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EFFECT OF VARIOUS CARBON SOURCES ON THE GROWTH AND REDIFFERENTIATION POTENCIAL OF SOYBEAN ROOT NODULE BACTERIOIDS.

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Abstract

The metabolic activity of soybean root nodule bacterioids, which are essential for biological nitrogen fixation, is highly dependent on the amount and kind of carbon sources provided by the host plant. This study looks at how different carbon sources affect the proliferation and redifferentiation capability of bacterioids in soybean root nodules. Bacterioid survival, metabolic activity, and transition between differentiated and free-living stages were assessed on several organic carbon substrates, including sugars, organic acids, and polyols. The results show that organic acids, especially dicarboxylic acids like succinate and malate, greatly improve bacterioid development and maintain nitrogen-fixing capability. This suggests that these acids are used more frequently in the symbiotic environment. While certain carbon sources encouraged partial redifferentiation into free-living bacterial forms under controlled circumstances, simple sugars demonstrated insufficient support for bacterioid metabolism. The importance of carbon molecules obtained from the host in controlling bacterioid physiology, growth dynamics, and developmental flexibility is emphasized by these results. Strategies to improve symbiotic efficiency and soybean output may be informed by a better understanding of the carbon source-dependent responses of bacterioids, which in turn gives important insights into the metabolic control of legume-rhizobium symbiosis.

Keywords: *redifferentiation, bacterioids, soybean root*

Introduction

There is a strong correlation between symbiotic nitrogen fixation and the productivity of soybeans (Glycine max L.), which are among the most significant leguminous crops globally because of their high oil and protein content. Root nodules, specialized organs produced by infected soybean roots, are mediated by Rhizobium and Bradyrhizobium species. These nodules are the sites of bacterial differentiation into bacteroids, a metabolically specialized form that can fix atmospheric nitrogen into ammonia, which the host plant may then absorb. In order for this symbiotic relationship to work, the host plant and bacterium companion must engage in intricate metabolic and physiological exchanges. The carbon molecules supplied by the host plant are an important component regulating bacteroid metabolism and nitrogen-fixing activities. Bacterioid respiration and nitrogenase activity rely on photosynthetically produced carbon, which is transferred from the shoots to the nodules during symbiosis. Bacteriooids share several metabolic features with free-living rhizobia, but they also have a marked predilection for certain carbon substrates. Soybean nodule bacteroids have a high level of metabolic specialization consistent with symbiotic differentiation, as shown by many investigations indicating dicarboxylic organic acids like fumarate, succinate, and malate are their primary carbon sources. Carbon sources affect bacteroids' development, survival, and growth plasticity; they also

support nitrogen fixation. Redifferentiation, in which bacteroids return in part or in whole to free-living bacterial forms, can occur under specific experimental or physiological circumstances. Because it sheds light on the regulatory mechanisms that govern bacteroid development and the reversibility of symbiotic differentiation, this redifferentiation potential is highly intriguing. It is believed that carbon composition and availability regulate cellular metabolism, gene expression, and energy status, playing a crucial part in this process. There is a lack of comparative data on the effects of various carbon sources on the growth and redifferentiation capacity of soybean root nodule bacterioids, even though carbon metabolism is known to play a significant role in the legume-rhizobium symbiosis. The metabolic needs of bacteroids and their ability to adapt to different dietary conditions can be better understood by conducting a comprehensive analysis of different carbon substrates. To further our understanding of carbon-regulated symbiotic functioning and its implications for improving nitrogen fixation efficiency in soybean, the present study seeks to investigate the effect of different carbon sources on the growth characteristics and redifferentiation behavior of soybean root nodule bacterioids.

Materials and methods

Situated in Xiangfang District, Harbin, Heilongjiang Province, China (126°43′E, 45°44′N), the experimental base of Northeast Agricultural University hosted the 2023 experiment. A technique based on sand culture was utilized. Dongda 1 was the nodulated soybean cultivar, while WDD01795, L8-4858 were the non-nodulated lines. The crop research institute of the Chinese academy of agricultural sciences and northeast Agricultural University provided the plant materials. The technique outlined in Zhang et al. was used to grow unilateral nodulated dual-root soybean plants. Each pot was seeded with two seedlings having dual roots. Nodules harvested from soybean plants growing in the field and then frozen to create the rhizobial inoculum. Approximately 5 g rhizobia per liter was obtained by washing, crushing, and suspending nodules in water. After the dual-rooted seedlings were successfully grafted, the supernatant was utilized to water the roots constantly for five days. Plants were watered once a day before to the VC stage, when their actual leaves began to expand. Daily applications of nutrient solution were made from the VC stage to the V4 stage. Starting at V4 and continuing until the completion of the experiment, 250 mL of the nutrient solution was applied to each side of the roots twice a day, in the morning and evening. The previously described process was used to generate a nutrition solution that did not include nitrogen. As a supply of nitrogen, potassium nitrate (KNO3) was utilized. To maintain consistent potassium levels across treatments, the non-nodulated root side and the control treatment were treated with potassium sulfate (K2SO4) at an equal concentration to the KNO3 administered to the nodulated side.

Experimental design

The experiment developed four treatments: CK, LG, HN, and HG. The first group, CK (Control), did not undergo girdling. There was an N-free fertilizer solution that was watered onto both sides of the dual-root system. nutritional solution that does not include nitrogen; (2) LG: the roots that are encircled by a ring. A sterile scalpel was used to girdle the non-nodulated root's base with a breadth of half a centimeter. Additionally, tin foil was used to protect the girdled root from infection. No girdling was done to (3) HN. A nitrate nutritional solution containing 200 mgN•L^{-1} was applied to the root side that did not have any nodules, while a nutrient solution devoid of nitrogen was given to the side that had nodules. (4) HG: Applying the identical technique as the LG treatment, the non-nodulated root's base

was girded. Nitrate nutritional solution containing 200 mgN•L^{-1} was irrigated onto the non-nodulated root side after girdling, whereas a nutrient solution devoid of nitrogen was applied to the nodulated side. Two and seven days following treatment, researchers took root samples. Figure 1 is a schematic depiction of the girdling sites and treatments.

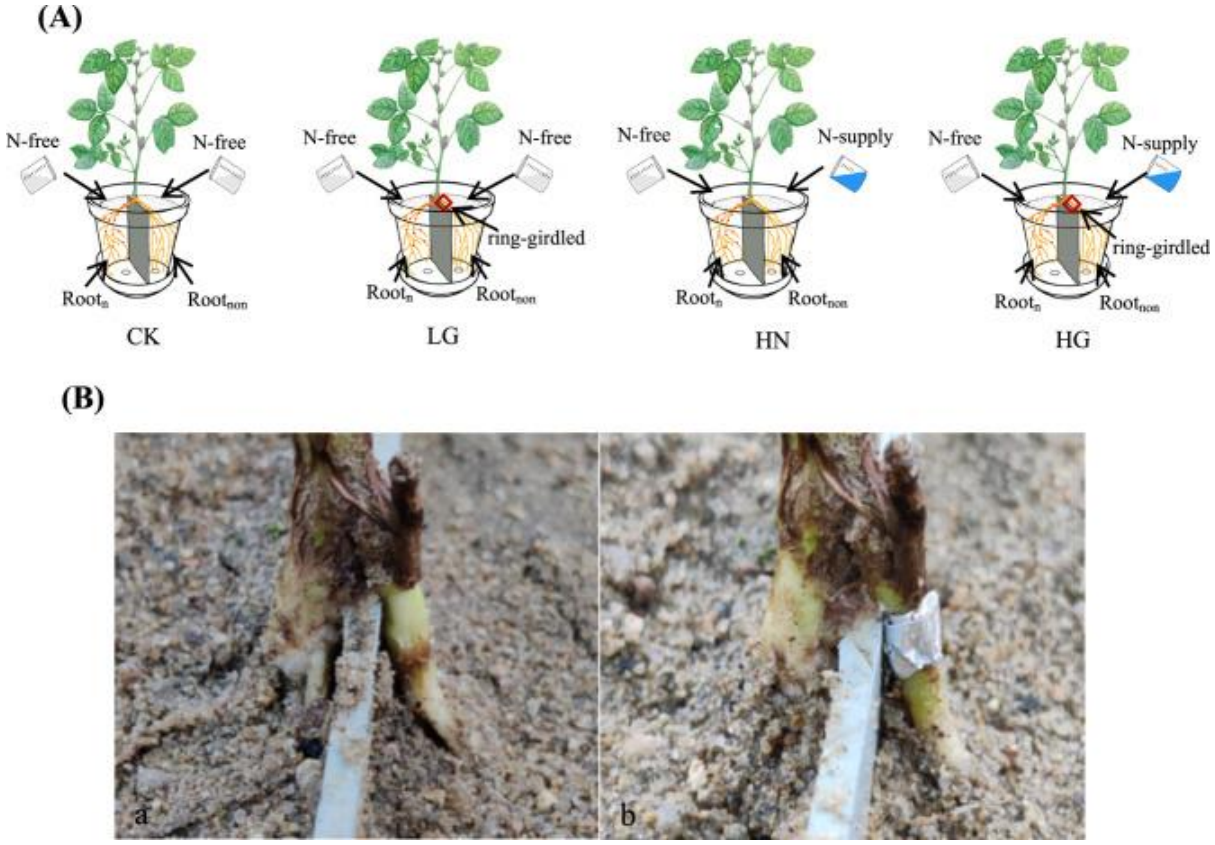

Fig. 1. Graphic depicting the treatments and the components with ring-girdles. The experimental treatments are shown by A. B The LG and HG treatments used ring-girded components. a: A half-meter-wide band was wound around the nodulated root's base.

Sampling and determination

Shoots were removed from the grafting location as the experiment came to a close. After choosing four containers, distilled water was used to delicately wash the nodulated lateral roots. Following the protocol outlined by Xia et al., we utilized the acetylene reduction test to determine the nodules' nitrogenase activity. Nodules were removed, tallied, oven-dried, and weighed after the measurement. Nodules, nodulated lateral roots, and non-nodulated lateral roots were the classifications used to divide another set of four containers. After being rinsed with distilled water and dried with filter paper, every component was quickly frozen in liquid nitrogen and kept at -80 °C for subsequent examination. Every tissue had its sucrose, starch, and soluble sugar concentrations measured. Eighty percent ethanol was used for sample extraction. A Multospher Sugar column, attached to a Waters 152 high-performance liquid chromatograph, was used to quantify the sucrose content. Using a BIOTEK-ELx800 microplate reader at a wavelength of 620 nm, the levels of starch and soluble sugars were measured using anthrone colorimetry. Also, six nodules of comparable size were chosen at random on the nodulated side. After slicing, they were submerged in a phosphate buffer solution (0.1 mol•L^{-1}, pH 7.0) that contained 2.5%

glutaraldehyde. The microstructures of nodules were studied following the steps outlined in the study by Li et al.

Date analysis

We used SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA) for all of our statistical analyses. We utilized Duncan's multi-range test to ensure that all of our data was normally distributed before running a one-way analysis of variance (ANOVA). $P < 0.05$ was the criterion of significance.

Results

N fixation activity of soybean nodules

Table 1 shows that the number of nodules and their dry weight were both affected by the indirect application of nitrate and the improved allocation of carbohydrates to soybean nodules. Nodule number increased by 37.5% after 2 days of therapy with LG treatment compared to CK, while nodule dry weight stayed the same. While there was no statistically significant change in nodule quantity between CK and HN, there was a 19.1% reduction in nodule dry weight. In terms of dry weight and nodule number, there was no statistically significant difference between the HG and HN treatments. Nonetheless, there was a notable decrease of 26.9% in nodule number and 24.3% in dry weight with the HG therapy as compared to LG. After 7 days of therapy, the number of nodules rose by 29.4% and the dry weight by 47.6% in the LG group compared to the CK group. Nodule dry weight was unaffected by HN therapy, while nodule number was decreased by 25.7%. Nodule number rose by 42.0% and dry weight increased by 45.5% relative to HN when treated with HG. There was no discernible change in dry weight after HG therapy, however the number of nodules was 18.5% lower than after LG. This data suggests that nodule formation was progressively inhibited when nitrate was applied to the non-nodulated side. Nodule growth on the nodulated side was stimulated by ring-girdling the non-nodulated side. Nitrate supply after girdling still had inhibitory effects, although they weren't as strong. Nitrate inhibits nodule formation, although improved glucose allocation from the shoot to the nodulated side makes this effect less noticeable.

Table 1. Number and dry weight of soybean nodules

	Treatments	Nodule Number (per plant)	Dry Weight ($g \cdot plant^{-1}$)
2 d	CK	317.5 ± 10.96 b	0.68 ± 0.11 a
	LG	436.5 ± 9.71 a	0.70 ± 0.05 a
	HN	292.0 ± 8.62 b	0.55 ± 0.04 b
	HG	319.2 ± 8.90 b	0.53 ± 0.04 b
7 d	CK	474.0 ± 12.49 b	0.82 ± 0.04 bc
	LG	613.5 ± 13.89 a	1.21 ± 0.05 a
	HN	352.2 ± 10.02 c	0.77 ± 0.03 c
	HG	500.0 ± 12.17 b	1.12 ± 0.02 ab

Two measures of nitrogenase activity—specific nitrogenase activity (SNA) per unit of nodule dry weight and total nitrogenase activity per plant (ARA)—were considerably affected by indirect nitrate application and improved carbohydrate allocation to soybean nodules, respectively (Table 2). After only two days of therapy, SNA and ARA were 34% higher in the LG group than in the CK group. The HN therapy resulted in a 36.6% reduction in ARA and no change to SNA. There was no statistically

significant change in SNA with HG therapy, whereas ARA decreased by 21.6%. The HG therapy raised SNA by 27.9% in comparison to HN, but had no discernible effect on ARA. Both SNA and ARA were significantly reduced by 23.1% and 41.5%, respectively, when treated with HG as compared to LG. The LG group showed a notable 15.8% rise in SNA and a 72.5% increase in ARA after 7 days of therapy, in comparison to the CK group. Both SNA and ARA were significantly reduced by 35.7% and 39.5% after receiving the HN therapy, respectively. In the HG treatment group, there was no discernible difference between CK and SNA or ARA. On the other hand, SNA was up 36.3% and ARA was up 97.4% after HG therapy compared to HN. Both SNA and ARA were considerably decreased by 24.3% and 30.8%, respectively, when treated with HG as opposed to LG. According to these results, nodules' N fixing activity is enhanced when carbohydrates are allocated more efficiently to the side of the root that has nodules. Additionally, the systemic inhibitory effects of nitrate on N fixing in soybeans might be mitigated by an increase in carbohydrate availability.

Table 2. Soybean nodule nitrogenase activity

	Treatments	SNA (C2H4μmol·g−1·nodule dry mass·h −1)	ARA (C2H4μmol·h−1·plant−1)
2 d	CK	39.0 ± 0.57 bc	26.8 ± 1.82 b
	LG	52.4 ± 3.84 a	35.9 ± 1.92 a
	HN	31.5 ± 1.25 c	17.0 ± 0.08 c
	HG	40.3 ± 3.26 b	21.0 ± 1.77 c
7 d	CK	47.6 ± 3.79 b	38.2 ± 2.74 b
	LG	55.1 ± 3.29 a	65.9 ± 3.54 a
	HN	30.6 ± 2.50 c	23.1 ± 1.10 c
	HG	41.7 ± 2.04 b	45.6 ± 2.17 b

Carbohydrate concentration in soybean roots and nodules

Soybean tissues' sucrose distribution changed significantly after nitrate injection (Table 3). A 63.0% increase in sucrose concentration in the nodulated lateral root (Rootn) and a 37.7% decrease in concentration in the non-nodulated lateral root (Rootnon) were observed after 2 days of treatment with LG compared to CK. The concentration of sucrose in the nodules did not alter. The results showed that the sucrose transport flux from the shoot to the nodulated side could be increased at the base of the non-nodulated root girdling, whereas the flux on the non-nodulated side may be reduced. The concentration of sucrose in Rootnon jumped 60.3% compared to CK in the HN treatment, but it fell 12.2% in Rootn. The content of sucrose in the nodules was 35.5% lower. Based on these findings, it appears that the nodules had less sucrose available and that the nitrate-treated side accumulated more sucrose due to indirect nitrate delivery. The HG therapy caused a 65.6% drop in Rootnon sucrose concentration compared to HN treatment, and a 55.3% increase in Rootn sucrose level and a 47.3% increase in nodule sucrose level. There was no discernible change in Rootnon's sucrose content between LG and HG treatments, whereas nodules and Rootn both showed decreases of 14.9% and 16.3%, respectively. The HG treatment significantly reduced Rootnon by 81.5% and increased Rootn by 36.5% compared to CK, but had no discernible effect on nodule sucrose content. The results show that when the non-nodulated root is girdled, the sucrose concentration in the nodules can be increased by the nitrate supply, which

reduces the competitive sucrose allocation that occurs between the two root systems due to indirect nitrate supply.

Table 3. Soybean root and nodule sucrose concentration (mg·g−1)

	Treatments	Nodules	Roots	
			$Root_{non}$	$Root_n$
2 d	CK	12.52 ± 0.40 ab	3.05 ± 0.08 b	3.62 ± 0.27 c
	LG	13.97 ± 0.08 a	1.90 ± 0.15 c	5.90 ± 0.29 a
	HN	8.07 ± 0.37 c	4.89 ± 0.13 a	3.18 ± 0.15 c
	HG	11.89 ± 0.78 b	1.68 ± 0.18 c	4.94 ± 0.21 b
7 d	CK	13.22 ± 0.38 b	4.11 ± 0.19 b	4.68 ± 0.31 bc
	LG	16.38 ± 0.54 a	3.08 ± 0.17 c	6.63 ± 0.15 a
	HN	10.25 ± 0.38 c	5.48 ± 0.26 a	3.91 ± 0.26 c
	HG	12.89 ± 0.44 b	3.95 ± 0.11 b	5.57 ± 0.45 b

The patterns seen across treatments at the 2-day mark were very similar with those at the 7-day mark. Nevertheless, after receiving LG therapy, the nodule sucrose concentration surpassed that of CK, suggesting that the nodules accumulated more sucrose due to the longer blocking of the C transport channel to the non-nodulated side. Rootn sucrose content was not significantly different from CK in the HN treatment, indicating that root-system sucrose distribution was affected by nitrate input to the non-nodulated side. Rivalry for sucrose supply between the nodules on the nodulated side and the nitrate-treated lateral roots most likely drove this shift. In general, the effects on starch content in soybean roots and nodules of nitrate supply and changed C transport were similar to those seen for sucrose (Table 4). Notably, compared to other treatments, the starch content in nodules treated with HG was twice as high after 7 days.

Table 4. Soybean root and nodule starch concentration (mg·g−1)

	Treatments	Nodules	Roots	
			$Root_{non}$	$Root_n$
2 d	CK	8.08 ± 0.22 b	2.10 ± 0.10 b	1.84 ± 0.08 c
	LG	10.42 ± 0.30 a	1.71 ± 0.10 c	3.66 ± 0.09 a
	HN	7.33 ± 0.49 b	2.57 ± 0.16 a	1.56 ± 0.16 c
	HG	10.25 ± 0.49 a	2.49 ± 0.15 a	3.03 ± 0.12 b
7 d	CK	8.91 ± 0.32 b	3.16 ± 0.18 b	3.90 ± 0.62 bc
	LG	10.88 ± 0.25 a	2.05 ± 0.11 c	5.10 ± 0.19 a
	HN	7.17 ± 0.36 c	3.83 ± 0.18 b	3.42 ± 0.62 c
	HG	19.18 ± 0.58 b	6.41 ± 0.34 a	5.20 ± 0.17 c

Table 5 shows that there was a somewhat distinct pattern for changes in soluble sugar content in nodules. After 2 days of treatment, Rootn and nodules treated with LG had increased soluble sugar contents compared to CK, but Rootnon shown a significant reduction. In Rootnon, Rootn, or nodules, there were no discernible changes in soluble sugar content between the CK and HN treatments. Soluble sugar levels were found to be higher in Rootn and nodules after HG treatment in comparison to HN and CK, but

Rootnon shown a notable reduction in comparison to HN but not CK. Furthermore, Rootnon's soluble sugar content was much greater under HG conditions compared to LG conditions. After 7 days of treatment, Rootn and nodules treated with LG had considerably reduced soluble sugar contents compared to CK, but Rootnon shown a large rise. In the HN treatment, similar patterns were noted: Rootn and nodules had much reduced soluble sugar levels compared to CK, but Rootnon showed a considerable rise. Nodule soluble sugar content in the HG treatment was substantially lower than in CK but was statistically indistinguishable from HN. When comparing Rootn to HN and CK, soluble sugar levels were much lower in Rootn, but greater in Rootnon. According to the data shown above, soluble sugar accumulation in Rootn and nodules was greatly enhanced when the base of the non-nodulated root was ring-girded for 2 days. After 7 days, though, the buildup slowed. Solvent sugar content in Rootn was raised when nitrate was supplied to the non-nodulated side, but no discernible change was seen in the nodules. There was an increase in soluble sugar accumulation in Rootnon and a decrease in Rootn and nodules when nitrate was given after the base of the non-nodulated root ring-girdled.

Table 5. Soybean root and nodule soluble sugar concentration (mg·g−1)

	Treatments	Nodules	Roots	
			$Root_{non}$	$Root_{n}$
2 d	CK	17.16 ± 0.23 b	6.33 ± 0.26 ab	5.79 ± 0.25 b
	LG	20.00 ± 0.55 a	3.35 ± 0.13 c	7.54 ± 0.37 a
	HN	18.42 ± 0.19 b	7.23 ± 0.21 a	6.06 ± 0.22 b
	HG	20.18 ± 0.79 a	5.54 ± 0.57 b	7.41 ± 0.10 a
7 d	CK	17.16 ± 0.23 b	6.33 ± 0.26 ab	5.79 ± 0.25 b
	LG	20.00 ± 0.55 a	3.35 ± 0.13 c	7.54 ± 0.37 a
	HN	18.42 ± 0.19 b	7.23 ± 0.21 a	6.06 ± 0.22 b
	HG	20.18 ± 0.79 a	5.54 ± 0.57 b	7.41 ± 0.10 a

Ultrastructure of soybean nodule

Two days after treatment, CK nodules had floccules packed tightly into the cytoplasm and bacteroids covering nearly every infected cell, pushing most organelles to the cell wall's perimeter. According to Figure 2A, the diseased cell walls seemed to be unbroken and smooth. Figures 2A and C show that the spherical bacteria were free in the cytoplasm. Once infected, bacteroids multiplied within vesicles, which led to their eventual elongation and enlargement. Symbiosomes, which consist of several bacteroids encased in PBM, develop within each vesicle throughout time (Fig. 2C). Fig. 2B and C show that bacteroids at this stage of development include PHB granules. In Figures 2A, 2B, and 2C, the area ratio of PHB in bacteroids was 15%, 20%, and 21%, respectively, as calculated using Photoshop and scale bars. The area ratio of PHB in bacteroids was 30%, which is an increase compared to CK (Fig. 2D). Some host cells showed a decrease in cytoplasmic floc density and infected cell walls looked uneven in the HN treatment, which involved indirectly supplying nitrate to nodules for two days (Fig. 2E and H). Bacteriooids were seen distributed outside infected cells, indicating the presence of loopholes (Fig. 2F and G). Organelle loss and a reduction in bacteroids were seen as signs of degeneration in certain cells (Fig. 2G). As seen in Figures 2G and H, bacteroids were discharged into the cytoplasm when the PBM was ruptured. Figures 2I and J show that although the PBS was larger in the HG treatment than in the CK treatment, no bacteroids were seen distributed outside of the infected cells.

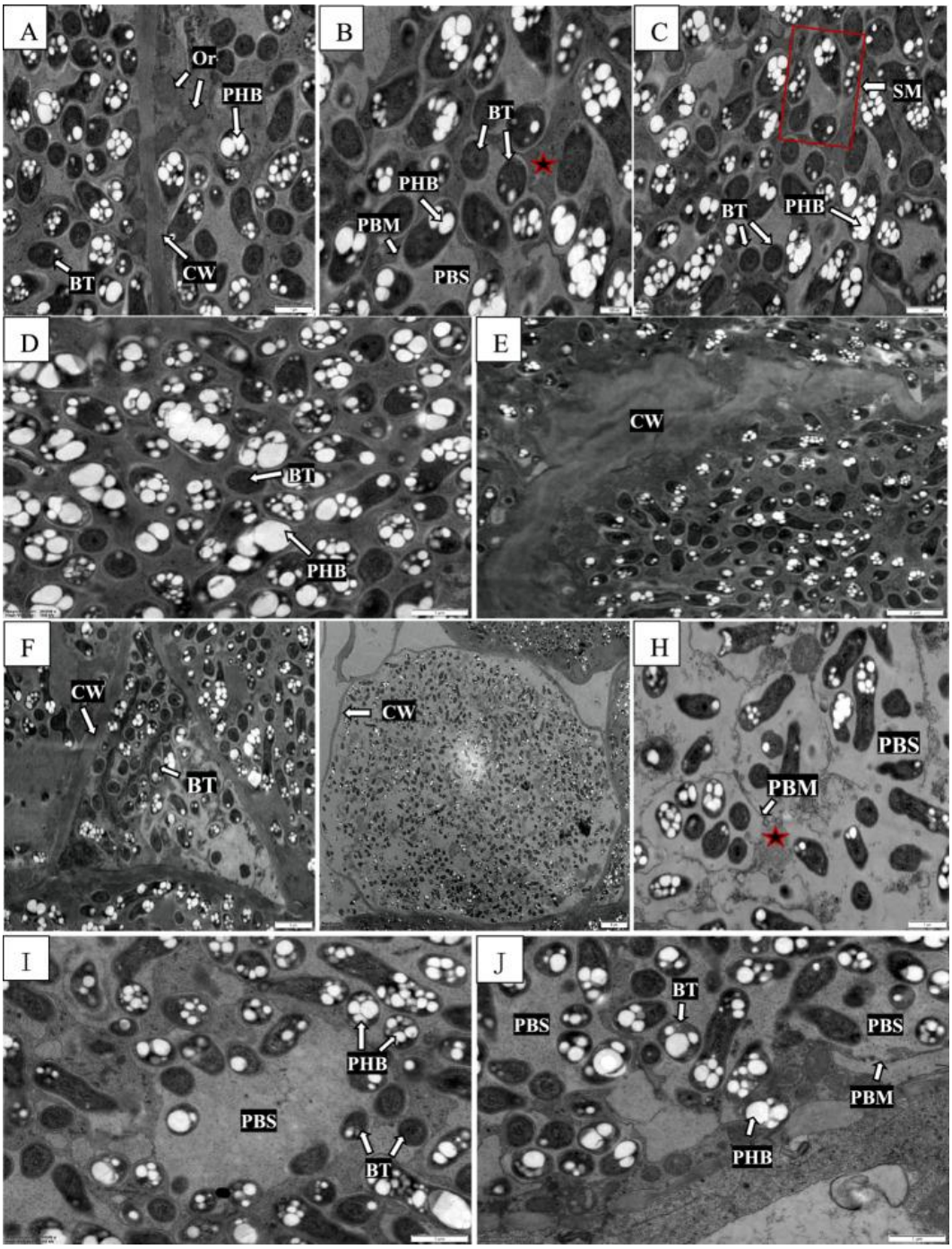

Fig Nodule ultrastructure following two days of therapy. Here we have the CK therapy represented by A, B, and C, the LG treatment by D, the HN treatment by E, F, G, and H, and the HG treatment by I and J. Cytoplasm, bacteroids, symbiosome, peribacteroid space, peribacteroid membrane, and cell wall are all abbreviated as VE, BT, PHB, SM, PBS, and PBM, respectively. Organelle, with flocculus shining brightest. Magnifications of A, B, E, and F are 30,000x, 10,000x, and 3,000x, respectively. Magnifications of C, D, H, I, and J are 20,000x.

There were very little structural changes in CK-infected cells after 7 days of therapy as compared to the 2-day time point (Fig. 3A and B). The LG treatment involved a dense filling of the spherical bacteria with PHB. The symbiosome structure was more ordered in comparison to CK due to the bactereroid arrangement in a ring-like pattern within the symbiosomes and the expanded PBS (Fig. 3C and D). In the HN therapy, the diseased cell structure underwent significant degeneration after 7 days of indirect nitrate supply to the nodules. Both the amount of bacteroids and the presence of cytoplasmic flocculent material were noticeably reduced. The host cytoplasm showed signs of symbiosome disruption, PBM lysis, and the dissociation and degradation of several bacteroids (Fig. 3E, F and G). The infection threads that brought the bacteroids into the host cells were still visible, even though the infected cells showed signs of premature senescence (Fig. 3H). Structure disruption, including vacuole formation, was seen in infected cells treated with HG (Fig. 3I). There were indications of freshly infected cells (Fig. 3K) and most bacteroids were contained within vesicles (Fig. 3J).

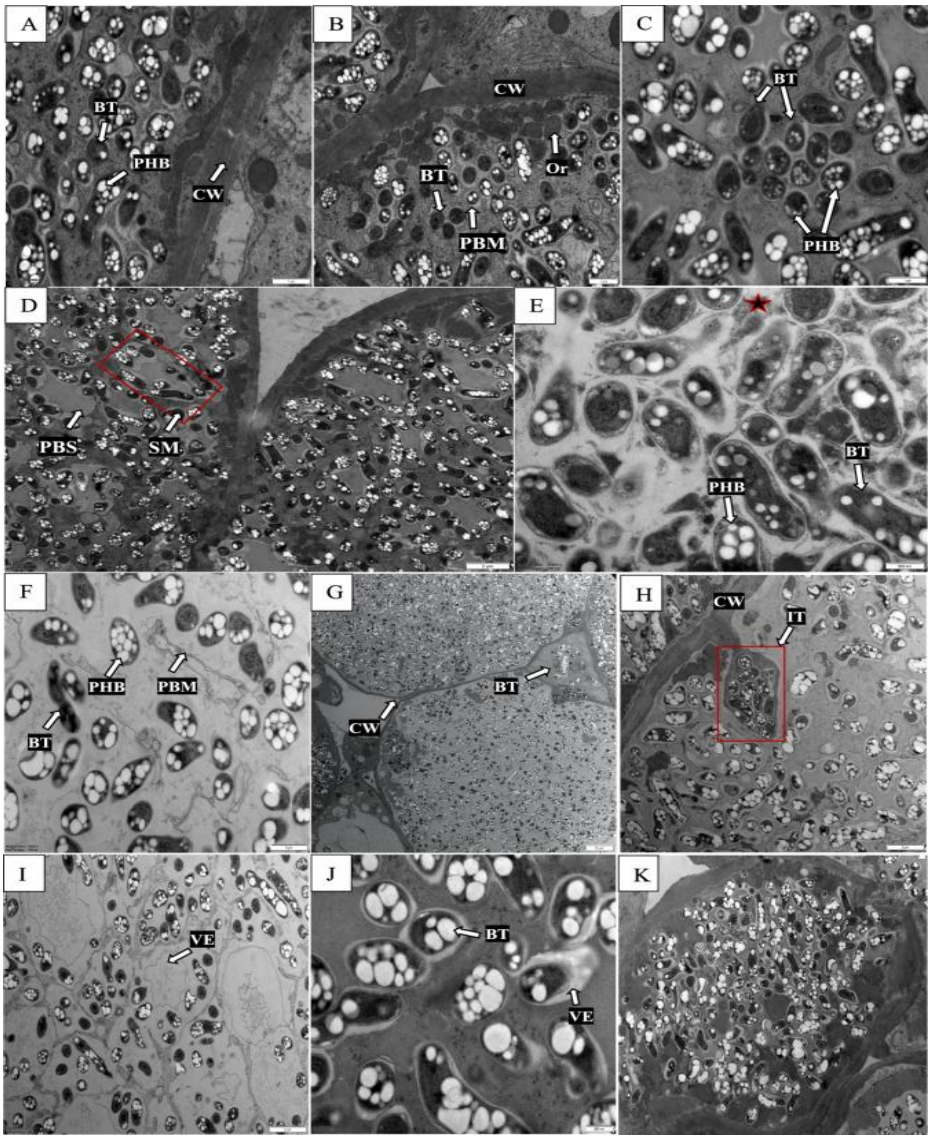

FIG The ultrastructure of nodules following a week of therapy. Here we have A and B for the CK therapy, C and D for the LG treatment, E, F, G, and H for the HN treatment, and I, J, and K for the HG

treatment, where K is an infection cell that has just developed. Vacuole, bacteroids, poly-β-hydroxybutyrate, symbiosome, peribacteroid space, peribacteroid membrane, and cell wall are all abbreviations used in the field of microbiology. Organelle, flocculus, and infecting thread are all parts of the same word. At 20,000 times magnification for A, C, and E; 15,000 times for B; 7,000 times for D; 30,000 times for E and J; 2,500 times for G; 10,000 times for H; 1,000 times for I; and 5,000 times for K.

Discussion

Plants primarily store carbon in starch, and they transfer carbon from shoots to roots mostly through sucrose. Soybean nodules include starch and sucrose, which are important sources of carbon for nodule metabolism, and account for a large amount of the carbon that is now being digested. Because of nitrate transport and C-N balance control across different organs, the contents of sucrose and starch in nodules dropped when nitrate was applied directly to the roots of soybeans. Even while nitrate supply has an immediate impact on soybean nodules, it doesn't mean the impacts won't be harmful. By selectively supplying high nitrate concentrations to the non-nodulated side of unilaterally nodulated dual-root soybean plants, the harmful consequences of direct nitrate interaction with nodules might be avoided. Nodule concentrations of sucrose and starch were lowered as a result of competition for photosynthetically produced C by roots from both sides (Tables 3 and 4). Lambert et al. state that nodule deactivation and reduced sugar buildup occur when internal N levels become adequate after a high dose of exogenous N is delivered. This leads to a drop in the nodules' sucrose and soluble sugar concentrations. Application of nitrate decreased starch concentration in nodules but had no effect on Rootn starch levels in the current investigation. This data points to the fact that C allocation to nodules was mostly decreased by indirect nitrate delivery. Hydrolysis of starch into sucrose proceeded unabated; nitrate supply drastically decreased Rootn and nodule sucrose concentrations while increasing Rootnon sucrose concentrations. Root and nodule soluble sugar concentrations did not differ significantly between CK and HN treatments. Starch intake may be an indication of C restriction, and research by Walsh et al. showed that nodule soluble sugar concentration was steady in dark settings. When Walsh et al. administered nitrate to soybean plants with just one root, they came to a similar finding. Thus, it's plausible that Rootn and nodules starch and sucrose levels decrease due to the indirect nitrate input, but soluble sugar levels remain essentially constant (Tables 4 and 5). Rootn and nodules had elevated quantities of sucrose, starch, and soluble sugars near the base of the non-nodulated root girdling. Nitrate treatment after girdling did not reduce the much increased carbohydrate concentrations compared to the CK and HN groups. After 7 days of therapy, there were no significant changes in SNA and ARA between the HG and CK groups, however the HG group showed considerably increased nodule dry weight and quantity when compared to HN. These results show that when the concentrations of sucrose, starch, and soluble sugars are increased and the allocation of carbohydrates from shoots to nodules is increased, nitrogenase activity may be enhanced and the detrimental effects of indirect nitrate input on N fixation activity and soybean nodulation can be mitigated.

discovered that nodule development and nitrogenase activity were both inhibited by three days of nitrate treatment; however, the effects of the nitrate-induced reduction of nitrogenase activity in nodules were eased by supplementing with sucrose. There was a 60% drop in ARA, a little fall in soluble sugar levels, and a serious compromise in nodule structural integrity when 50 mM nitrate was supplied to chickpea (Cicer arietinum) plants, according to reports. In light of these results, the author draws the conclusion

that sugar restriction may not be the main factor causing the decrease in ARA. As a result, we looked at the nodule ultrastructure in two different settings: one with an indirect nitrate supply, which eliminated the harmful consequences of direct contact, and another with improved carbohydrate allocation from the shoot to the nodules.

Determinate nodules are the soybean variety. Cells in the center zone of mature nodules can be classified as either infected or uninfected, depending on whether they have been invaded by bacteroids. The regular network arrangement of uninfected cells allows sucrose to be transported into the core infected area. Infected cells get their C from healthy cells, which they do via the uptake of sucrose or other metabolic byproducts of this sugar. While healthy cells store some carbon as starch granules, infected cells need most of their carbon to power the energy-intensive nitrogen fixation process. One important measure of nodules' N fixing activity is the availability of carbon. In this investigation, nitrogenase activity was shown to be boosted when the percentage of carbon provided to nodules was increased. Infected cells exhibited an augmented buildup of PHB. Increases in C allocation to nodules were correlated with higher PHB levels in freshly produced bacteroids, suggesting that bacteroids are able to synthesize PHB in the presence of C surplus. During times of low carbon levels, these PHB stores might be used to keep metabolism going. Research has demonstrated that PHB can extend the time it takes for soybean nodules to fixate nitrogen. Bacterioid development and maturity may be sped up by PHB buildup; mature bacteroids are the main sources of N fixation. The nitrogenase activity of nodules is, thus, dramatically enhanced by an increase in exogenous C.

Partridge pea bacteroids were discovered to mobilize PHB for usage when nitrate was applied to the plant. Increased PHB buildup was seen in soybeans when nitrate input was increased. Infected cells degraded PHB when unilaterally treated with nitrate in a prior work on dual-rooted soybeans. In addition to PHB alterations, the current investigation found that infected cells' structural integrity deteriorated. Infected cells that were dying off had their cell walls significantly thinner, and in some areas they had completely dissolved. Bacteroids, unprotected by cell walls and membranes, were discharged into the intercellular space and subsequently faded away with time (Fig. 2F). Degenerating infected cell counts increased in correlation with nitrate treatment time. The symbiosomes completely broke down, the PBS vanished, and the PBM lysed in old infected cells. Bacterioid particles scattered across the cytoplasm and a steadily decreasing cytoplasmic electron density indicated that PBMs had been significantly damaged after 7 days of treatment. Because ribosome distribution is positively correlated with cytoplasmic electron density, the decrease seen in infected cells could be an indication of ribosomal organization problems. Protein synthesis relies on ribosomes, which are plentiful in the cytoplasm of host cells. Between bacteroids and host cytoplasm, the PBM mediates material exchange, energy metabolism, and signal transmission. Bacterioid microbes rely on the PBM for their carbon supply. Root nodule organelles and cells are encased with PBM, which coordinates metabolism both inside and between cells. Nutrient recycling within the nodule and regulation of nutrient distribution to bacteroids are both facilitated by its membrane transporters. Iron, in particular, must be transported to bacteroids via PBM transfer. Reduced C transport across the PBM under high nitrate conditions inhibits N fixation by reducing C absorption by bacteroids. Nitrogasase activity in bacteroids is affected by the volume of the PBS, which also serves as a reservoir for ions and metabolites. Bacteriooids are able to transfer energy from the host cytoplasm to themselves because the PBS has a high concentration of sugar. Soybean nodule bacteroids' sugar profiles are influenced by the PBS's sugar content. As a medium for

the transfer of nutrients and signaling chemicals, PBS is also involved in C metabolism. Leghemoglobin coupled to the PBM releases oxygen, which diffuses through the PBS and reaches the bacteroid membrane. There was a correlation between the decline in nodule sucrose and starch concentrations and the structural deterioration of infected cells. Nodule metabolism relies on sucrose, which is produced during shoot photosynthesis, as its primary supply of reduced carbon. Hence, infected cells' energy depletion, which resulted in PBM and cell wall rupture, was probably caused in part by decreased sucrose availability. One important aspect of bacteroid breakdown seems to be the loss of structural integrity and protection for organelles.
We girdled the base of Rootnon and indirectly administered nitrate via Rootnon to validate the link between nitrate-induced structural changes in nodules and C supply. The ratio of shoot-to-nodule transfer of sucrose and starch was enhanced by this modification. The repair of the cell wall and PBM, as well as the promotion of the production of additional infected cells, were all unfavorable structural effects of nitrate that were mitigated by the higher C allocation to nodules (Fig. 3I, J and K). said that by decreasing nitrate reduction activity in the leaves, the inhibitory effects of high nitrate concentrations on nitrogen fixation in lentils might be alleviated by applying exogenous sucrose. The concentration of nitrates, according to some studies, decreases the availability of carbohydrates to nodules, which in turn suppresses N fixing activity. Hence, nitrogenase activity, which is strongly linked to the repair of nodule membrane structures and the creation of new infected cells, is enhanced when the quantity of carbon given to nodules is increased.

Conclusion

This study shows that the redifferentiation capability, metabolic activity, and proliferation of bacterioids in soybean root nodules are all affected by the carbon supply type. Organic acids, and especially dicarboxylic acids like succinate and malate, are the best carbon substrates for keeping bacterioid viable and facilitating symbiotic metabolic processes, according to the results. Since the principal energy source for nitrogen fixation is host-derived organic acids, this choice mimics the natural nutritional situation within soybean nodules. The restricted consumption routes in the differentiated state were suggested by the fact that simple sugars and some alternative carbon compounds were less effective in fostering bacterioid growth and metabolic stability. The metabolic flexibility and developmental plasticity of the symbiotic bacteria were demonstrated when some of these carbon sources allowed bacterioids to partially or completely redifferentiate into free-living bacterial forms under controlled conditions. These findings highlight how carbon availability affects the reversibility of bacteroid differentiation and how it affects the nitrogen-fixing state. In sum, the research sheds light on the ways in which certain carbon substrates affect bacterioid physiology and developmental destiny and highlights the critical role of carbon metabolism in legume-rhizobium symbiosis. To improve nitrogen fixation, symbiotic efficiency, and soybean output in a variety of agricultural settings, it is important to have a better grasp of these carbon-dependent reactions.

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