ORIGINAL ARTICLE

Plant growth regulators improve nitrogen metabolism, yield, and quality of soybean–rhizobia symbiosis

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Abstract

Purpose In recent years, considerable research has been conducted on the use of plant growth regulators (PGRs) to improve crop yields. Large amounts of PGRs are applied to regulate crop growth. However, few studies have assessed the impact of PGRs, which leave soil residues, on soil microorganisms, especially rhizobia.

Methods In this study, the influence of inoculation of soybean roots with *Sinorhizobium fredii* and *Bradyrhizobium japonicum* exposed to gibberellin A3 (GA$_3$) and mepiquat chloride (MC) on nodule number, nitrogenase activity, and a symbiotic nitrogen fixation gene (*fixA*) expression was studied. The nitrate content, free amino acids, and nitrate reductase activity of the roots, and seed yield and quality of soybean were measured.

Result Nodulation was promoted to some extent, whereas nitrogenase activity and *fixA* expression were inhibited to some extent by treatment with GA$_3$; however, these effects were reversed by treatment with MC. In a pot experiment to study phenotypic characteristics, rhizobia treatment in combination with GA$_3$ or MC significantly improved soybean yield and seed nitrogen content, and increased the root nitrate content, free amino acid content, and nitrate reductase activity.

Conclusion The results indicated that PGRs, which leave soil residues, have significant positive effects on the growth and activity of soybean and rhizobia.

Keywords Plant growth regulator, Rhizobia, Nitrogen metabolism, Soybean, Gibberellins, Mepiquat chloride

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Significance and impact of study

Although plant growth regulators (PGRs) are important factors in regulating legume growth and development

Limited information is available on their effects on legume symbioses with rhizobia. in this study

The impact of the PGRs gibberellin A3 (GA$_3$) and mepiquat chloride (MC) on rhizobia-induced effects in soybean was examined. an understanding of the effects of PGR residues on the soybean–rhizobia symbiosis will be helpful in the evaluation of soil ecological security and rational application of these regulators

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Introduction

The legume–rhizobia symbiosis is a specialized plant–microbial symbiotic system in the rhizosphere that helps to supply nitrogen nutrition to the host plant. Legumes can provide a stable environment and energy for microbes. It has been reported that 50–60% of nitrogen nutrition in soybean stems from its symbiosis with rhizobia (Rellán-Alvarez et al. 2010). Nitrogenases, a group of metalloenzymes, play a crucial role in the rhizobia–legume symbiosis (Dixon and Kahn 2004). Normally, rhizobia nitrogenase consists of two components: the homodimeric Fe protein and the tetrameric Mo–Fe protein, which contains the Mo–Fe cofactor. Many studies have reported that the nitrogenase activity of rhizobia is significantly influenced by external factors. A high concentration of NO_3^-, Fe^{2+}-free (LeVier et al. 1996), or Cu^{2+}-free (Maas et al. 1979) can suppress the nitrogenase activity of nodules in different manners. It has been found that indole acetic acid (IAA), which is secreted by rhizosphere microbes, can improve the nitrogen-fixation ability, thereby increasing the dry weight and yield of sorghum (Ashraf et al. 2011). Therefore, it is important to consider how to improve the nitrogenase activity to increase soybean yield. As early as the 1980s, some researchers found that host plants could secrete flavonoids when infected by rhizobia, which can induce the synthesis and release of nod factors (NFs) in the rhizobia. Many studies have shown that plant growth regulators (PGRs) regulate plant nodulation and nitrogen metabolism. For example, ethylene (ET), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), and brassinolide (BR) negatively affect nodule formation (Tirichine et al. 2007). The root and shoot dry weights, nodule number, nodule weight, nodule nitrogen content, and nitrogenase activity of soybean were improved significantly after foliar spray application of BR and SHK-6 (a type of novel plant growth regulator, the main component is diethyl aminoethyl hexanoate mepiquat). It was also found that the nitrogenase activity of soybean roots was increased by cytokinin (CTK)/gibberellic acid (GA) and CTK/IAA treatments, while the effect of IAA/ABA and GA/ABA treatments was the opposite. It has been shown that pea mutants deficient in GA_3 biosynthesis could not form nodules with rhizobia, but the addition of exogenous GA_3 in moderation can restore nodule formation (Ferguson et al. 2005). Moreover, it has been suggested that in aluminium (Al)-treated roots, nitrate reductase (NR) activity is increased but the IAA concentration is maintained at the same level as in the pretreatment (Tomioka et al. 2012). However, indole-3-acetyl-L-aspartic acid (IA-Asp), which is a metabolic intermediate of IAA degradation, is not detected in the roots. In calcium-treated roots, NR activity and IA-Asp concentration increases, but the IAA concentration decreases. It has been found that the activity of NR, nitrite reductase, glutamine synthetase, and glutamate synthase decreases significantly (Gangwar et al., 2011); however, the ammonium content and activity of glutamate dehydrogenase are increased by chromium and 100 µM IAA treatments in pea seedlings. The effects of PGRs on rhizobia cells in free-living cultures have also been confirmed. It has been reported that 0.1% D1 (a synthetic phytohormone analogue) suppresses bacterial growth (Kosenko et al. 2001; 2003); however, bactozole (a plant growth stimulant of bacterial origin) at different concentrations (0.001%, 0.01%, and 0.1%) exerted similar effects on the growth of bacteria when grown under a low nitrate concentration (6 mM). There is also evidence for the role of conventional PGRs in nodule structure (Brewin 1993). PGRs play an important role in regulating the grain-filling process and protein accumulation. Yang et al. (2013) demonstrated that the grain-filling rate is positively and significantly dependent on the contents of GA, IAA, and ABA and that the content of free amino acids and activity of glutamine synthetase is dependent on the contents of ABA and GA. In addition, the content of soluble proteins is significantly affected by the contents of ABA and IAA (Yang et al. 2013). Thus, it could be concluded that exogenous hormones can affect the grain-filling process and nitrogen metabolism characteristics by changing the contents of endogenous hormones.

Several genes and proteins have been identified as essential for symbiotic nitrogen fixation by the bacterium *Sinorhizobium meliloti* and are termed symbiotic nitrogen fixation (*fix*) genes. *Fix* genes were first identified in *S. meliloti* (Mueller and Gonzalez 2010) and later in *Bradyrhizobium japonicum*, *Azorhizobium caulinodans* (Wu 2012), *Rhizobium leguminosarum* bv. *viciae* (Elsayed et al. 2013), *R. leguminosarum* bv. *trifolii* (Miller et al. 2007), and *R. leguminosarum* bv. *phaseoli* (Dombrecht et al. 2002). Mutations in any one of the *fixA/B/C/X* genes of *R. meliloti*, *B. japonicum*, or *A. caulinodans* completely abolish nitrogen fixation. It has been proposed that *fixA/B/C/X* gene products may be involved in electron transport to nitrogenase (Dai et al. 2014). In a study using mutant plants, it has been suggested that, in addition to the electron transfer flavoprotein, the *fixA* locus is required for symbiotic efficiency (Delmotte et al. 2014). It has been shown that application of IAA and 2,4-dichlorophenoxyacetic acid in a symbiotic environment can significantly increase the mRNA transcript levels of *fix* genes in *S. meliloti* (Bianco, 2010).

In our previous work, we confirmed that PGRs could change the growth rate and structure of rhizobia cells in free-living cultures. However, the effect of PGRs on the influence of rhizobia on nitrogen metabolism, yield,

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and quality of soybean remains unclear. In this study, we investigated the effect of soybean root inoculation with *S. fredii* and *B. japonicum* exposed to GA_3 and MC on nodule number, nitrogenase activity, *fixA* expression, nitrate content, free amino acid content, NR activity, and seed yield and quality.

Materials and methods

Rhizobial strains and PGRs

Two strains of rhizobia, *Bradyrhizobium japonicam* (strain number GIM 1.94) and *Sinorhizobium fredii* (strain number GIM1.227), were obtained from the China Agricultural Culture Collection Center (ACCC). GA_3 and MC were obtained from our laboratory. Uniform soybean "Suinong 28" seeds were obtained from YiBin University.

Culture media

Yeast mannitol agar (YMA) liquid medium was prepared. The nitrogen-free nutrient solution contained 0.15 g $Na_2HPO_4·2H_2O$, 0.1 g $CaCl_2·2H_2O$, 0.12 g $MgSO_4·7H_2O$, 0.1 g K_2HPO_4, 5 mg ferric citrate, and 1 mL minor elements and diluted with distilled water to 1000 mL, sterilized at 121 °C for 15 min. The stock of minor elements was prepared using 2.86 g H_3BO_3, 2.03 g $MnSO_4·4H_2O$, 0.22 g $ZnSO_4·7H_2O$, 0.13 g $Na_2MoO_4·2H_2O$, and 0.08 g $CuSO_4·5H_2O$ and diluted with distilled water to 1000 mL. The GA_3 mother solution comprised 10.0 g GA_3 diluted with absolute ethyl alcohol (100 mL) and was stored at 4 °C until use. The MC mother solution contained 10.0 g MC diluted with distilled water to 100 mL and was stored at 4 °C until use.

Soybean pot experiments

Plant growth regulator GA_3 and MC mother solutions at 10^{-3} and 10^{-2} (*v/v*) concentrations, respectively, were added to the YMA liquid medium, then *S. fredii* and *B. japonicum* were inoculated and cultured at 28 °C with shaking at 170 rpm for 3 days. The strains in each treatment were collected after centrifugation of the 1 mL bacterial solution at $8000 \times g$ for 10 min, rinsed with sterile water to remove PGRs in the YMA liquid medium, and then suspended in 1 mL sterile water. Soybean seeds were surface-sterilized with 70% ethanol (*v/v*) for 5 min followed by extensive rinsing with sterile water. The surface-sterilized seeds were inoculated individually with 1 mL bacterial suspension, then planted in an autoclaved mixture (1:1) of perlite and vermiculite in pots (25 cm diameter × 30 cm height). Each pot contained three plants and was supplied daily with 500 mL nitrogen-free nutrient solution. Each treatment was replicated five times. Plants were grown in an artificial climate with sunlight and 60% relative humidity at 28 °C.

Root nodule number and nitrogenase activity in acetylene reduction assay

Plants were carefully uprooted from 08:00 to 09:00 during the soybean pod stage, and the root systems were rinsed thoroughly with sterile water. Nodules of similar number and size in each treatment were counted and detached carefully, rinsed with sterile water, and pooled. Ten nodules, selected at random from the pooled sample, were placed in serum bottles. After removal of 10% of the volume of air from the flask and injection with an equal volume of C_2H_2 30 min after removing the reaction gases, a gas chromatograph (Agilent 7890A) was used to determine the amount of C_2H_4 (Kurz, et al., 1975). Each treatment was replicated five times.

Real-time PCR assay for fixA gene expression

Total RNA of rhizobia in the collected nodules (as described in the preceding section) was extracted using the QIAGEN RNeasy Mini Kit. Reverse transcription was performed using the TransScript II First-Strand cDNA Synthesis Super Mix in accordance with the manufacturer's instructions. All reagents were treated with DEPC on ice to ensure that the entire process was performed on a clean bench. The cDNA was reverse-transcribed and diluted 30 times as a template for the real-time PCR experiment. The PCR mixture was prepared to a total volume of 12.5 µL and included 6.25 µL Eco GreenI fluorescent dye MIX, 0.25 µL forward primer, 0.25 µL reverse primer, 2 µL cDNA first reaction product, and 3.5 µL ddH_2O. The *fixA* gene primers used were forward 5′-CTATGATCTGTTCGCGCTTG-3′ and reverse 5′-AGACGATATCGGGCGTACC-3′. The reference gene primers were forward 5′-CCTACGGGAGGCAGCAG-3′ and reverse 5′-ATTACCGCGGCTGCTGG-3′. Amplifications used the following program: 95 °C for 5 min, 1 cycle; 95 °C for 15 s, 55 °C for 30 s, 5 cycles. After the amplification, melting curve analysis was performed.

Nitrogen metabolism-related indicators

Root samples were collected and frozen in liquid nitrogen for 1 h and then stored at −40 °C. The frozen samples were used for determination of physiological indicators. Free amino acids were determined using the ninhydrin method (Gerhard et al. 2002), nitrate-nitrogen was determined using the salicylic acid method (Ratushnyak et al. 2011), and NR was determined using the reference method (Yin et al. 2015).

Soybean yield and quality assessment

The yield and quality of soybean were assessed by measuring plant height, pod number per plant, seed number

per plant, and 100-seed weight. The nitrogen content of the seeds was determined using the Kjeldahl method.

Data analysis

Data processing and mapping were performed using Microsoft Excel 2003, *fixA* relative gene expression levels were analysed using SDS 2.3 software, and the SPSS statistical analysis system was used for analysis of variance and significance tests.

Results and discussion

Effects of PGRs and rhizobia on nodule number and nitrogenase activity

Leguminous plants can form a symbiotic relationship with rhizobium and capture nitrogen in the atmosphere to provide to host plants by forming nodules in the roots. Plant hormones play a key role in this process. GA_3 is a kind of plant hormone known to be involved in a variety of biological processes (such as cell growth and germination). Previous studies have also shown that gibberellin is involved in the formation and maturation of root nodules in legumes (Liu H et al., 2018). To study the effects of PGRs on nodulation and nitrogen fixation of soybean infected by rhizobia, GA_3 and MC were incorporated separately in combination with *S. fredii* and *B. japonicum* in the culture medium. Inoculation with GA_3–*S. fredii* and GA_3–*B. japonicum* increased the nodule number of soybean roots by 12.9% and 10.5% compared with the control, respectively, but the increase was not significant. Many studies have found that plant hormones such as ethylene, jasmonic acid, abscisic acid, and gibberellin can negatively regulate the formation of infection lines and the development of root nodules (Li et al., 2022; Velandia et al., 2022; Lin et al., 2020). Exogenous application of GA_3 can restore the phenotypic changes of these plants,

suggesting that the formation of root nodules in leguminous plants depends on the effect of GA_3. However, in vitro studies have shown that high concentrations of GA_3 above the threshold level will in turn inhibit nodulation (Ferguson et al. 2005). These data suggest that the dependence of nodulation on GA_3 is regulated by its concentration. It was also reported that exogenous GA_3 treatment inhibited ammonium nitrate-induced root hair deformation, infection line formation and nodule development, and the application of GA_3 biosynthesis inhibitors saved this phenotypic change. The results showed that GA_3 played a negative role in the formation of infection lines and nodule development (Maekawa et al., 2009; Fonouni-Farde et al., 2016; Hung et al., 2016). In contrast, inoculation with MC–*S. fredii* and MC–*B. japonicum* decreased the nodule number of soybean roots by 15.8% and 5.6% compared with the control, respectively (Fig. 1A).

Although the differences were statistically non-significant, the results were consistent. This study represented a preliminary exploration and the sample size was relatively small. Therefore, a larger sample size in a future study will provide a better understanding of the effect of PGRs on nitrogen fixation by rhizobia and infected legumes.

In contrast to the nodule number, the nitrogenase activity of soybean roots inoculated with GA_3–*S. fredii*, GA_3–*B. japonicum*, MC–*S. fredii*, or MC–*B. japonicum* were decreased by 11.1% and 5.9% and increased by 18.1% and 6.2%, respectively, compared with the control. However, the changes in nitrogenase activity were not significant (Fig. 1B). These findings were consistent with previous studies. There are many potential reasons for the differences being not statistically significant. The main reason may be that the sample size was small and
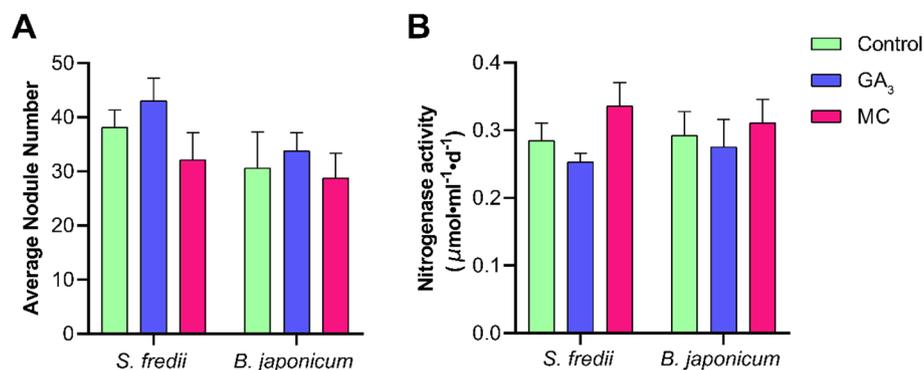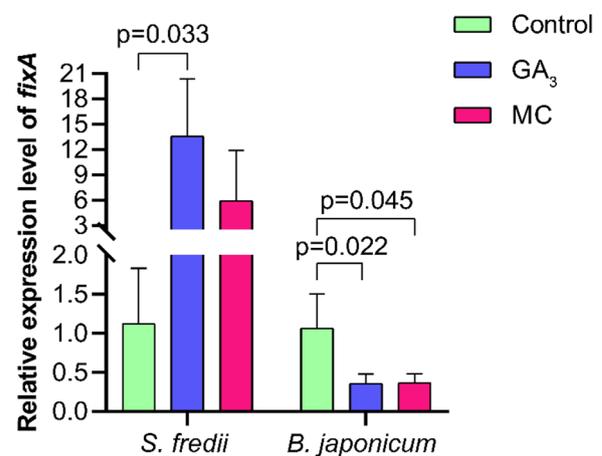

Fig. 1 Effects of plant growth regulators (PGRs) and rhizobia on nodule number and nitrogenase activity. **A** Influence of PGRs (gibberellin A3 [GA_3] and mepiquat chloride [MC]) and inoculation with rhizobia (*Sinorhizobium fredii* and *Bradyrhizobium japonicum*) on the nodule number of soybean roots. **B** Influence of rhizobial inoculation combined with PGRs (GA_3 and MC) on the nitrogenase activity of soybean roots. Each bar is the mean of eight measurements of nodule number and nitrogenase activity. Student's *t* test was used to evaluate the difference between the control and the treatments

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that variation among individuals may have impacted on the statistical results. Previous studies have shown that the growth rate and cell structure of free-living rhizobia cultures may be regulated by GA₃ and MC, and the nodule number and nitrogenase activity of soybean roots were affected after inoculation with the rhizobia. It has been observed that *R. japonicum* and *R. phaseoli* differ in their responses to PGRs in a manner depending on the type and concentration (Stearn et al. 1980). Furthermore, it has been demonstrated that auxin, which is produced by growth-promoting bacteria, significantly increases nitrogenase activity (Egamberdiyeva et al., 2004). In contrast to previous studies, to avoid the interference of plant secretions and exogenous sources that were produced by the applied rhizobia, in the present study rhizobia were applied simultaneously with PGRs. The present study showed that nodulation of soybean roots was promoted by inoculation with GA₃–rhizobia to varying degrees, but nitrogenase activity in the soybean roots was inhibited. The opposite results were observed for MC. This might be because the expression of certain regulatory signals of rhizobia is stimulated or inhibited by PGRs. It has been demonstrated previously that expression of the receptor for activated protein C kinase 1 mRNA, which is responsible for nodule meristem initiation and rhizobia infection, is induced by PGRs (namely, auxins, ABA, CTK, and GA) (Islas-Flores et al., 2012). In a previous study, *S. meliloti* wild-type cells were treated with 0.5 mM IAA or a derivative *S. meliloti* strain that overproduced IAA (RD64) was used. *Medicago truncatula* plants inoculated with RD64 (*Mt*-RD64) showed increases in acetylene reduction activity and root dry weight (Imperlini et al. 2009).

Effect of PGRs–rhizobia on *fixA* gene expression

The expression levels of *fixA* from *S. fredii* were significantly upregulated by GA₃ and MC treatment compared with that of the *S. fredii* control (Fig. 2). However, *fixA* was significantly downregulated by MC treatment in combination with *B. japonicum*. It is evident that PGRs have different effects on the transcription level of *fixA* among different rhizobia, and the present experiments showed that this effect is significant. In previous studies, we have preliminarily showed that GA₃ and MC have the same effect on the level of nodulation nitrogen fixation among rhizobia, although not with statistical support. The difference in *fixA* transcription level shows the specificity of PGRs. If studies with a large sample size support this conclusion, the mechanism of PGR-specific regulation of the *fixA* transcription level should be investigated in future studies. Previous research has demonstrated that the operon formed by the *fixA*, *fixB*, *fixC*, and *fixX* genes (Earl et al. 1987) is essential for nitrogen fixation
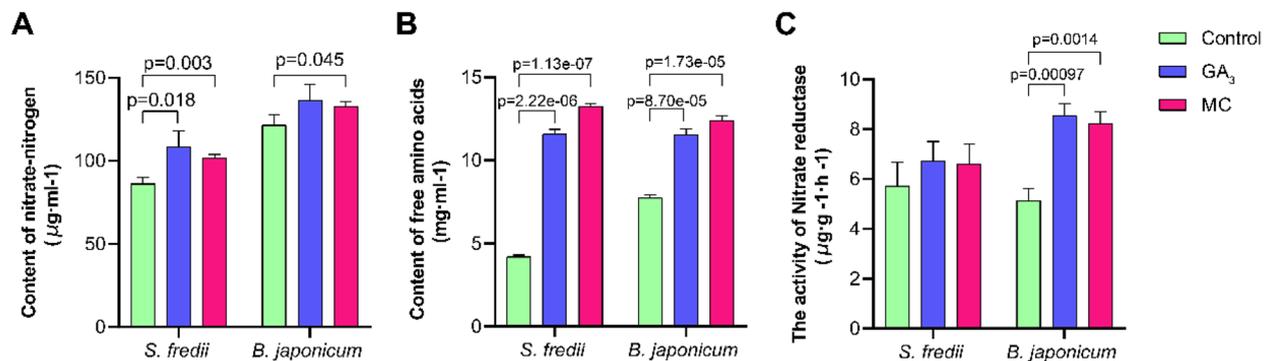

Fig. 2 Effect of plant growth regulators in combination with inoculation with *Sinorhizobium fredii* or *Bradyrhizobium japonicum* on the *fixA* transcription level in soybean. The results shown are representative of three independent experiments. Error bars represent the standard error of the mean

by *S. meliloti* and that homologous genes are present in *Sinorhizobium* and *Bradyrhizobium*. The present results showed that expression of *fixA* of *S. fredii* was stimulated by GA₃, but significantly inhibited by MC in *B. japonicum*. Wheatley et al. (2020) also found not only 27 genes were annotated as *nif* and *fix* in *Rhizobium leguminosarum*, but also 603 genetic regions (593 genes, 5 transfer RNAs, and 5 RNA features) related to nitrogenase activity and symbiotic nitrogen fixation using mariner-based transposon insertion sequencing. This demonstrated that the nitrogenase activity of soybean roots is regulated by PGRs by regulation of the expression of symbiotic nitrogen-fixation genes in the rhizobia. However, differences between the nitrogenase activity and the expression of *fixA* were observed.

Effect of PGR–rhizobia on nitrogen metabolism-related indicators

As the main form of nitrogen absorbed by plants, the content of nitrate-nitrogen in soybean roots was increased compared with that of the control in all PGR–rhizobia treatments (Fig. 3A). Inoculation with GA₃–*S. fredii* and MC–*S. fredii* significantly ($P < 0.05$) increased the nitrate–nitrogen content of soybean roots by 26.0% and 18.1%, respectively. Inoculation with MC–*B. japonicum* significantly increased the nitrate-nitrogen content in soybean roots by 12.3%, whereas inoculation with GA₃–*B. japonicum* increased the nitrate-nitrogen content by 9.3% but this was not statistically significant.

Free amino acid content is an important indicator of the nitrogen content and nutrient metabolism in plants. Inoculation with GA₃–*S. fredii* and MC–*S. fredii*

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Fig. 3 Effect of plant growth regulators and rhizobia on nitrogen metabolism-related indicators. **A** Content of nitrate nitrogen. **B** Content of free amino acids. **C** Activity of nitrate reductase. Each bar is the mean of three independent experiments. Student's *t* test was used to evaluate the difference between the control and the treatments

significantly ($P < 0.05$) increased the free amino acid content in soybean roots by 175.3% and 215.4%, respectively. In addition, the inoculation with GA_3–*B. japonicum* and MC–*B. japonicum* significantly ($P < 0.05$) increased the content of free amino acids in soybean roots by 49.0% and 60.2%, respectively (Fig. 3B).

Nitrate reductase is a rate-limiting enzyme involved in the nitrate assimilation process in plants. Inoculation with GA_3–*S. fredii* and MC–*S. fredii* increased the NR activity by 17.4% and 15.5%, respectively. However, inoculation with GA_3–*B. japonicum* and MC–*B. japonicum* significantly ($P < 0.05$) increased NR activity, respectively (Fig. 3C).

Nitrate and free amino acids are the main substances involved in physiological metabolism in the roots of plants and play an important role in the regulation of plant metabolism. The nitrate metabolic rate was determined based on the activity of NR in plants. Salem et al. (2017) analyzed the phenotype of the mutant of raptor1b, a protein related to TOR1B regulation. The results showed that the mutant of raptor1b led to delayed seed germination and poor resistance to exogenous stress. At the same time, studies at the molecular level showed that these phenotypic changes were accompanied by an increase in the level of free amino acids and a decrease in protective secondary metabolites and storage proteins. In addition, the levels of plant hormones also changed significantly, and the levels of abscisic acid, auxin, and jasmonic acid increased significantly. The supply of exogenous gibberellin could restore the above phenotypic and molecular changes. Wang et al. (2023) found that nitrogen application can promote nitrogen absorption and utilization by increasing the expression of genes related to the absorption and transport of NH_4^+ and NO_3^- and increasing the activities of nitrate reductase

and glutamine synthetase in rice. At the same time, it was also found that nitrogen could affect the levels of GA_3 and ABA by regulating the synthesis and metabolism of GA_3 and ABA. Evensen et al. (1981) showed that the nodule strains lacking nitrate reductase were inoculated with Lima bean. Compared with the control group, they all responded to exogenous GA_3. After 3–5 weeks of inoculation, the response of the control group to GA_3 was weakened, while that of the NR deficient strain did not change. After the application of GA3 biosynthesis inhibitor, the height of Lima bean inoculated with NR deficient strain decreased by 20%, while that of the control group was affected. In addition, it was also found that Lima bean inoculated with NR-deficient strain would form nodules, and the content of extractable gibberellic acid analogues was 4–50 times higher than that of the control group. Previous studies have shown that the root activity and NR of soybean are increased by 2-*N*,*N*-diethyl aminoethyl hexanoate (DTA-6) and (DTA-6)–GA_3 treatment (He et al., 2014; Hassan et al., 2021). However, in the present study, the accumulation and output of free amino acids were increased by GA_3, whereas the nitrate content and NR activity were reduced. It has been shown that the nitrogen fixation ability of soybean roots is well maintained by inoculation with different rhizobia under salt, drought, acid, alkaline, nutrition, fertilizer, pesticide, and heavy-metal stresses (Zahran 1999). The contents of seed protein and amino acids and NR activity of soybean are increased significantly in response to treatment with rhizobia with nitrogen and phosphorus fertilizers (Sital et al. 2011). The NR activity in rhizobia is largely suppressed by cadmium (Bianucci et al. 2013). In the present study, we speculated that the efficiency of root uptake and accumulation of nitrogen were improved with increase in the number of root nodules increased or nitrogenase activity of rhizobia.

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Table 1 Effect of PGRs and rhizobia on yield components, yield, and growth quality of soybean

Groups	Plant height (cm)		Pods per plant		Grains per plant		Total N (%)	
	AVE ± SD	P	AVE ± SD	P	AVE ± SD	P	AVE ± SD	P
S. fredii								
Control	37.9 ± 3.6		13.6 ± 2.6		22.1 ± 2.4	/	7.4 ± 0.01	
GA_3	46.6 ± 3.8	0.000891	18.4 ± 2.6	0.004131	32.6 ± 6.4	0.00604	7.5 ± 0.03	0.005064
MC	44.3 ± 3.4	0.005578	19.1 ± 2.8	0,002,185	31.1 ± 6.9	0.01310	7.5 ± 0.03	0.039737
B. japonicum								
Control	40.0 ± 3.1		12.6 ± 1.3		15.3 ± 2.7		7.0 ± 0.01	
GA_3	44.2 ± 3.0	0.023268	16.9 ± 2.2	0.562065	19.1 ± 3.4	0.037503	7.4 ± 0.02	3.027E − 06
MC	45.6 ± 2.7	0.003607	17.4 ± 2.8	0.005278	21.7 ± 2.6	0.000636	7.4 ± 0.03	2.563E − 05

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Effect of PGRs–rhizobia on seed yield and quality of soybean

Inoculation with GA$_3$–rhizobia or MC–rhizobia significantly increased the plant height, number of pods per plant, seed number per plant, and total nitrogen content of soybean (Table 1). Inoculation with GA$_3$–*S. fredii* treatment increased the plant height and number of seeds per plant by 22.9% and 47.1% compared with the *S. fredii* control, respectively. Inoculation with MC–*S. fredii* increased the number of pods per plant by 41.1% and 19.7% compared with the *S. fredii* control, respectively. The total quality of soybean seeds was increased by inoculation with GA$_3$–rhizobia or MC–rhizobia. Inoculation with GA$_3$–*B. japonicum* or MC–*B. japonicum* significantly increased the total nitrogen content of the soybean seeds by 5.1 or 6.0% compared with the *B. japonicum* control, respectively.

As a type of protein-rich food, the yield and quality of soybean seeds are intimately linked with nitrogen metabolism and thus is affected by many external factors. We found that the yield components and quality of soybean were regulated by inoculation with PGRs–rhizobia. These results indicated that nitrogen metabolism in soybean roots was regulated by inoculation with rhizobia under treatment with GA$_3$ and MC, thereby affecting the yield and quality of soybean; however, the mechanism was not clear. Roy Choudhury et al. (2019) have shown that plant hormones such as abscisic acid and jasmonic acid have an overall inhibitory effect on nodulation in leguminous plants, while plant hormones such as gibberellin and brassine steroids play a positive role in promoting nodulation. Tatsukami et al. (2016) showed that the hosts inoculated with *M. loti* mutants with insufficient GA$_3$ synthesis formed more nodules 4 weeks after inoculation than those inoculated with wild type, which indicated that GA$_3$ from added rhizobium could prevent the formation of new nodules. These studies partly explain some of the conclusions of this study that the regulatory mechanism between legume growth and nodulation induced by plant hormones needs to be further studied. In the follow-up work, we will focus on the role of plant hormones as a bridge between plant growth and nodulation.

Conclusion

The present study examined the effects of PGRs on the growth and physiological activity of soybeans and rhizobia by simulating PGR soil residues. It was indicated that a suitable concentration of PGRs played an active role in soybean production. Interestingly, the regulation differed when soybeans were inoculated with different genera of rhizobia in combination with PGRs. This difference might be due to physiological diversity among rhizobium genera, which requires further tests.

Availability of data and materials
The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References

Ashraf MA, Rasool M, Mirza MS (2011) Nitrogen fixation and indole acetic acid production potential of bacteria isolated from rhizosphere of sugarcane (*Saccharum officinarum* L.). Adv Biol Res 5(6): 348–355.

Bianco C, Defez R (2010) Auxins upregulate *nif* and *fix* genes. Plant Signal Behav 5(10):1290–1294

Bianucci E, Fullana C, Furlan A, Castro S (2013) Antioxidant defense system responses and role of nitrate reductase in the redox balance maintenance in *Bradyrhizobium japonicum* strains exposed to cadmium. Enzyme Microb Technol 53(5):345–350

Brewin NJ (1993) The Rhizobium-legume symbiosis: plant morphogenesis in a nodule. Semin Cell Biol 4(2):149–156

Dai Z, Guo X, Yin H, Liang Y, Cong J, Liu X (2014) Identification of nitrogen-fixing genes and gene clusters from metagenomic library of acid mine drainage. PLoS ONE 9(2):e87976

Delmotte N, Mondy S, Alunni B, Fardoux J, Chaintreuil C, Vorholt JA, Giraud E, Gourion B (2014) A proteomic approach of *Bradyrhizobium/Aeschynomene* root and stem symbioses reveals the importance of the *fixA* locus for symbiosis. Int J Mol Sci 15(3):3660–3670

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2(8):621–631

Dombrecht B, Tesfay M, Verreth C, Heusdens C, Nápoles M, Vanderleyden J, Michiels J (2002) The *Rhizobium etli* gene *iscN* is highly expressed in

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bacteroids and required for nitrogen fixation. Mol Genet Genomics 267(6):820–828

Earl CD, Ronson CW, Ausubel FM (1987) Genetic and structural analysis of the *Rhizobium meliloti fixA, fixB, fixC,* and *fixX* genes. J Bacteriol 169(3):1127–1136

Egamberdiyeva D, Hoeflich G (2004) Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. J Arid Environ 56(2):293–301

Elsayed BB, Hassan MM, Ramady EHR (2013) Phylogenetic and characterization of salt-tolerant rhizobial strain nodulating faba bean plants. African Journal of Biotechnology 12(7).

Evensen KB, Blevins DG (1981) Differences in Endogenous Levels of Gibberellin-Like Substances in Nodules of Phaseolus lunatus L. Plants Inoculated with Two Rhizobium Strains. Plant Physiol 68(1):195–198

Ferguson NM, Cummings DA, Cauchemez S, Fraser C, Riley S, Meeyai A, Iamsirithaworn S, Burke DS (2005) Strategies for containing an emerging influenza pandemic in Southeast Asia. Nature 437(7056):209–214

Fonouni-Farde C, Diet A, Frugier F (2016) Root Development and Endosymbioses: DELLAs Lead the Orchestra. Trends Plant Sci 21(11):898–900

Gangwar S, Singh VP (2011) Indole acetic acid differently changes growth and nitrogen metabolism in *Pisum sativum* L. seedlings under chromium (VI) phytotoxicity: Implication of oxidative stress. Scientia Horticulturae 129(2): 321–328.

Gerhard H, Peter Z, Gert B (2002) Plant species affect the concentration of free sugars and free amino acids in different types of soil. J Plant Nutr Soil Sci 165(5):557–565

Hassan M, Israr M, Mansoor S, et al (2021) Acclimation of cadmium-induced genotoxicity and oxidative stress in mung bean seedlings by priming effect of phytohormones and proline. PLoS One 16(9):e0257924

He S, Wu Q, He Z (2014) Synergetic effects of DA-6/GA$_3$ with EDTA on plant growth, extraction and detoxification of Cd by Lolium perenne. Chemosphere 117:132–138

Hung CY, Qiu J, Sun YH, et al (2016) Gibberellin deficiency is responsible for shy-flowering nature of Epipremnum aureum. Sci Rep 6:28598

Imperlini E, Bianco C, Lonardo E, Camerini S, Cermola M, Moschetti G, Defez R (2009) Effects of indole-3-acetic acid on *Sinorhizobium meliloti* survival and on symbiotic nitrogen fixation and stem dry weight production. Appl Microbiol Biotechnol 83(4):727–738

Islas-Flores T, Guillén G, Sánchez F, Villanueva MA (2012) Changes in RACK1 expression induce defects in nodulation and development in Phaseolus vulgaris. Plant Signal Behav 7(1):132–134

Kosenko LV, Krugova ED, Mandrovskaia NM, Okhrimenko SM (2001) Effect of plant growth stimulators on *Rhizobium leguminosarum* BV. VICIAE 263b and efficiency of symbiotic nitrogen-fixation in peas. Mikrobiolohichny Zhurnal 63(5): 59–66.

Kosenko LV, Mandrovskaya NM, Krugova ED, Varbanets LD (2003) The effect of the plant growth stimulant bactozole on *Rhizobium leguminosarum* bv. viciae 250a and its nitrogen-tolerant mutant M-71 under different nitrogen supply conditions. Microbiology 72(1), 30–36.

Kurz WGW, LaRue TA (1975) Nitrogenase activity in rhizobia in absence of plant host. Nature 256(5516):407–409

LeVier K, Day DA, Guerinot ML (1996) Iron uptake by symbiosomes from soybean root nodules. Plant Physiol 111(3):893–900

Li Y, Pei Y, Shen Y, et al (2022) Progress in the Self-Regulation System in Legume Nodule Development-AON (Autoregulation of Nodulation). Int J Mol Sci 23(12):6676

Lin J, Frank M, Reid D (2020) No Home without Hormones: How Plant Hormones Control Legume Nodule Organogenesis. Plant Commun 1(5):100104

Liu H, Zhang C, Yang J, Yu N, Wang E (2018) Hormone modulation of legume-rhizobial symbiosis. J Integr Plant Biol 60(8):632–648

Maas MR, Post FJ, Salunkhe DK (1979) Effect of inorganic nutrients on production of steroid glycoalkaloids by *Phytophthora infestans* race 1.2.4 In Vitro. J Food Prot 42(1): 27–30.

Maekawa T, Maekawa-Yoshikawa M, Takeda N, Imaizumi-Anraku H, Murooka Y, Hayashi M (2009) Gibberellin controls the nodulation signaling pathway in Lotus japonicus. Plant J 58(2):183–194

Miller SH, Elliot RM, Sullivan JT, Ronson CW (2007) Host-specific regulation of symbiotic nitrogen fixation in *Rhizobium leguminosarum* biovar trifolii. Microbiology 153(9):3184–3195

Mueller K, Gonzalez JE (2010) Complex regulation of symbiotic functions is coordinated by MucR and quorum sensing in *Sinorhizobium meliloti*. J Bacteriol 193(2):485–496

Ratushnyak AP, Vi A, Ki A, Trushin MV (2011) The effect of nitrate nitrogen and salicylic acid on aerenchyma formation in *Typha angustifolia* grown in mesocosms. Plant Root 5:25–30

Rellán-Alvarez R, Andaluz S, Rodríguez-Celma J, Wohlgemuth G, Zocchi G, Alvarez-Fernández A, Fiehn O, López-Millán AF, Abadía J (2010) Changes in the proteomic and metabolic profiles of *Beta vulgaris* root tips in response to iron deficiency and resupply. BMC Plant Biol 10(1):1–15

Roy Choudhury S, Johns SM, Pandey S (2019) A convenient, soil-free method for the production of root nodules in soybean to study the effects of exogenous additives. Plant Direct 3(4):e00135

Salem MA, Li Y, Wiszniewski A, Giavalisco P (2017) Regulatory-associated protein of TOR (RAPTOR) alters the hormonal and metabolic composition of Arabidopsis seeds, controlling seed morphology, viability and germination potential. Plant J 92(4):525–545

Sital JS, Kaur K, Sharma S, Sandhu JS, Singh S (2011) Effect of *Rhizobium* inoculation, phosphorus and nitrogen supplements on protein quality in developing lentil (*Lens culinaris*) seeds. Indian J Agricult Biochem 24:17–22

Stearn WC, Miller RH, Cakmakci L (1980) Possible effects of PGRs on competitiveness of *Rhizobium* spp.

Tatsukami Y, Ueda M (2016) Rhizobial gibberellin negatively regulates host nodule number. Sci Rep 6:27998

Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 315(5808):104–107

Tomioka R, Takenaka C, Maeshima M, Tezuka T, Kojima M, Sakakibara H (2012) Stimulation of root growth induced by aluminum in *Quercus serrata* Thunb. Is related to activity of nitrate reductase and maintenance of IAA concentration in roots. American J Plant ences 3(11): 1619.

Velandia K, Reid JB, Foo E (2022) Right time, right place: The dynamic role of hormones in rhizobial infection and nodulation of legumes. Plant Commun 3(5):100327

Wang H, Zhong L, Fu X, et al (2023) Physiological analysis reveals the mechanism of accelerated growth recovery for rice seedlings by nitrogen application after low temperature stress. Front Plant Sci 14:1133592

Wheatley RM, Ford BL, Li L, Aroney ST, Knights HE, Ledermann R, Easta AK, Ramachandrana VK, Poole PS (2020) Lifestyle adaptations of *Rhizobium* from rhizosphere to symbiosis. Proc Natl Acad Sci 117(38):23823–23834

Wu L (2012) Mutants of the hybrid sensor kinase CstA affect cyst cell development in *Azospirillum brasilense* Sp7. Afr J Microbiol Res 6(2):348–354

Yang W, Cai T, Li Y, Guo J, Peng D, Yang D, Yin Y, Wang Z (2013) Effects of exogenous abscisic acid and gibberellic acidonfilling process and nitrogen metabolism characteristics in wheat grains. Aust J Crop Sci 7(1):58–65

Yin X, Liang X, Zhang R, Yu L, Xu G, Zhou Q, Zhan X (2015) Impact of phenanthrene exposure on activities of nitrate reductase, phosphoenolpyruvate carboxylase, vacuolar H$^+$-pyrophosphatase and plasma membrane H$^+$-ATPase in roots of soybean, wheat and carrot. Environ Exp Bot 113:59–66

Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63(4):968–989

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