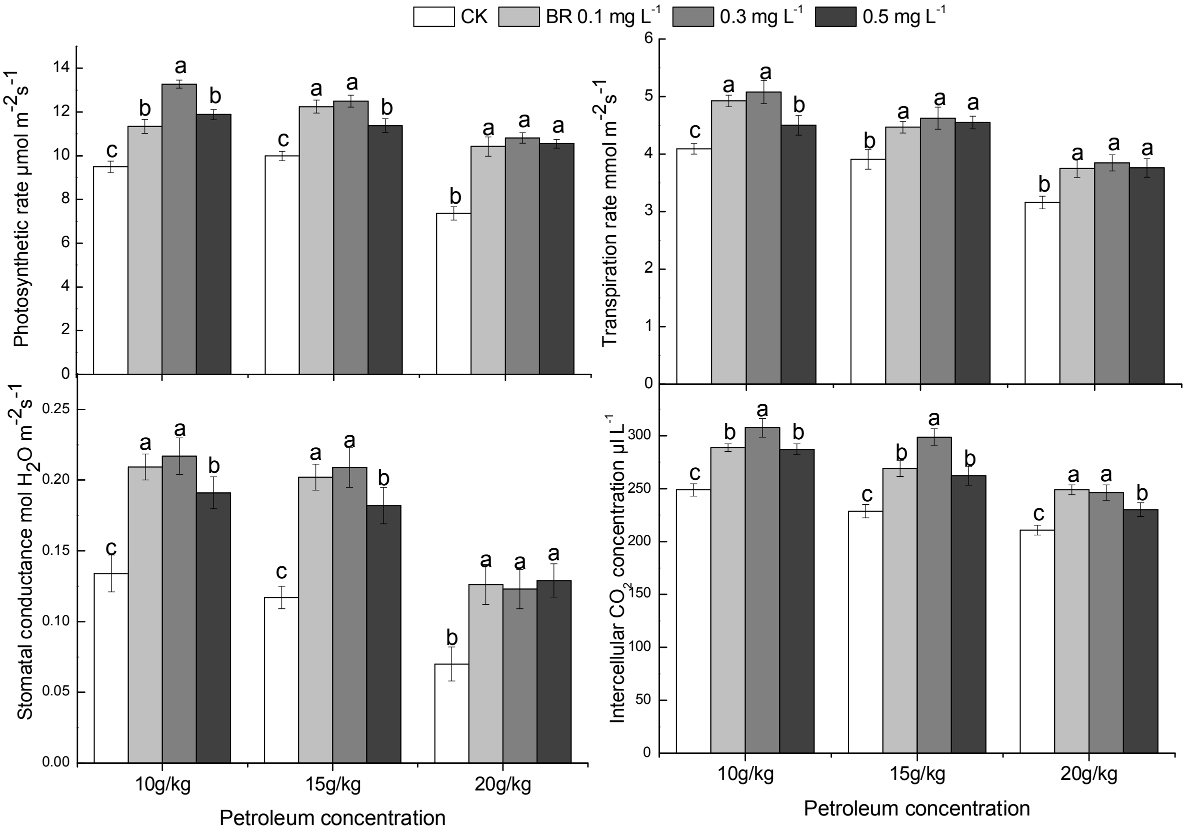

Fig.1: Responses of gas exchange abilities of seedlings to brassinolide treatments in petroleum polluted soil, the different letter indicated significant difference at 0. 05 level at the same pollution degree.

olide on the P_n, T_r and G_s exhibited no significant difference among the 3 treatments. In 20 g kg^{-1} polluted soil, 0.3 mg L^{-1} brassinolide treatment exhibited the most significant ($P< 0.05$) improving effects on the P_n, T_r and G_s of the seedlings, while 0.1 and 0.3 mg L^{-1} brassinolide treatments exhibited the most significant ($P< 0.05$) improving effects on the C_i of the seedlings.

Effects of brassinolide on the chlorophyll fluorescence characteristics of seedlings: Similar to its effects on the gas exchange abilities, brassinolide significantly ($P< 0.05$) counteracted the inhibitory effects of petroleum pollution on the chlorophyll fluorescence characteristics of *R. pseudoacacia* seedlings as well (Fig. 2). In 10 and 15 g kg^{-1} polluted soil, all 3 brassinolide treatments significantly ($P< 0.05$) increased the F_v/F_m, Φ_{PSII}, *qP* and *ETR* of the seedlings, and the improving effects of brassinolide on these indices exhibited no significant differences among the 3 treatments. In 20 g kg^{-1} polluted soil, only 0.1 and 0.3 mg L^{-1} brassinolide treatments significantly ($P< 0.05$) increased the F_v/F_m of seedlings. All 3 brassinolide treatments significantly increase the Φ_{PSII}, *qP* and *ETR* of the seedlings, while the 0.3 mg L^{-1} brassinolide treatment exhibited the most significant improving effects ($P< 0.05$).

DISCUSSION

Previous studies demonstrated that brassinolide can effectively remit the stomata limitations caused by stressful environments. For instance, Fariduddin et al. (2009) indicated that brassinolide can increase the G_s and C_i of *Brassica juncea* in Cu stress conditions, Zou (2001) reported that the brassinolide treated *Zea mays* exhibits a significantly higher P_n in drought conditions. Similarly with the aforementioned literatures, our results indicated that brassinolide treatments could significantly increase the stoma opening (the G_s increased by 14.79%-38.17%) and accelerate the entrance of CO_2 into plant tissue (the C_i increased by 10.04%-24.69%), and thus increase the photosynthetic rate (13.72%-38.54%), which indicated that brassinolide could effectually counteract the stomatal limitation caused by petroleum stress. However, the effects of brassinolide were relatively weaker at the 0.5 mg L^{-1} concentration. Because brassinolide at low concentration can accelerate the biosynthesis of glutath-

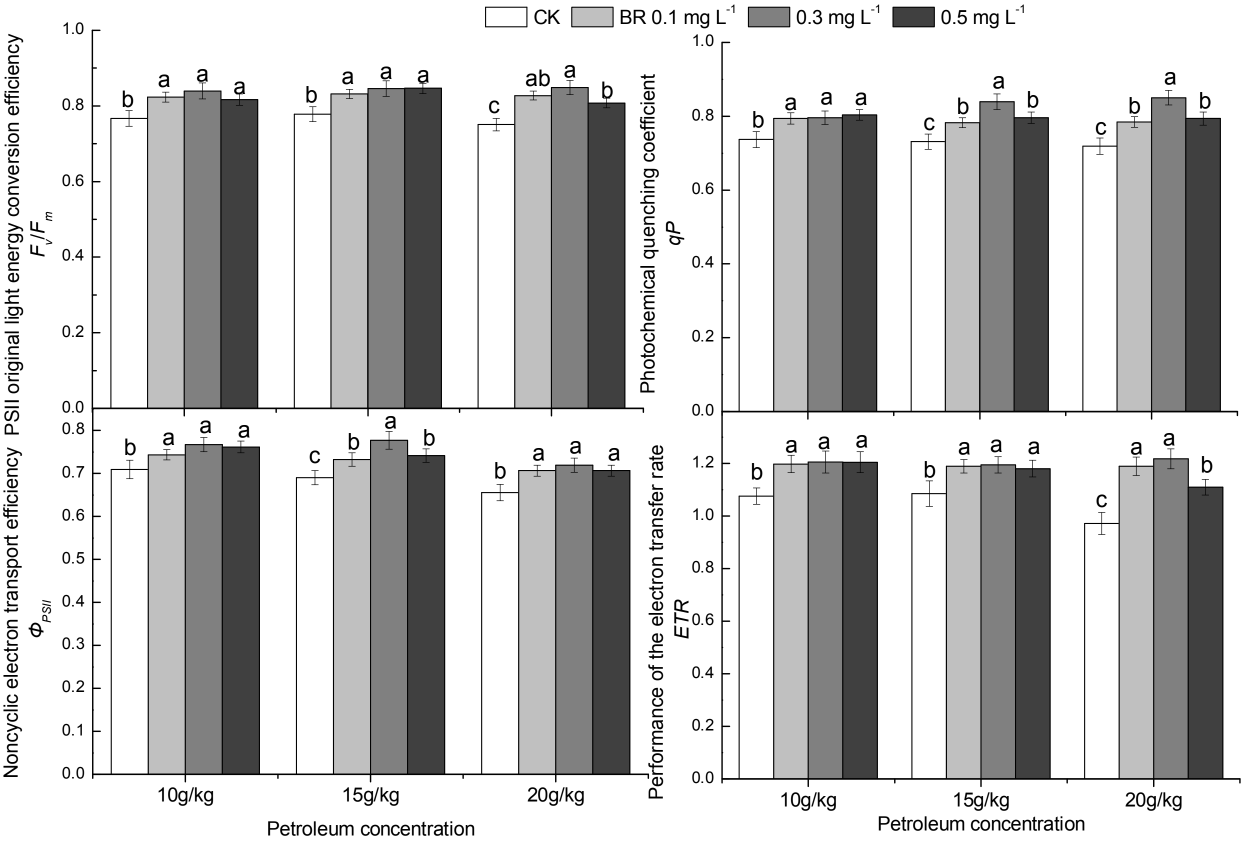

Fig. 2: Response of chlorophyll fluorescence characteristics of seedlings to brassinolide treatments in petroleum polluted soil.

ione, change the redox status of cells and thus promoting the stomatal opening (Gao 2012). In addition, brassinolide at low concentration can also inhibit the inducing effects of abscisic acid (ABA) to prevent the stomatal closure (Gao 2012). In contrast, brassinolide with a high concentration can accelerate the biosynthesis of ABA and thus promote the stomatal closure (Gao 2012). Hence, in this study, the best improving effects of brassinolide on gas exchange abilities occurred on the seedling treated by the 0.3 mg L^{-1} brassinolide solution.

Except for the aforementioned stomata factors, petroleum stress can also inhibit the photosynthesis by non-stomata factors, that is, by inhibiting the carboxylation of mesophyll cells (such as RuBP activity, photosynthetic electron transport, photophosphorylation and RuBP regeneration). Many studies have demonstrated that petroleum pollution can cause significant injury to the chloroplast, increase the initial fluorescence, and decrease the contents of photosynthetic pigments, the maximal fluorescence and the PSII photochemical efficiency, and thus hinder the photosynthesis (Chaîneau et al. 2003; Lin et al. 2002; Lu et al. 2009). Previous studies have demonstrated that brassinolide can recover the decreased PSII photochemical efficiency, PSII noncyclic electron transport efficiency and photochemical quenching in the stressful conditions (Dong et al. 2008; Hu et al. 2006), our results were inconsistent with these studies. As shown in Fig. 2, the F_v/F_m of seedlings was decreased to approximately 0.80 in the petroleum polluted soil, while after being treated by brassinolide, the F_v/F_m increased to approximately 0.85, which indicated that the light energy converting efficiency was significantly improved, and almost eliminated the photoinhibition caused by petroleum (Kong et al. 2011). In addition, the qP increased by 4.46%-9.37%, which demonstrated that more light energy can be used in photochemical reactions, thus increased the photosynthesis potentiality of the seedlings. Furthermore, the Φ_{PSII} and ETR were also increased by 4.49%-15.61% and 9.95%-21.29% after the brassinolide treatment respectively, these indicated that the photosynthetic electron transport was promoted, and the carboxylation ability of mesophyll cells was improved as well (Baker 2008). That might be caused by (1) the inducing effects of brassinolide on photosynthetic gene expression, in addition, brassinolide can increase the activity of Rubisco to increase the carboxylation rate, and thus increase the assimilation rates (Xia et al. 2009); (2) brassinolide can inhibit the excessive accumulation of reactive oxygen species by stimulating the antioxidant enzyme activities to prevent the peroxidating damages of photosynthetic apparatus (Ahammed et al. 2012b); (3) brass-inolide can increase the contents of photosynthetic pigment in stressful conditions (Hasan et al. 2011; Hayat et al. 2012).

In general, the improving effects of brassinolide on the gas exchange abilities (especially the G_s and C_i) of *R. pseudoacacia* seedlings were more obvious than its effects on the carboxylation capacity of mesophyll cells, that is, brassinolide mainly counteracts the stresses of petroleum on *R. pseudoacacia* by adjusting the stomatal limitation factors. Of course, the promoting effects brassinolide on the integrated photosynthetic capacity can significantly accelerate the growth of *R. pseudoacacia* seedlings, the results of this study might support the previous findings of us (Han et al. 2015b).

CONCLUSIONS

In polluted soil with different petroleum concentrations, 0.1-0.5 mg L^{-1} could significantly increase the gas exchange abilities (P_n, T_r, G_s and C_i) and the chlorophyll fluorescence characteristics (F_v/F_m, Φ_{PSII}, qP and ETR) of the *R. pseudoacacia* seedlings. Hence, brassinolide treatment could counteract the stomatal limitation caused by petroleum stress, accelerate the light energy conversion efficiency and the carboxylation ability of mesophyll cells. In general, brassinolide could promote the photosynthesis of *R. pseudoacacia* seedlings in petroleum stress conditions. Comprehensively considering the effects of brassinolide on the photosynthesis indices of seedlings, 0.3 mg L^{-1} was the favourable concentration to be used for counteracting the adverse effects of petroleum pollution on *R. pseudoacacia*.

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