Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°5, 995-1003
DOI: 10.24205/03276716.2020.1096

Effect of Gibberellin and Mepiquat Chloride on Cell Growth and Structure of Rhizobia

Wenhao Chen[a], Ling You[b], Tao Wang[c], Qin Wei[d], Marwan M. A. Rashed[e]

ABSTRACT

Plant growth regulators (PGRs) which have characteristics of improving crop resistance are used in agricultural production widely. However, there is rarely related research about PGRs on phenotype and molecular mechanism of free-living rhizobia. This is a key issue in evaluating the ecological role of PGRs. A comparative study is conducted on the effect of different concentration (10^{-1}-10^{-6} v/v) of gibberellin (GA_3) and mepiquat chloride (PIX) on the cell growth, pH, microstructure and *nod* gene expression of *Ensifer fredii* and *Bradyrhizobia japonicum*. The results show that the cell growth rate, pH and expression level of *nod*A and *nod*D are accelerated, reduced and up-regulated respectively, when adding GA_3 10^{-3} v/v into the culture medium of *E. fredii* and *B. japonicum*. However, the cell growth rate, pH and expression level of *nod*A and *nod*D are decelerated, increased and down-regulated respectively, when adding 10^{-2} v/v PIX into the culture medium of *E. fredii* and *B. japonicum*. Meanwhile, the microstructure of *E. fredii* and *B. japonicum* is altered respectively by GA_3 and PIX. It is indicated that PGRs could change a series of rhizobia cell phenotype through regulating expression of *nod* gene. These findings establish a foundation for understanding of PGRs in soil ecological security evaluation and rational application.

Keywords: Rhizobia, Plant growth regulators, *nod* gene, microstructure

INTRODUCTION

Plant growth regulators (PGRs), exogenously applied to improve crop production, stress resistance, and overall crop quality, have played an increasingly important role in agriculture. The more common PGRs include Gibberellins (GA_3), Mepiquat chloride (PIX), Indoleacetic acid (IAA) and Cytokinins (CTK) (Alayón-Luaces *et al.* 2012, Rademacher 2018). Foliar application of GA_3 is often used in various crops to stimulate growth and development (Chandran *et al.*, 2020), for instance, plant cell, stem, and stalk elongation (Sharifuzzaman *et al.* 2011); leaf enlargement (Maggio *et al.* 2010); early crop maturation, breaking of seed dormancy, seed germination (Gupta and Chakrabarty 2013); leaf abscission and increases in dry weight and yield (Arora *et al.* 2012). Conversely, PIX, a growth retardant, is used to weaken terminal buds, inhibit cell elongation and reduce overall plant vigor (Chen *et al.* 2015, Jaidka 2016). It has also been revealed to regulate color properties of leaves, photosynthetic ability, resists cold, drought and saline-alkali soil (Yang *et al.* 2019, Zhao *et al.* 2019). Most PGRs had been demonstrated that possess a long residual period, which exerted an important impact on a variety of soil microbial activities (Singh and Bhattacherjee 2005).

Nodulation (*nod*) gene, which located in the sym-plasmid of rhizobia, the major function were regulating cell growth of free-living rhizobia, forming and exchanging of diffusible signal molecules at initial of symbiotic plant-nodules (Guasch-Vidal *et al.* 2013). It was including that the shared *nod* genes (*nod*ABC), regulatory genes (*nod*D) and the host-specificity of nodulation genes (*hsn*), e.g. *nod*H. Thereinto, *nod*ABC with highly homology are functionally interchangeable between different rhizobia species, and they are required to induce the plant root hairs curling and meristematic cells differentiation (Liu *et al.* 2018). The reason why *nod*ABC are named common *nod* gene is because they are structurally conserved, and the function of host will not be changed by *nod*ABC exchanged during each rhizobia species. If *nod*ABC inactivation, the induction of mutualistic symbiosis on plant of rhizobia will be lost, including the curling of plant root hairs, the formation of infection threads, differentiation of meristematic

[a]Scientist (84460462@qq.com), [b]Associate Professor (23686037@qq.com), [c,d]Professor (289615848@qq.com, weiqin2001-67@163.com), [e]Assistant Professor(m72rashed@yahoo.com)
College of Life Science and Food Engineering, Yibin University, Yibin 644 000, P.R. China
Solid- state Fermentation Resource Utilization Key Laboratory of Sichuan Province, Yibin 644000, Sichuan, China

cells and the formation of root nodules (Okazaki *et al.* 2016). The *nod*D is transcribed divergently from the *nod*ABC operon. Beside this operon, *nod*D activates the transcription of all three other nodulation operons (Banfalvi *et al.* 1988). Mulligan and Long (1985) had confirmed that *nod*D was essential for the transcriptional activation of shared *nod* genes. The most important nodulation genes *nod*ABC and *nod*D, as well as the main promoter region between them, had been analyzed in details.

Although much work has been conducted with use of compounds in leguminous crops, the treatment of PGRs is daily foliar application or soaking seeds in most research. Therefore, it is not rule out that the formation of root nodule was affected by root exudates such as *nod* gene. Little is known about PGRs effects on growth, *nod* gene-expressing and cell morphology-changing of free-living rhizobia. Many studies have provided evidence for the effect of PGRs on formation of nodule, for instance, supplementing rhizobia with L-Tryptophan could be an effective way for improving growth, nodulation and production of legumes (Zahir *et al.* 2010). And some flavonoids act as antagonists (anti-inducers) of *nod* gene transcriptional activation that is triggered by inducing flavonoids (Peck *et al.* 2006). So, whether the PGRs with the same effect on flavonoids? These data suggest the importance of further exploring the role such compounds have in host-bacterium relationships in leguminous crops. To understand such, we need to specifically determine the direct effect of such compounds on rhizobia cell.

MATERIALS AND METHODS

Materials

Rhizobia samples: *Ensifer fredii* (GIM 1.227) and *Bradyrhizobia japonicum* (GIM 1.94) acquired from the Agricultural Culture Collection of China. PGRs samples: GA_3 and PIX from the Life Science and Food Engineering College, Yibin University (Yibin, China).

Culture media: yeast-malt extract agar (YMA)

3.0 g yeast, 10.0 g mannitol, 0.1 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.25 g K_2HPO_4, 0.25 g KH_2PO_4, 20.0 g agar, diluted with DI water to 1,000 mL, sterilized at 121℃ for 20 min. YMA liquid medium: Same as YMA without agar. GA_3 solution: weigh accurately 10.0 g GA_3 in a 100 mL volumetric flask, diluted with absolute ethyl alcohol. PIX solution: weigh accurately 10.0 g PIX in a 100 mL volumetric flask, diluted with sterile water.

Cell growth and pH of free-living rhizobia

Mother solution of GA_3 and PIX were added into YMA liquid medium, and the concentration would be 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} v/v, respectively. Then 5% aliquots of bacterial solution of *E. fredii* and *B. japonicum* were inoculated and cultured on sterilized 40 mL of YMA liquid medium with GA_3 and PIX of different concentrations at 28℃ using a 170 rpm shaker. The cell growth and pH of rhizobia were determined by spectrophotometer (VIS-723G) at 600 nm and compact pH meter B-212 (NORIBA) at time points as follows: 2 h, 4 h, 8 h, 16 h, 24 h, 36 h, 48 h, 72 h, and 96 h, 3 times of repetition.

Microstructure analysis

E fredii and *B japonicum* colonies were first excised and trimmed to approximately 10 mm × 10 mm specimens (1-2 mm, as thin as possible). Each specimen was fixed in a solution containing (2.5% glutaraldehyde). After fixation the specimen was washed repeatedly with double-distilled sterile water to remove salt crystals. The specimen was dehydrated in different concentration of ethanol (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 96%, each change 5 min) and subsequently in HMDS (hydroxyl mexamethyl disilazane). After drying, the specimen was placed to scanning electron microscope (SEM) stubs by double-sided conductive tape and sputter-coated with gold. The specimen was examined by SEM (Hitachi S3400N); the acceleration tension was 5.0 kV (Ho *et al.* 2015).

E. fredii and *B. japonicum* were collected by centrifugation at 8000 rpm. Then the bacteria that were fixed for 20 min in the 2% glutaraldehyde in 0.1M PBS were washed with distilled water (×6). Then the bacteria were stained with 2% uranyl acetate for 5 min and washed with distilled water (×3). The stained bacteria were exposed to osmium tetraoxide for 5 min and the excess osmium tetraoxide was then discarded. The dehydration of the exposed bacteria to osmium tetraoxide was conducted in different concentration of ethanol (50%, 70%, 90%, and 100%, each change 5 min). Polymerization was done with pure epoxy resin in the embedding oven at 75℃/2 h and 90℃/2 h, after the bacteria were infiltrated by mixture of acetone and epoxy resin (1:1) for 15 min. The blocks were trimmed to 90 nm, ultra-thin sections and mounted on 200-mesh, thin-bar copper grids (Agar). Each specimen was examined by transmission electron microscope (TEM) (Hitachi H600) at an accelerating voltage of 100 kV (Tan *et al.* 2017). The length and width of the bacteria were measured by TEM, and an SPSS significance test was performed.

RT-qPCR assay for expression of nodA and nod

Total RNA was extracted with the RNeasy Mini kit (QIAGEN, Hilden, Germany). Finally, the concentration of total RNA was quantified at OD_{260}. cDNA was synthesized with the TransScript Ⅱ First Strand cDNA SynthesisSuperMix (TRANSGEN, China): cDNA was made from 8 μL total RNA (1000 μg/μL) in a volume of 20 mL containing 1 μL oligo (dT18), 10 μL 2×TS Ⅱ RT Reaction Mix and 1 μL TS Ⅱ RT Enzyme Mix for centrifuging 30 s followed by 30 min at 42°C and 5 min at 85°C. The PCR was performed with 2 μL of the cDNA solution in 0.5 μL primer (10 μM) and 6.25 μL Eco GreenI fluorochrome MIX (TRANSGEN) in a total volume of 12.5 μL. The 16S rDNA V3 region gene which was amplified by universal oligonucleotide primer (338F, 518R) was used to normalize levels. The primers used for RT-qPCR are listed in Table 1. RT-qPCR (95℃ for 5 min, 40 cycles at 95℃ for 15 s, 55℃ for 30 s) was performed with ABI PRISM 7000 (Applied Biosystems, USA). The ΔΔCT (threshold cycle) method of comparing expression data was applied and the relative quantitative value was expressed as $2^{-\Delta\Delta CT}$. The specificity of the amplification was confirmed with the presence of a single peak in a dissociation curve. All reactions were done in triplicate. SDS version 2.3 was used for Data analysing.

Table 1. **Primers used for RT-qPCR**

Primer name	Primer sequence (5'-3')	Fragment length
nodA F	CGCAGCGGAATCTACGAG	226 bp
nodA R	GGTCGATTACGGCCTTAGGT	226 bp
nodD F	ATCTGTTGGTGGCGGAACT	242 bp
nodD R	CGGCAGGTTGAGATAGACATC	242 bp
338 F	CCTACGGGAGGCAGCAG	180 bp
518 R	ATTACCGCGGCTGCTGG	180 bp

RESULTS AND DISCUSSION

Effect of PGRs on the cell growth of rhizobia

Fig. 1 showed that the cell growth of *E. fredii* and *B. japonicum* increased in GA_3 solutions over control from 10^{-3} v/v to 10^{-6} v/v. The absorbance reached 2.32 and 2.15 with the 10^{-3} v/v GA_3 of *E. fredii* and *B. japonicum*, respectively. The absorbance was higher than other treatments. However, the absorbance was very low when the concentration of GA_3 was 10^{-1} v/v and 10^{-2} v/v (Fig. 1 A and C). It revealed that the optimum concentration of GA_3 could induce an increase in the amount of rhizobia. The cell growth of *B. japonicum* was reduced to varying degrees for all the concentrations of PIX over the control except 10^{-1} v/v. The cell growth rate of *B. japonicum* was lower than other treatments. The absorbance of *B. japonicum* reached 1.57, which was the lowest value compared to other treatments when the concentration of PIX was 10^{-2} v/v (Fig 1 D). With these results, we chose the concentration of 10^{-3} v/v GA_3 and 10^{-2} v/v PIX, respectively, as the optimum concentration for the following experiment. It is interesting to note that the cell growth of *B. japonicum* did not change significantly with the PIX except 10^{-1} v/v over the control (Fig. 1 B). Explaining such differences could prove an interesting subject for further research.

Although PGRs were applied intensively, comprehensive results of its biological impact on rhizobia were very limited. In our study, *E. fredii* and *B. japonicum* were selected as test strains due to their distinguishing physiological features of when grown in YMA, and nodulated with plant hosts subsequently. Our results indicated that the cell growth rate of rhizobia was significantly induced following exposure to a GA_3 concentration of 10^{-3} v/v, whereas lower or higher concentrations had either negative or inhibitory effects, respectively. These data confirm that presented in Hasan (2014) in which GA and IAA also induced a significant increase in rhizobia growth. Differences in nodulation responses in field-grown leguminous crops may therefore be related to genotypic differences in endogenous gibberellins production within these crops, further confounding data observed following foliar application of such compounds. PIX had been previously demonstrated to suppress vegetative growth (Wang *et al.* 2014). However, our study exposed free-living rhizobia with 10^{-2} v/v PIX, inducing a significant deceleration in the quantity, nod gene expression and growth-rate. To our knowledge, this was the first report of the direct effect of PIX on free-living rhizobia.

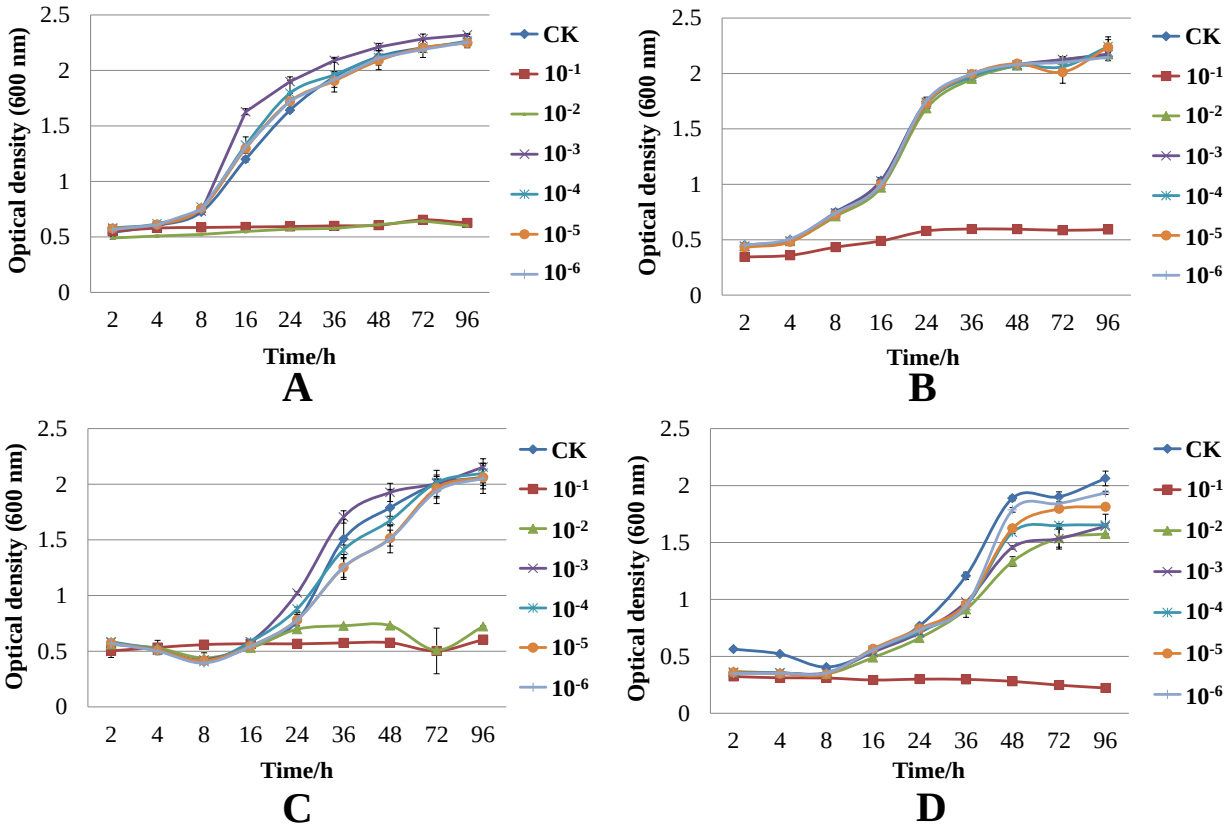


Figure 1. **Cell growth of rhizobia in different concentration PGRs.A: Effect of different concentration GA_3 on *E. fredii* cell growth. B: Effect of different concentration PIX on *E. fredii* cell growth. C: Effect of different concentrations GA_3 on *B. japonicum* cell growth. D: Effect of different concentrations PIX on *B. japonicum* cell growth.**

Effect of PGRs on pH of rhizobia

As shown in Fig. 2 A and C, within 96 h, the pH was less than 6 when the concentration of GA_3 was 10^{-1} v/v and 10^{-2} v/v. However, the pH of *E. fredii* and *B. japonicum* under 10^{-3} v/v GA_3 stabilized between 6 and 7, and the rate of pH decrease was faster than other treatments including control. The pH of *E. fredii* and *B. japonicum* under 10^{-3} v/v GA_3 reached the lowest point at 36 h, 5.9 and 7, respectively, and then remained stable. This is one reason why the GA_3 induced an increase in the amount of rhizobia. As Fig. 2 B and D indicated, within 96 h, the pH was consistently above 7 when the concentration of PIX was 10^{-1} v/v. Inhibition of cell growth is evident above pH 7 as shown in Fig. 1 B and D. The pH of *E. fredii* and *B. japonicum* increased to varying degrees for other treatments of different concentrations of PIX over control throughout the 96 h growth period. During testing, the pH of *E. fredii* and *B. japonicum* under 10^{-2} v/v PIX reached the highest point at 16 h, 7.2 and 7.8, then fell gradually. Even so, the pH was still higher than for other treatments at the end of culture. This was considered one reason why PIX inhibited cell growth of rhizobia.

Among the many soil properties that influenced the growth rate of rhizobia, soil pH was of fundamental importance. As we known, very acidic or very alkaline environments were inhibitory to both nodulation and subsequent growth of the rhizobia, and the optimum pH of the growth of rhizobia was between 6 and 7 (Głodowska *et al.* 2017). Furthermore, pH also had a role in inducing expression of *nod* genes, and on the structures of nodulation factors (Andrés *et al.* 2012). In our study, cell growth rate of rhizobia accelerated following addition of GA_3, which also resulted in a rapid decrease of pH. Conversely, when exposed to PIX the cell growth rate of rhizobia decelerated, subsequently increasing pH. Many rhizobia strains, IAA producing, isolated from the rhizosphere by revealed that these species produce relatively high amounts of IAA during growth in the proper pH (Mohite 2013, Shoukry *et al.* 2018). It will likely be of value to explore these phenomena in greater detail in subsequent studies.

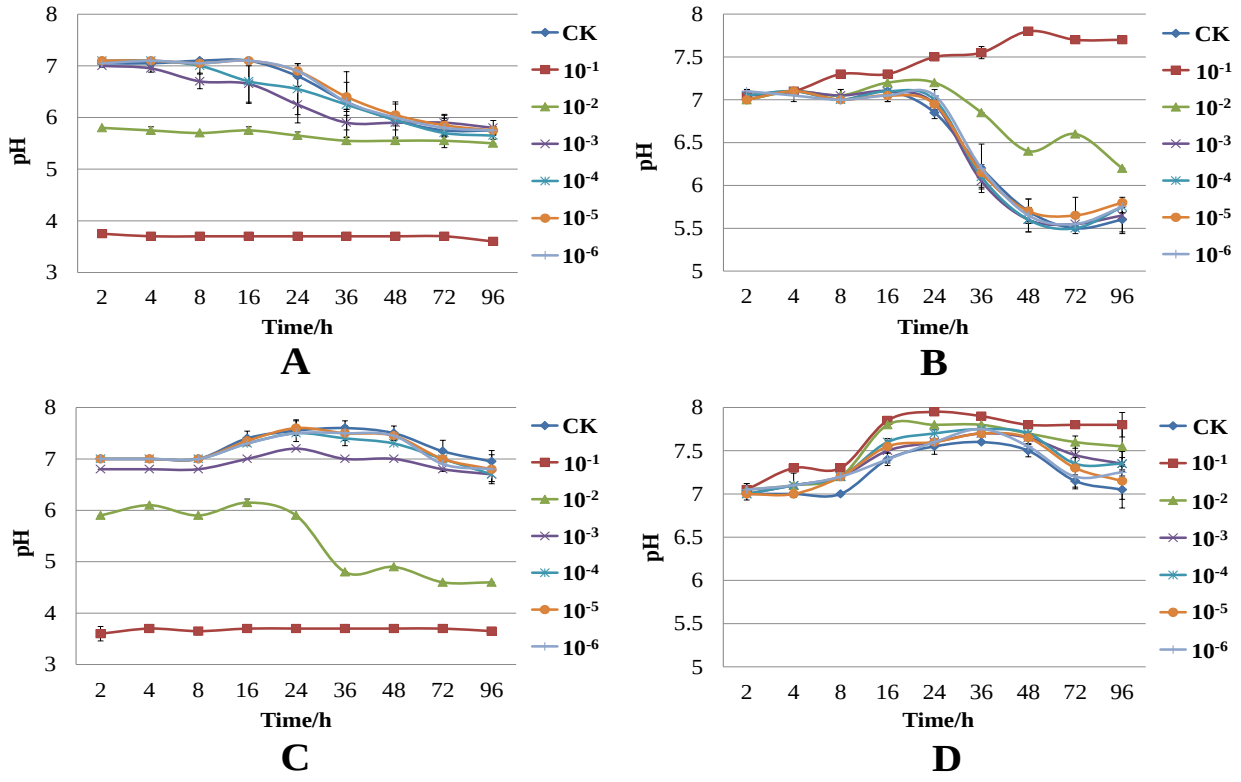


Figure 2. **pH during the process of rhizobia culturing in different concentration PGRs. A: Effect of different concentration GA_3 on pH of E. fredii. B: Effect of different concentration PIX on pH of E. fredii. C. Effect of different concentration GA_3 on pH of B. japonicum. D. Effect of different concentration PIX on pH of B. japonicum.**

Effect of PGRs on microstructure of rhizobia

Most of *E. fredii* under 10^{-3} v/v GA_3 were round (Fig. 3 B) and the quantity of polyhydroxybutyrate granules (Fig. 4 PHB) was induced to increase by GA_3. Results showed that the length of *E. fredii* cells decreased from 2793 nm to 1928 nm and the width increased from 684 nm to 1240 nm by inoculating 10^{-3} v/v GA_3, which reached a very significant level ($P < 0.01$) in Table 3. The change of width from 684 nm to 1007 nm in *E. fredii* cells by inoculating 10^{-2} v/v PIX was very significant ($P < 0.01$). However, the length increase of *E. fredii* cells from 2793 nm to 3023 nm was less remarkable at 5% and 1% level of probability (Table 2). As Fig. 3 A and C showed, both end of *E. fredii* cells under 10^{-2} v/v PIX were sharper than control. That may be because large amounts of exopolysaccharides (Fig. 4 EPS) were induced on the cell surface by PIX, thereby altering the shape. This also explained the phenomenon that the absorbance at 600 nm did not changed markedly compared with the control. In addition, the quantity of PHB in *E. fredii* was reduced by PIX, as shown in Fig. 4 A, C. The effect of PGRs on *B. japonicum* was different from *E. fredii*. As shown in Figs. 3 and Table 3, the length of *B. japonicum* cells increased and the width decreased with 10^{-3} v/v GA_3, the change in length from 2246 nm to 2844 nm was very significant ($P < 0.01$). The width change from 815 nm to 719 nm was less remarkable at 1% level of probability. However, the size of almost all *B. japonicum* cells with 10^{-2} v/v PIX was quite small compared with the control (Fig. 3 D, F). The change of length from 2246 nm to 1607 nm based on Table 3 was very significant at 5% and 1% level of probability, but the width change from 815 nm to 829 nm was less remarkable.

Previous research had indicated that the phytohormone gibberellin promoted plant growth by stimulating cellular expansion, and the growth retardants cycocel and mepiquat chloride inhibited cell elongation. Most scholars have drawn similar conclusions (Olanrewaju *et al.* 2017, Rademacher 2018, Small and Degenhardt 2018). In addition, it was of interest to us that based on the transmission electron micrographs the amount of PHB in *E. fredii* was changed by PGRs. There had been no such reports to date although elevated levels of NADPH significantly enhanced PHB accumulation by using *Ralstonia eutropha* (Choi *et al.* 2003). The effect of different PGRs on rhizobia merits further study.

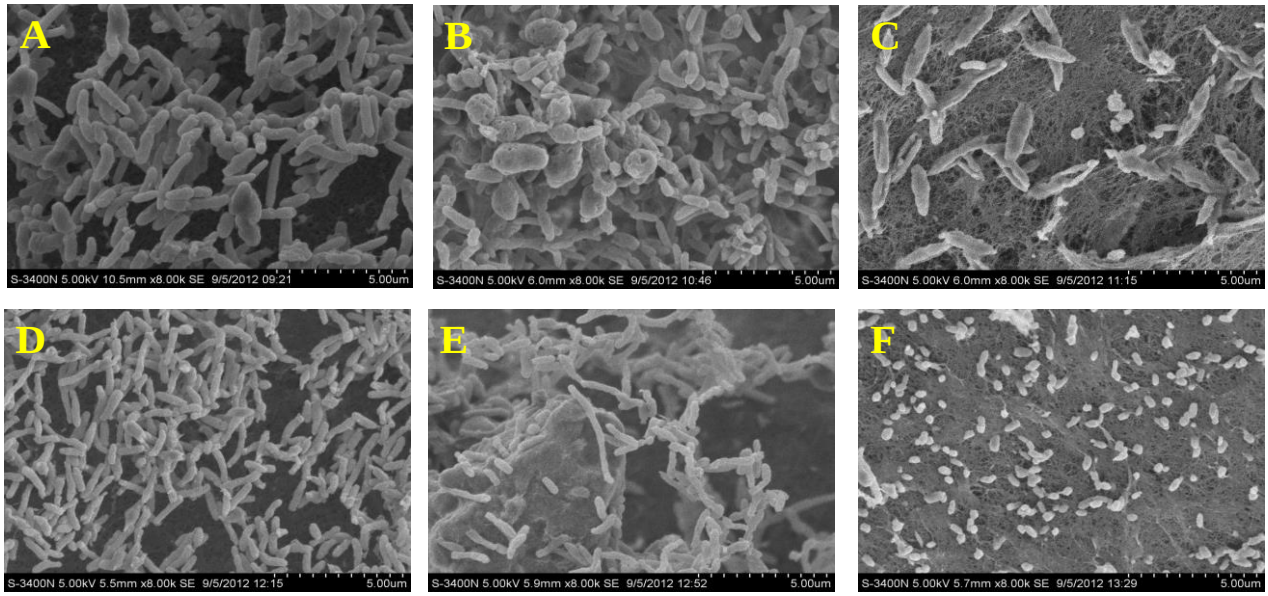
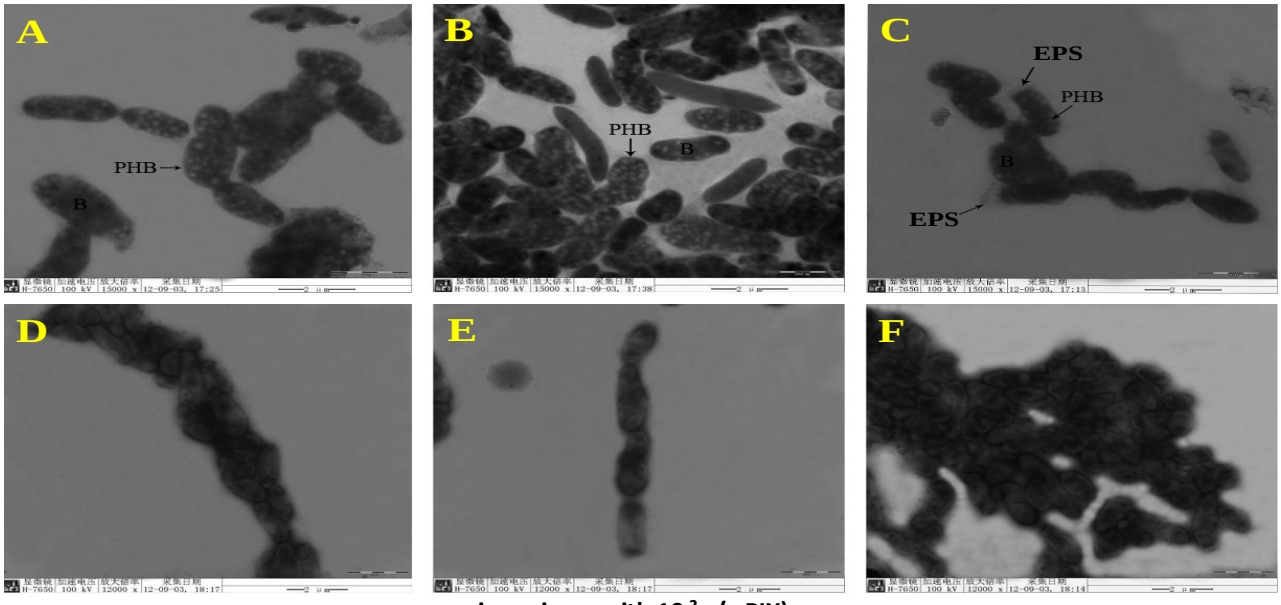


Figure 3. **Scanning electron micrograph of rhizobia in different concentration PGRs (A: Microstructure of E. fredii, B: Microstructure of E. fredii with 10^{-3} v/v GA_3, C: Microstructure of E. fredii with 10^{-2} v/v PIX, D: Microstructure of B. japonicum, E: Microstructure of B. japonicum with 10^{-3} v/v GA_3, F: Microstructure of B.**



japonicum with 10^{-2} v/v PIX)

Figure 4. **Transmission electron micrograph of rhizobia in different concentration PGRs (A: Microstructure of E. fredii, B: Microstructure of E. fredii with 10^{-3} v/v GA_3, C: Microstructure of E. fredii with 10^{-2} v/v PIX, D: Microstructure of B. japonicum, E: Microstructure of B. japonicum with 10^{-3} v/v GA_3, F: Microstructure of B. japonicum with 10^{-2} v/v PIX, PHB: β-polyhydroxybutanic acid granule, EPS: exopolysaccharides)**

Table 2. **Effect of different concentration GA_3 and PIX on length and width of *E. fredii* and *B. japonicum* cell**

Treatment	Length (L) or Width (W) -rhizobia			
	L- *E. fredii* (103 nm)	W- *E. fredii* (103 nm)	L- *B. japonicum* (103 nm)	W- *B. japonicum* (103 nm)
CK	2.79±0.15 bB	0.68±0.13 aA	2.25±0.26 bB	0.82±0.05 bA
10-3 v/v GA3	1.93±0.35 aA	1.24±0.13 cC	2.84±0.17 cC	0.72±0.08 aA
10-2 v/v PIX	3.02±0.41 bB	1.01±0.12 bB	1.61±0.19 aA	0.83±0.06 bA

Values followed by a different lower- or uppercase letter within each column are

significantly different at 0.05 and 0.01 probability levels respectively, using Duncan's multiple range test.

Effect of PGRs on nod gene expression

As *nod* gene was involved in the growth of cell and development of nodule formation, the relative transcript abundance of *nod*A and *nod*D were determined. To assay the selectivity of RNA extracted from rhizobia, the presence of spurious amplification product was checked by qualitative analysis of melting curves on the amplicon. Only single melting main peaks with the same Tm (75.4℃ for *nod*A, 89.5℃ for *nod*D) were scored from all assays in Fig. 5, it was indicate that the spurious amplification product was inexistent. The cell growth and nodule formation of rhizobia was believed to be regulated by PGRs. To search for PGRs with a similar regulated pattern on *nod* gene of rhizobia, we analyzed the expression level of the *nod*A and *nod*D genes at 96 hours after inoculation (the cell growth of both *E. fredii* and *B. japonicum* in the most vigorous stage) with 10^{-3} v/v GA_3 and 10^{-2} v/v PIX respectively. From Fig. 6, the level of *nod*A and *nod*D expression of *E. fredii* and *B. japonicum* under 10^{-3} v/v GA_3 were up-regulated, but not significantly. It was indicated that the *nod* gene expression could be induced by proper concentration of GA_3. Conversely, the situation of 10^{-2} v/v PIX was down-regulated, thereinto, the suppressive effects of *nod*A and *nod*D expression of *E. fredii* was not significantly. But, *nod*A and *nod*D expression of *B. japonicum* under 10^{-2} v/v PIX were almost not detected (Fig. 6 C). It was also indicated that the *nod* gene expression could be inhibited by proper concentration of PIX.

Islas-Flores *et al.* (2011) had found that expression of PvRACK1 which responsible for rhizobia infecting and nodule meristem initiating was regulated by PGRs. Our results suggested the expression level of *nod*A and *nod*D in 10^{-3} v/v GA_3 treated free-living *E. fredii* and *B. japonicum* were inordinately up-regulated. It was directly demonstrated that the cell growth of rhizobia were significantly induced following exposure to the *nod* gene of rhizobia overexpression were induced by the suitable concentration of GA_3. However, the expression of *nod* gene was regulated by PIX negatively, which associated with the decrease of the rhizobia growth. Interestingly, the expression of *nod*D of free-living cultures *B. japonicum* under 10^{-2} v/v PIX was rarely, consequently, the expression of *nod*A was suppressed (*nod*A expression regulated by *nod*D) (Podlešáková *et al.* 2013). However, *B. japonicum* still grew in the culture medium. These results suggest that *nod* gene was not the only factor that affected the growth of rhizobia.

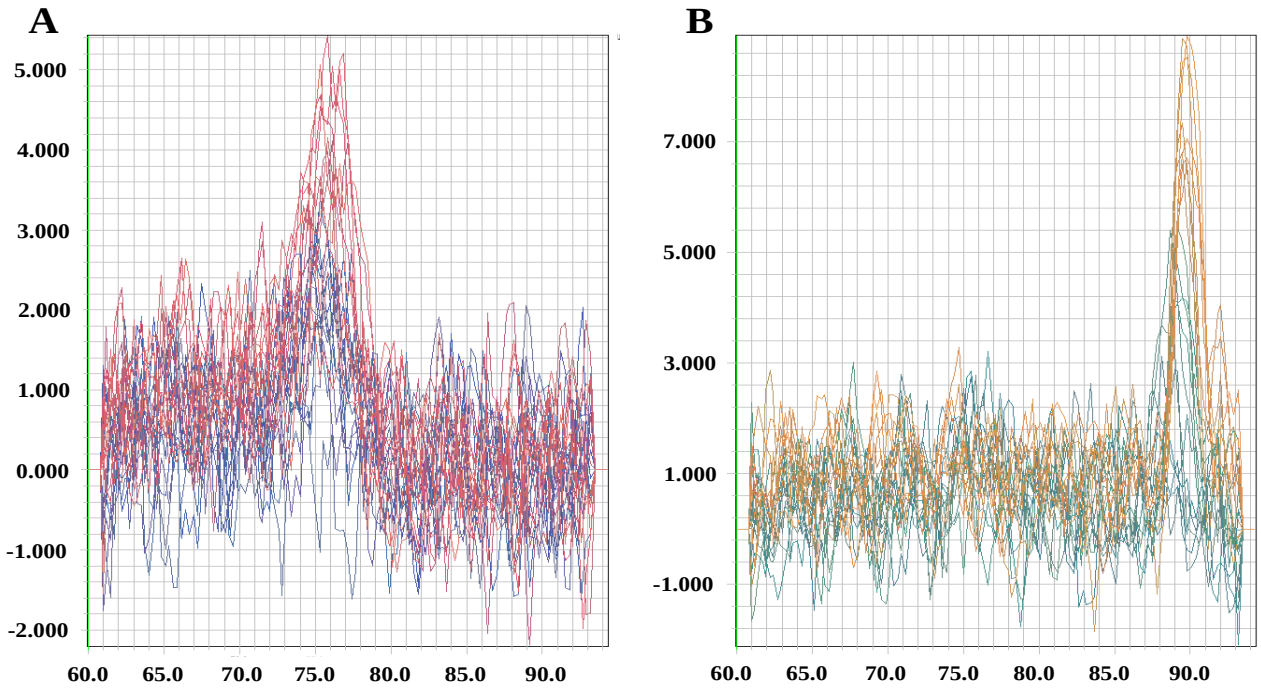


Figure 5. **Melting curve of quantitative RT-qPCR for *nod*A and *nod*D (A: Melting curves for *nod*A amplicons from *E. fredii* (red curve) and *B. japonicum* (blue curve), B: Melting curves for *nod*D amplicons from *E. fredii* (green curve) and *B. japonicum* (orange curve))**

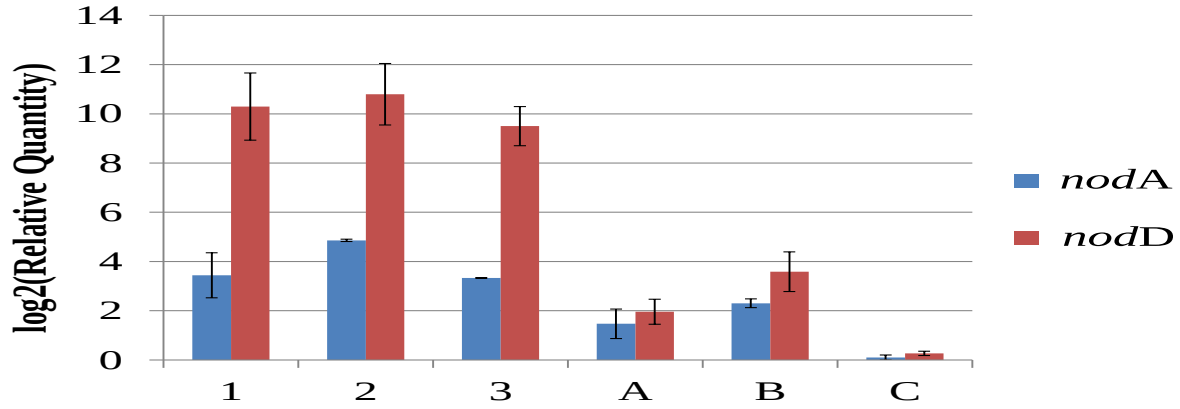


Figure 6. **Expression level of nodA and nodD (X axis-1: Gene expression of E. fredii, X axis-2: Gene expression of E. fredii with 10^{-3} v/v GA_3, X axis-3: Gene expression of E. fredii with 10^{-2} v/v PIX, X axis-A: Gene expression of B. japonicum, X axis-B: Gene expression of B. japonicum with 10^{-3} v/v GA_3, X axis-C: Gene expression of B. japonicum with 10^{-2} v/v PIX)**

Through this study, reliable evidence was provided regarding the important role played by PGRs in regulating cell growth and microstructure of rhizobia cell. Moreover, the response of different *Rhizobia* sp. to PGRs type and concentration were various. Our RT-qPCR results indicated that PGRs could change a series of rhizobia cell proliferation and differentiation through regulating expression of *nod* gene. However, further study will be needed to determine whether there are other genes involved in this regulation pathway.

ACKNOWLEDGEMENTS

This work was supported financially from Solid-state Fermentation Resource Utilization Key Laboratory of Sichuan Province (No. 2018GTY001), Scientific Research Project of Yibin University (No. 2017RC03) and Key Lab of Aromatic Plant Resources Exploitation and Utilization in Sichuan Higher Education (No. 2018XLZ00301). We also appreciate Wuhan for its contribution in the fight against the 2019-nCoV very much.

REFERENCES

[1] Alayón-Luaces P, Ponce N M, Mroginski L A, Stortz CA and Sozzi G O. 2012. Compositional changes in cell wall polysaccharides from apple fruit callus cultures modulated by different plant growth regulators. *Plant Science* 185: 169-75.

[2] Andrés J A, Rovera M, Guinazú L B, Pastor N A and Rosas S B. 2012. *Bacteria in Agrobiology: Stress Management*, pp77-94. Springer.

[3] Arora S, Sharma P, Kumar S, Nayan R, Khanna PK and Zaidi M. 2012. Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. *Plant Growth Regulation* 66(3): 303-10.

[4] Banfalvi Z, Nieuwkoop A, Schell M, Besl L and Stacey G. 1988. Regulation of *nod* gene expression in *Bradyrhizobium japonicum*. *Molecular and General Genetics* 214(3): 420-24.

[5] Chandran V, Shahena S, Rajan M and Mathew L. 2020. *Controlled Release of Pesticides for Sustainable Agriculture*, pp253-66. Springer.

[6] Chen W, Zheng D, Feng N, Liu T, Liu Y, Gong S, Cui H and Xiang H. 2015. The effects of gibberellins and mepiquat chloride on nitrogenase activity in *Bradyrhizobium japonicum*. *Acta physiologiae plantarum* 37(1): 1723.

[7] Choi J, Shin H and Lee Y. 2003. Modulation of 3-hydroxyvalerate molar fraction in poly (3-hydroxybutyrate-3-hydroxyvalerate) using *Ralstonia eutropha* transformant co-amplifying *phbC* and NADPH generation-related *zwf* genes. *Enzyme and microbial technology* 32(1): 178-85.

[8] Głodowska M, Schwinghamer T, Husk B and Smith D. 2017. Biochar based inoculants improve soybean growth and nodulation. *Agricultural Sciences* 8(9): 1048-64.

[9] Guasch-Vidal B, Estévez J, Dardanelli M S, Soria-Díaz M E, de Córdoba F F, Balog C, Manyani H, Gil-Serrano A, Thomas-Oates J and Hensbergen P J. 2013. High NaCl concentrations induce the *nod* genes of *Rhizobium tropici* CIAT899 in the absence of flavonoid inducers. *Molecular plant-microbe interactions* 26(4): 451-60.

[10] Gupta R and Chakrabarty S K. 2013. Gibberellic acid in plant: still a mystery unresolved. *Plant signaling and behavior* 8: e25504.

[11] Hasan M, Bano A, Hassan SG, Iqbal J, Awan U,

[12] Rong-ji D and Khan K A. 2014. Enhancement of rice growth and production of growth-promoting phytohormones by inoculation with Rhizobium and other rhizobacteria. *World*

Applied Sciences Journal 31: 1734-43.

[13] Ho BJ, Tsoi JK, Liu D, Lung CY, Wong H and Matinlinna J P. 2015. Effects of sandblasting distance and angles on resin cement bonding to zirconia and titanium. *International Journal of Adhesion and Adhesives* 62: 25-31.

[14] Islas-Flores T, Guillén G, Alvarado-Affantranger X, Lara-Flores M, Sánchez F and Villanueva M A. 2011. PvRACK1 loss-of-function impairs cell expansion and morphogenesis in *Phaseolus vulgaris* L. root nodules. *Molecular plant-microbe interactions* 24(1): 819-26.

[15] Jaidka M. 2016. Growth regulation and defoliation studies for source-sink optimization and synchronized maturity in soybean (*Glycine max* L. Merrill): Punjab Agricultural University, Ludhiana.

[16] Liu Y H, Jiao Y S, Liu LX, Wang D, Tian C F, Wang E T, Wang L, Chen W X, Wu S Y and Guo B L. 2018. Nonspecific symbiosis between *Sophora flavescens* and different rhizobia. *Molecular plant-microbe interactions* 31(2): 224-32.

[17] Maggio A, Barbieri G, Raimondi G and De Pascale S. 2010. Contrasting effects of GA_3 treatments on tomato plants exposed to increasing salinity. *Journal of plant growth regulation* 29(1): 63-72.

[18] Mohite B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of soil science and plant nutrition* 13: 638-49.

[19] Mulligan J T and Long S R. 1985. Induction of *Rhizobium meliloti nodC* expression by plant exudate requires *nodD*. *Proceedings of the National Academy of Sciences* 82(19): 6609-13.

[20] Okazaki S, Tittabutr P, Teulet A, Thouin J, Fardoux J, Chaintreuil C, Gully D, Arrighi J, Furuta N and Miwa H. 2016. Rhizobium-legume symbiosis in the absence of Nod factors: two possible scenarios with or without the T3SS. *The ISME journal* 10(1): 64-74.

[21] Olanrewaju O S, Glick B R and Babalola O O. 2017. Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology* 33(11): 197.

[22] Peck M C, Fisher R F and Long S R. 2006. Diverse flavonoids stimulate NodD1 binding to *nod* gene promoters in *Sinorhizobium meliloti*. *Journal of bacteriology* 188(15): 5417-27.

[23] Podlešáková K, Fardoux J, Patrel D, Bonaldi K, Novák O, Strnad M, Giraud E, Spíchal L and Nouwen N. 2013. Rhizobial synthesized cytokinins contribute to but are not essential for the symbiotic interaction between photosynthetic bradyrhizobia and *Aeschynomene* legumes. *Molecular plant-microbe interactions* 26(10): 1232-38.

[24] Rademacher W. 2018. Chemical regulators of gibberellin status and their application in plant production. *Annual Plant Reviews online*: 359-03.

[25] Sharifuzzaman S M, Ara K A, Rahman M H, Kabir K and Talukdar M B. 2011. Effect of GA_3, CCC and MH on vegetative growth, flower yield and quality of chrysanthemum. *International Journal of Experimental Agriculture* 2(1): 17-20.

[26] Shoukry A A, El-Sebaay H H and El-Ghomary A E. 2018. Assessment of Indole Acetic Acid Production from *Rhizobium leguminosarum* Strains. *Current Science International* 7: 60-69.

[27] Singh V K and Bhattacherjee A K. 2005. Genotypic response of mango yield to persistence of paclobutrazol in soil. *Scientia Horticulturae* 106(1): 53-59.

[28] Small C C and Degenhardt D. 2018. Plant growth regulators for enhancing revegetation success in reclamation: a review. *Ecological engineering* 118: 43-51.

[29] Tan Y, Xu S, Wu R, Du J, Sang J and Wang J. 2017. A gradient Laponite-crosslinked nanocomposite hydrogel with anisotropic stress and thermo-response. *Applied Clay Science* 148: 77-82.

[30] Wang L, Mu C, Du M, Chen Y, Tian X, Zhang M and Li Z. 2014. The effect of mepiquat chloride on elongation of cotton (*Gossypium hirsutum L.*) internode is associated with low concentration of gibberellic acid. *Plant Science* 225: 15-23.

[31] Yang S, Zhao L, Yan J, Zhang J, Guo F, Geng Y, Wang Q, Yang F, Wan S and Li X. 2019. Peanut genes encoding tetrapyrrole biosynthetic enzymes, AhHEMA1 and AhFC1, alleviating the salt stress in transgenic tobacco. *Plant physiology and biochemistry* 137: 14-24.

[32] Zahir Z A, Yasin H M, Naveed M, Anjum M A and Khalid M. 2010. L-Tryptophan application enhances the effectiveness of Rhizobium inoculation for improving growth and yield of mungbean (*Vigna Radiata* L. Wilczek). *Pakistan Journal of Botany* 42(3): 1771-80.

[33] Zhao Q, Sun Q, Dong P, Ma C, Sun H and Liu C. 2019. Jasmonic acid alleviates boron toxicity in *Puccinellia tenuiflora*, a promising species for boron phytoremediation. *Plant and Soil* 445: 397-07.