agronomy

MDPI

Article

Evaluation of the Symbiotic Effects of *Bradyrhizobium elkanii* Y63-1 Inoculation on Soybean Zhongdou 63

Lu Lu, Piao Leng †, Fuxiao Jin, Jiayu Lu, Qianqian Hu, Wanwan Liang, Yi Huang, Chanjuan Zhang, Chao Li, Zhuang Xu, Zhonglu Yang, Shuilian Chen, Songli Yuan * and Haifeng Chen

Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs, Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan 430062, China; jdufnfm@163.com (L.L.); lengpiao1997@163.com (P.L.); jinfuxiao0610@163.com (F.J.); 13050767620@163.com (J.L.); m13477402361@163.com (Q.H.); liangwanwan@caas.cn (W.L.); huangyi@caas.cn (Y.H.); zhangchanjuan@caas.cn (C.Z.); lichao@caas.cn (C.L.); xuzhuang@caas.cn (Z.X.); yangzhonglu@caas.cn (Z.Y.); chenshuilian@caas.cn (S.C.); chenhaifeng@caas.cn (H.C.)

* Correspondence: songliyuan@caas.cn; Tel.: +86-27-86735887

† Current address: Nanchong Academy of Agricultural Sciences, Nanchong 637000, China.

Abstract

Our previous studies identified a new efficient and broad-spectrum rhizobium strain *Bradyrhizobium elkanii* Y63-1. This study evaluated the symbiotic effects of Y63-1 inoculation on Zhongdou 63 (ZD63) in native environments and under different nitrogen levels. The evaluation of symbiotic effects in native environments was conducted through pot experiments and field trials. Pot experiments were performed in greenhouse using three soil types. Field trials were conducted in three regions with different soil nitrogen levels. The symbiotic effect of soybean ZD63 inoculated with Y63-1 under different nitrogen levels was investigated through pot experiments in greenhouse. The results showed that Y63-1 is more competitive than the indigenous rhizobia of the three soil types in the nodulation of soybean ZD63. The nodulation ability and yield-related traits of soybean ZD63 were improved after inoculation with Y63-1 in the three regions, especially in Hanchuan, where the soil nitrogen level is relatively rich. The symbiotic effect of soybean ZD63 inoculated with Y63-1 in a pot experiment with four levels of N from 0 to 3.75 mmol/L was superior at N 2.81 mmol/L. Our findings provided technical support for the application of Y63-1 in China, and a theoretical basis for increasing the yield potential of soybean through inoculation with highly efficient rhizobia in agricultural production.

Keywords: ZD63; Y63-1 rhizobium; symbiotic nitrogen fixation; nodulation phenotype; yield; nitrogen level

check for updates

Academic Editors: Jerzy Wielbo and Caterina Morcia

Received: 16 October 2025
Revised: 6 November 2025
Accepted: 11 November 2025
Published: 19 November 2025

Citation: Lu, L.; Leng, P.; Jin, F.; Lu, J.; Hu, Q.; Liang, W.; Huang, Y.; Zhang, C.; Li, C.; Xu, Z.; et al. Evaluation of the Symbiotic Effects of *Bradyrhizobium elkanii* Y63-1 Inoculation on Soybean Zhongdou 63. *Agronomy* **2025**, *15*, 2649. https://doi.org/10.3390/agronomy15112649

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Soybean (*Glycine max*) originated in China and is one of the most important sources of plant-based protein and edible vegetable oil [1]. Soybean has elevated protein content, so it necessitates higher nitrogen inputs compared to other crops for optimal production [2,3]. The nitrogen resources required for soybean production mainly come from three aspects, including free nitrogen in the soil, artificially applied chemical nitrogen fertilizers [4] and symbiotic nitrogen fixation (SNF) [5]. In China, excessive use of industrial nitrogen fertilizers is a prevalent issue in soybean production, which, combined with the slow growth rate of the yield, results in a phenomenon of "fertilizer increase without yield increase" and environmental pollution [6]. SNF by rhizobium is a sustainable, green way to

maintain soil fertility without chemical energy consumption, and approximately 40–70% of nitrogen sources in soybean production are derived from SNF [5]. Therefore, maximizing the role of SNF in soybean production is crucial for reducing costs, improving yields, and protecting the environment.

It is estimated that approximately 70% of global nitrogen resources come from biological nitrogen fixation [7], over 60% of which are from SNF between legumes and rhizobia [8]. Currently, soybean-producing countries inoculate rhizobia in approximately 80% of their soybean production processes [9], such as in Brazil, the United States, and Argentina [10], and the inoculation coverage reaches 70–95% of the total cultivated area. Although these countries have industrialized systems for rhizobial production, they apply only a small amount of basal nitrogen fertilizer or no nitrogen fertilizer [11]. This planting mode has significantly reduced nitrogen fertilizer usage, alleviating the problems of greenhouse gas emissions and water eutrophication caused by excessive fertilization [12]. However, in China, the application of rhizobial inoculants accounts for only 1–2% of the total soybean planting area, and nitrogen supplementation still relies heavily on the usage of chemical fertilizers [13], suggesting that soybean rhizobial inoculants have great application prospects in China.

Previous studies have shown that inoculating with rhizobia can increase soybean yields [14,15]; however, there are also some rhizobial inoculants that do not exert positive effects on yield and can even reduce it [16,17], especially rhizobia with weaker nodulation competition abilities [18,19]. The main reason is that a large number of indigenous rhizobia, with low nitrogen fixation efficiency and high nodulation competitiveness, are distributed in the soil of various soybean producing areas and often lead to sub-optimal inoculation effects of endogenously added rhizobia [20]. The studies on the symbiotic compatibility between rhizobia isolated from Xinjiang and different soybean varieties revealed significant differences in nodulation ability among different rhizobium–soybean combinations [21]. In general, it is important to identify soybean rhizobia that have high affinity with the main local soybean varieties and are adaptable to the local soil and climate conditions. Three *Mesorhizobium huakuii* strains isolated from the local area of Inner Mongolia significantly increased the biomass accumulation of local soybean cultivars under low-nitrogen conditions [1]. The salt–alkali-tolerant rhizobia isolated from wild soybean nodules in saline alkali soil can tolerate 2% NaCl, alleviate salt stress on soybean seedlings and improve soybean productivity in saline–alkali soil [22].

In our previous studies, we isolated and identified a new *Bradyrhizobium elkanii* strain Y63-1 from a high-yield demonstration field of soybean variety soybean ZD63 in Honghu, Hubei Province [12], and the nodulation experiments in greenhouse indicated that Y63-1 is a highly efficient broad-spectrum soybean rhizobium [23]. In the present study, we evaluated the competitive nodulation ability of Y63-1 inoculation on soybean ZD63 in different soils, and the symbiotic effects of soybean ZD63 inoculated with Y63-1 under different nitrogen levels. The results provide technical support for the practical application of Y63-1 strain and its corresponding field management strategies.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions in Pot Experiments

The seeds of ZD63 were surface sterilized and grown in a greenhouse with a 12/12 or 13/11 h day/night regime and 60–70% relative humidity (RH) at 25~28 °C. For pot experiments in three soil types, the seedlings were grown in pots (diameter 15 cm, height 20 cm) filled with soil and sterilized vermiculite, and among them, each soil was mixed with sterilized vermiculite pre-inoculated with Y63-1 at a 10:1 (*w*/*w*) ratio (total weight

2.5 kg). For pot experiments under different nitrogen levels, the seedlings were grown in pots filled with sterilized vermiculite.

2.2. Preparation of Rhizobial Inoculant

The *B. elkanii* Y63-1 strain was stored at −80 °C in our laboratory. The Y63-1 strain was streaked on YMA (Yeast Extract Mannitol Agar) plates supplemented with 100 μg/mL ampicillin and 100 μg/mL carbenicillin and cultured at 28 °C for about 4 days. We selected a small number of colonies and cultured them in the corresponding YMA liquid medium at 28 °C for 3–5 days. For pot experiments, we collected Y63-1 strain cells by centrifugation and re-suspended them in sterilized water until the OD value was 0.6~1.0 and then inoculated soybean ZD63. For field trials, the cultured Y63-1 strains with OD value > 1.0 were mixed with sterilized vermiculite and soybean ZD63; the volume ratio of vermiculite to seeds was 2:1, and the mass ratio of the Y63-1 strain suspension to seeds was 1:3. After thoroughly mixing the three components, the mixture was air-dried for approximately 30 min, and then the seeds and vermiculite were evenly sown together.

2.3. Culture Medium and Reagents

YMA medium composition (except for yeast extract, the chemicals were obtained from Sunopharm Chemical Reagent Co., Ltd., Shanghai, China) (1 L): Yeast extract 0.4 g/L (Sigma-Aldrich, St. Louis, MO, USA), Sucrose 10 g/L, $MgSO_4$ 0.2 g/L, K_2HPO_4 0.5 g/L, NaCl 0.1 g/L, $CaCl_2 \cdot 6H_2O$ 0.1 g/L, 4 mL trace element solution (H_3BO_3 5 g/L, Na_2MnO_4 5 g/L).

Liquid Fertilizer (CHINOOK, Ararat (Canton) Biotechnology Co., Ltd., Guangzhou, China) (1 L): Nutrient base powder (KH_2PO_4 0.5 mmol/L, $MgSO_4$ 0.25 mmol/L, K_2SO_4 0.25 mmol/L, Fe-citrate 0.05 mmol/L, H_3BO_3 2 mmol/L, $ZnSO_4$ 0.5 mmol/L, $CuSO_4$ 0.2 mmol/L, $CoSO_4$ 0.1 mmol/L, Na_2MoO_4 0.1 mmol/L, $MnSO_4$ 1 mmol/L), $CaCl_2$ 1 mmol/L, NH_4NO_3 content for different nitrogen concentration gradient experimental groups: 0 mmol/L (N1), 1.82 mmol/L (N2), 2.81 mmol/L (N3), 3.75 mmol/L (N4).

2.4. Determination of Nitrogen, Phosphorus, and Potassium Content in Soil

For pot experiments in greenhouse, soil samples were collected from Ezhou (Hubei, HB), Xuchang (Henan, HN), and Dengping (Shandong, SD). The soil samples were collected using a three-point sampling method. For each point, we chose approximately one square meter plot of land and mixed the soils together after sampling using the five-point method. The soil was collected from the surface (between 0 and −20 cm) using a soil auger and analyzed. The test results are as follows: total nitrogen 1.79 g/kg, total phosphorus 0.62 g/kg, and total potassium 20.27 g/kg in Hubei soil; total nitrogen 1.20 g/kg, total phosphorus 1.06 g/kg, and total potassium 21.04 g/kg in Henan soil; total nitrogen 1.18 g/kg, total phosphorus 0.97 g/kg, and total potassium 19.53 g/kg in Shandong soil.

For field trials, the sampling method for the soil was the same as mentioned above. The test results are as follows: pH 4.68, organic matter 15.2 mg/kg, available N 116.1 mg/kg, available P 60.0 mg/kg, and available K 111.9 mg/kg in Zhumadian (ZMD) soil; pH 6.71, organic matter 16.0 g/kg, available N 99.2 mg/kg, available P 17.5 mg/kg, and available K 200.6 mg/kg in Hanchuan (HC) soil; the Ezhou (EZ) soil sample is the same as the Hubei soil sample mentioned above. According to the nutrient classification standard of the second national soil survey (Table 1) [24], the soil nitrogen contents at Zhumadian and Hanchuan are classified as relatively rich, while the soil at Ezhou is classified as rich.

Table 1. Nutrient classification standard of the second national soil survey.

Indicator	I (Extremely Rich)	II (Rich)	III (Relatively Rich)	IV (Moderate)	V (Poor)	VI (Extremely Poor)
Organic matter (g/kg)	>40	30~40	20~30	10~20	6~10	<6
Total N (g/kg)	>2	1.5~2	1~1.5	0.75~1	0.5~0.75	<0.5
Total P (g/kg)	>1	0.8~1	0.6~0.8	0.4~0.6	0.2~0.4	<0.2
Total K (g/kg)	>25	20~25	15~20	10~15	5~10	<5
Available N (mg/kg)	>150	120~150	90~120	60–90	30~60	<30
Available P (mg/kg)	>40	20~40	10~20	5~10	3–5	<3
Available K (mg/kg)	>200	150~200	100~150	50~100	30–50	<30

2.5. Pot Experiments in Three Soil Types

The three soil types were collected from Ezhou (HB), Xuchang (HN), and Dengping (SD). Two treatments were established: water-inoculated control and Y63-1 rhizobial inoculation, each with three replicates. Twenty-four plants per replicate were grown, half for nodulation and half for yield evaluation. The soils were mixed with vermiculite pre-inoculated with Y63-1 at a 10:1 (*w*/*w*) ratio. The compound fertilizer was applied at a rate of 0.208 g to 1 kg soil, and no other nitrogen fertilizer was added. The plants were grown in a greenhouse under short-day conditions (12 h light/12 h dark, 25~28 °C). At the flowering stage (35 days), we cut the plants from the cotyledons to measure the fresh weights of above-ground parts and plant heights, removed the root systems from the pots with soil, cleaned the root systems and separated the nodules, then measured the numbers and dry weights of the root nodules after air drying. At the mature stage, each plant was harvested individually, and the grain weight of each plant was measured. Subsequently, we mixed the grains with the same treatment and measured the hundred-grain weight three times.

For the nodulation competitiveness testing of Y63-1 strain, 5–8 larger nodules within 0–3 cm of each of the root systems were randomly selected and washed with sterile water. On a sterile workbench, the separated nodules were surface-sterilized in 75% ethanol for 5 min, followed by 3.5% sodium hypochlorite for 6 min. After sterilization, the nodules were rinsed thoroughly with sterile water to remove any residual disinfectant, then cut open and gently crushed to obtain the nodule extracts. We mixed the nodule extracts and then coated them on YMA plates with or without Amp and Car antibiotics. The plates were incubated at 28 °C for 4–6 days to observe the growth of the rhizobia.

2.6. Pot Experiments Under Different Nitrogen Levels

The experiment was conducted in vermiculite with eight groups, including two treatments (a water-inoculated control and Y63-1 rhizobial inoculation) and four nitrogen levels (N1, N2, N3, and N4). Each treatment consisted of seven replications with eight plants per replication. Among them, two replications were used for sampling, and five replications were used for yield trait evaluation. ZD63 soybeans were grown in a greenhouse under a 13/11 h day/night cycle, with 60–70% relative humidity (RH) at 25–28 °C. From cotyledon abscission to pod maturity, 2 L of liquid fertilizer was supplied weekly, directly applied into every pot (8 plants). Chlorophyll SPAD values were measured 17 days after inoculation. Nodulation phenotypes, including plant height, shoot fresh weight, root length, root dry weight, nodule number, and nodule dry weight, were quantified at the flowering stage (40 days after inoculation), and stem node number, number of fruiting branches, grain weight, and grain number per plant were measured at maturity.

2.7. Field Experiment Design and Indicator Measurement

The field trial in Ezhou was conducted from June to October 2023 at the experimental base of the Hubei Academy of Agricultural Sciences. Two treatments included a control (no inoculation) and Y63-1 inoculation, with three replications for each treatment. Each

plot (8.4 m^2) had 7 rows (3 m length, 0.4 m line spacing, 0.1 m plant spacing). There were 5 protective rows surrounding the trial. At the pod-setting stage, 10 plants from the second row were sampled per plot to measure plant height, nodule number, and nodule dry weight. At the mature stage, the middle 5 rows (about 5 m^2) were harvested to detect the yield-related traits.

The field trial in Hanchuan was conducted from June to October 2024 at the Hanchuan base of the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences. The two treatments included a control (no inoculation) and Y63-1 inoculation, with 12 replications for each treatment. Each plot (6 m^2) had 5 rows (3 m length, 0.4 m line spacing, 0.1 m plant spacing). We removed the border plants and also selected 5 m^2 to detect the yield-related traits. The methods and period for measuring relevant indicators were as described in the field trial in Ezhou.

The field trial in Zhumadian was conducted from June to October 2024 at the base of the Zhumadian Academy of Agricultural Sciences. Two treatments included a control (no inoculation) and Y63-1 inoculation, with 9 replications for each treatment. Each plot (7.2 m^2) had 6 rows (3 m length including furrow). The methods and period for measuring relevant indicators were as described in the field trial in Ezhou or Hanchuan.

2.8. Statistical Analysis

According to the central limit theorem, the collected field experimental data was assumed to follow a normal distribution. Statistical significance was evaluated using the *t*-test, with all analyses conducted under the assumption of equal variances between two samples. Differences were considered significant at $p \leq 0.05$. Graphs were generated using GraphPad Prism 9.5.0, and data analysis and tabulation were performed in Microsoft Excel 2016.

3. Results

3.1. The Nodulation Phenotype of Soybean ZD63 Was Significantly Improved After Inoculation with Y63-1 in Three Soil Types from Different Regions

In order to investigate the competitive nodulation ability of the Y63-1 strain, we first conducted a pot experiment on Y63-1 inoculation in ZD63 using soils from three soybean planting areas in Henan (HN), Hubei (HB), and Shandong (SD). The original nitrogen levels of these three soil types are nearly the same, and the irrigation levels of the nutrient solution in these three soil types also remained consistent during the planting process (Section 2). The results showed that the number and dry weight of nodules in soybean ZD63 significantly increased after inoculation with Y63-1 in these three soil sources (Figure 1). Among them, the nodule numbers of potted soybean ZD63 in the Henan soil significantly increased by 30.9%, and the dry weights of the root nodules significantly increased by 21.5%. The nodule numbers of potted soybean ZD63 in the Hubei soil increased significantly by 40.4%, and the dry weight of the root nodules increased significantly by 23.3%. The nodule numbers of potted soybean ZD63 in Shandong soil significantly increased by 24.9%, and the dry weight of the root nodules significantly increased by 30.0% (Figure 1). Collectively, these results indicate that Y63-1 inoculation significantly improved the nodulation ability of soybean ZD63 grown in three different native environments (soils) with the same nitrogen level.

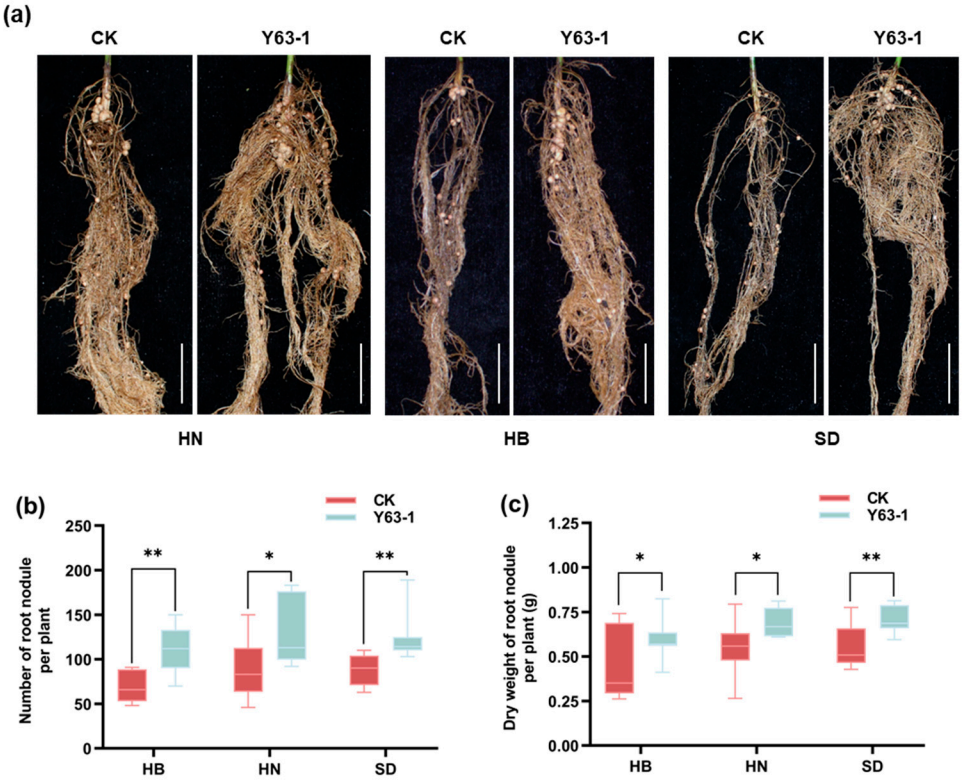


Figure 1. Nodulation phenotype of soybean ZD 63 inoculated or uninoculated with Y63-1 in soil samples from Hubei (HB), Henan (HN), and Shandong (SD). (**a**) Representative images of roots and nodules of ZD63 inoculation with Y63-1 and control groups in three soil types. Photographs were taken 35 days after inoculation. Bars, 5 cm. (**b**) Mean numbers of nodules per plant. The numbers of plants measured in the control groups in HB, HN, and SD were 8, 10, and 11, respectively, and for Y63-1, the number of plants in the inoculation groups were 11, 6, and 11, respectively. (**c**) The mean dry weight per plant. The numbers of plants measured in the control groups in HB, HN, and SD were 10, 10 and 11, respectively, and the number of plants in the Y63-1 inoculation groups were 11, 6, and 11, respectively. The boxes represent the interquartile range (middle 50% of the data), the horizontal line within each box indicates the median, and the whiskers denote the data range. Pairwise *t*-tests (equal variance assumed) were used to determine significant differences. Significant differences are indicated by asterisks (*, $p \leq 0.05$; **, $p \leq 0.01$).

3.2. The Yield-Related Traits of Soybean ZD63 Were Improved After Inoculation with Y63-1 in Three Soil Types from Different Regions

In order to further evaluate the yield-related traits of ZD63 after inoculation with Y63-1, we conducted statistical analyses of plant height, fresh weight of above-ground parts, grain weight per plant, and hundred-grain weight in the above-mentioned pot nodulation experiment (Figure 2). The results revealed that the plant height of potted ZD63 in Henan soil significantly increased by 9.3% after inoculation with Y63-1, whereas no significant differences were observed in potted ZD63 in soils from Hubei and Shandong. The above-ground fresh weight of potted ZD63 increased by 11.5%, 6.1%, and 9.6% after inoculation with Y63-1 in Henan, Hubei, and Shandong soils, respectively. The grain weight per plant of potted ZD63 was also enhanced by 12.3%, 15.7%, and 1.1% in these three soil types, respectively. Notably, the hundred-grain weight of ZD63 exhibited significant increases of 4.0%, 3.7%, and 4.1% after inoculation with Y63-1 in Henan, Hubei, and Shandong soils, respectively (Figure 2). Collectively, these results suggest that Y63-1 inoculation significantly improved the yield-related traits of ZD63 grown in different native environments (soil).

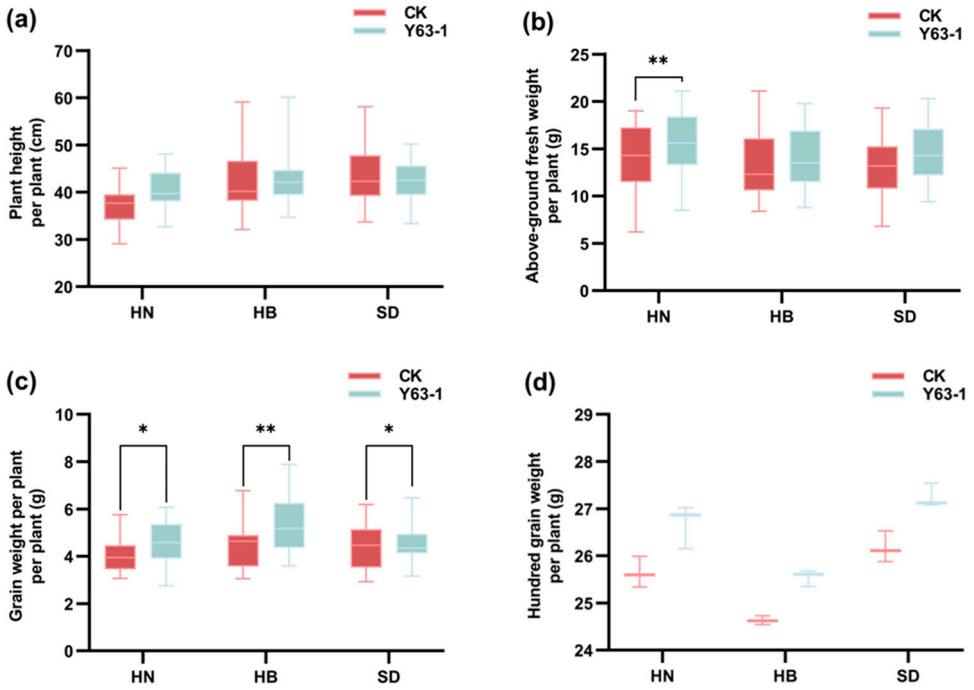


Figure 2. Yield-related traits of soybean ZD 63 inoculated or uninoculated with Y63-1 in three soil samples from Hubei (HB), Henan (HN), and Shandong (SD). (**a**) The mean plant height per plant with standard error (SE). The numbers of plants measured in the control groups in HN, HB, and SD were 21, 24, and 25, respectively, and in the Y63-1 inoculation groups were 19, 23, and 23, respectively. (**b**) The mean above-ground fresh weight per plant with SE. The numbers of plants measured in the control groups in HN, HB, and SD were 21, 23, and 25, respectively, and in the Y63-1 inoculation groups were 19, 19, and 23, respectively. (**c**) The mean grain weight per plant with SE. The number of plants measured in the two groups in HN, HB, and SD was 11 for each group. (**d**) The mean hundred-grain weight per plant with SE. The number of samples for the two groups in HN, HB, and SD was 3 for each group. The boxes represent the interquartile range (middle 50% of the data), the horizontal line within each box indicates the median, and the whiskers denote the data range. Pairwise *t*-tests (equal variance assumed) were used to determine significant differences. Significant differences are indicated by asterisks (*, $p \leq 0.05$; **, $p \leq 0.01$).

3.3. Y63-1 Strain Is More Competitive in Nodulation of Soybean ZD63 Compared to Indigenous Rhizobia of the Three Soil Types from Different Regions

To assess whether Y63-1 strain is the advantageous strain in the nodules of potted soybean ZD63 in the three soil types, we isolated and purified the rhizobia from the nodules of potted soybean ZD63 inoculated with Y63-1. According to our previous study, Y63-1 strain has resistance to four antibiotics, including Car, Cep, Amp, and Rif [12]; we thus screened the isolated rhizobia on YMA media with or without antibiotics. As shown in Figure 3, for the rhizobia isolated from the nodules of soybean ZD63 uninoculated with Y63-1 (CK), very few colonies appeared on the YMA plates with two antibiotics (Amp+Car). Rhizobia isolated from the nodules of soybean ZD63 inoculated with Y63-1 grew well on non-antibiotic YMA plates, and most of these rhizobia could be grown on antibiotic YMA plates (Figure 3). These results imply that the Y63-1 strain is more competitive than the indigenous rhizobia in the three different soils during the nodulation of soybean ZD63.

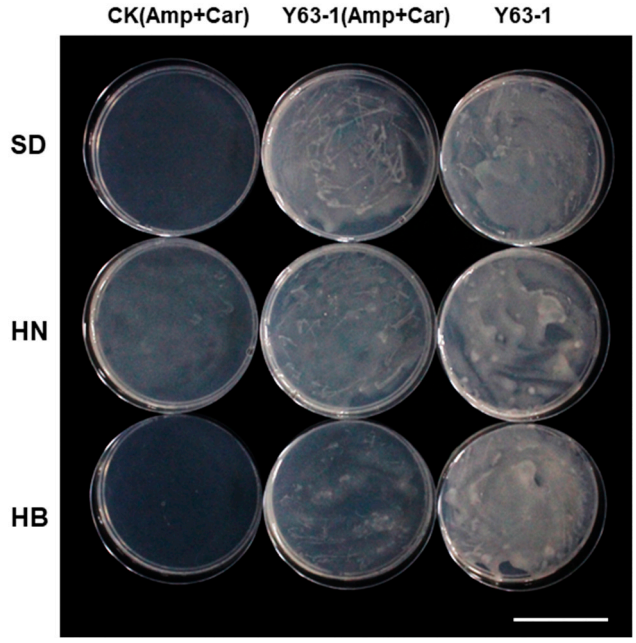


Figure 3. The growth status of rhizobia isolated from the nodules of potted soybean ZD63 inoculated or uninoculated with Y63-1. SD, Shandong. HN, Henan. HB, Hubei. For CK (Amp+Car), the rhizobia were isolated from the nodules of potted soybean ZD63 uninoculated with Y63-1 and screened on YMA media with Amp and Car antibiotics. For Y63-1 (Amp+Car), the rhizobia were isolated from the nodules of potted soybean ZD63 inoculated with Y63-1 and screened on YMA media with Amp and Car antibiotics. For Y63-1, the rhizobia were isolated from the nodules of potted soybean ZD63 inoculated with Y63-1 and screened on YMA media with no antibiotics. The white bar represents 5 cm.

3.4. Symbiotic Effects and Plot Yields of Soybean ZD63 Were Improved After Inoculation with Y63-1 in Three Areas with Different Nitrogen Levels

To evaluate the application potential of Y63-1 inoculated in soybean ZD63 in the field, we conducted field trials to detect the symbiotic effects of soybean ZD63 inoculated with Y63-1 in Zhumadian (ZMD), Hanchuan (HC), and Ezhou (EZ) soil. The soil nitrogen level in EZ is classified as "II (Rich)", the soil nitrogen level in ZMD is close to the upper limit of the "III (Relatively rich)" classification, and the soil nitrogen level in HC is near the lower limit of the "III (Relatively rich)" classification (Section 2). The results revealed that the nodulation ability and plot yield of soybean ZD63 increased after inoculation with Y63-1 in these three areas (Figure 4). The nodulation phenotypes were measured at pod-setting stage. Soybean ZD63 inoculated with Y63-1 showed significantly higher nodule numbers than the controls in all three areas. Among them, the nodule numbers per plant of soybean ZD63 increased by 111.4% after inoculation with Y63-1 in ZMD, 15.9% in HC, and 107.9% in EZ. The nodules' dry weight per soybean ZD63 plant increased by 52.9% after inoculation with Y63-1 in ZMD, 3.7% in HC, and 88.7% in EZ. The plant height per soybean ZD63 plant increased significantly by 15.9% after being inoculated with Y63-1 in HC, while only smaller increases of 1.8% and 2.1% were observed in ZMD and EZ, respectively. Correspondingly, the plot yield of soybean ZD63 increased significantly by 5.6% after inoculation with Y63-1 in HC, 2.4% in ZMD, and 2.8% in EZ (Figure 4). Together, these results demonstrate that the symbiotic effects and plot yields of soybean ZD63 can be significantly improved by inoculation with Y63-1 in diverse native environments with varying nitrogen contents, highlighting the adaptability of Y63-1 to high nitrogen soils and complex indigenous rhizobium populations in soybean production areas in China.

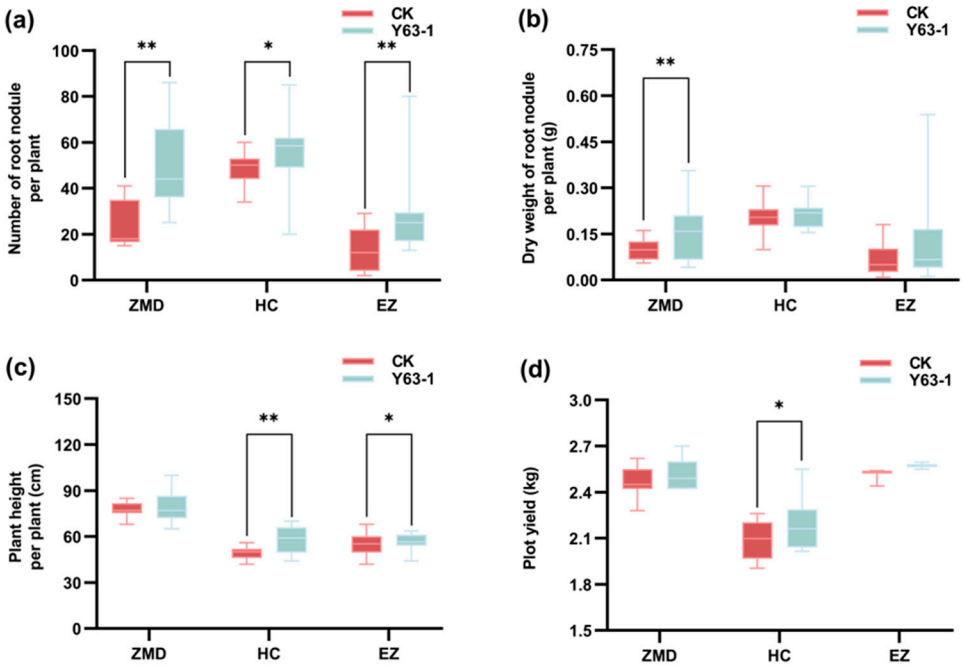


Figure 4. Yield-related traits and nodulation phenotypes of soybean ZD63 in Zhumadian (ZMD), Hanchuan (HC), and Ezhou (EZ) with different nitrogen levels. (**a**) The mean number of root nodules per plant with SE. The numbers of plants measured in the control groups in ZMD, HC, and EZ were 9, 19, and 17, respectively, and in the Y63-1 inoculation groups were 10, 14, and 17, respectively. (**b**) The mean dry weight of the root nodules per plant with SE. The numbers of plants measured in the control groups in ZMD, HC, and EZ were 9, 23, and 16, respectively, and in the the Y63-1 inoculation groups were 12, 17, and 17, respectively. (**c**) The mean plant height per plant with SE. The numbers of control plants for control groups in ZMD, HC, and EZ were 28, 23, and 24, respectively, and for Y63-1 inoculation groups were 29, 21, and 23, respectively. (**d**) The mean plot yield with SE. The numbers of plots for two groups in ZMD, HC, and EZ were both 7, 12, and 3, respectively. The boxes represent the interquartile range (middle 50% of the data), the horizontal line within each box indicates the median, and the whiskers denote the data range. Pairwise *t*-tests (equal variance assumed) were used to determine significant differences. Significant differences are indicated by asterisks (*, $p \leq 0.05$; **, $p \leq 0.01$).

3.5. Symbiotic Effects of Y63-1 Inoculation on Soybean ZD63 Grown in Greenhouse Under Different Nitrogen Levels

As shown in Figure 4, soybean ZD63 inoculated with Y63-1 displayed the best symbiotic effects in Hanchuan, where the soil nitrogen level is relatively lower than in the other two areas, suggesting that nitrogen level is a critical factor affecting the symbiotic effects of soybean ZD63 inoculated with Y63-1. To investigate the impact of nitrogen level on the symbiotic effects of soybean ZD63 inoculated with Y63-1, we conducted a pot experiment at different nitrogen concentrations (N1, 0 mmol/L; N2, 1.82 mmol/L; N3, 2.81 mmol/L; N4, 3.75 mmol/L) in greenhouse. Chlorophyll content, above-ground part biomass, root development, nodulation ability, and yield-related traits were statistically analyzed in the groups of soybean ZD63 inoculated and un-inoculated with Y63-1.

The chlorophyll content increased with the increase of nitrogen level, both in the group of ZD63 inoculated with Y63-1 and the group without Y63-1. At the same nitrogen level, the chlorophyll content in the inoculated group was significantly higher than in the un-inoculated group (Figure 5a). Similarly to the chlorophyll content, the above-ground plant biomass also increased with the increase in nitrogen level, and at N4, the above-ground plant biomass of the inoculated group was significantly higher than the un-inoculated group (Figure 5b). To investigate the inoculation effects of Y63-1 on the root development

and symbiotic phenotype of ZD63 under different nitrogen concentrations, we statistically analyzed root dry weight and nodule dry weight (Figure 5c,d). The root dry weight of the inoculated plants increased with the increase in nitrogen level, and at N1, N3, and N4, the inoculated group was significantly higher than the un-inoculated group. The nodule dry weight decreased with the increase of nitrogen level in the un-inoculated group, while there was no significant change with the increase in nitrogen level in the inoculated group. Specifically, the nodule dry weight per plant increased from 0.98 g in the un-inoculated group and 1.10 g in the inoculated group at N1, to 0.77 and 0.97 at N3, and to 0.79 and 0.95 at N4 (Figure 5d). These results expose that the symbiotic effects of ZD63 inoculated with Y63-1 at N3 and N4 are superior to the other two nitrogen concentrations, highlighting the strong adaptability of Y63-1 to high nitrogen environments. The results are consistent with the conclusions drawn from the field trials.

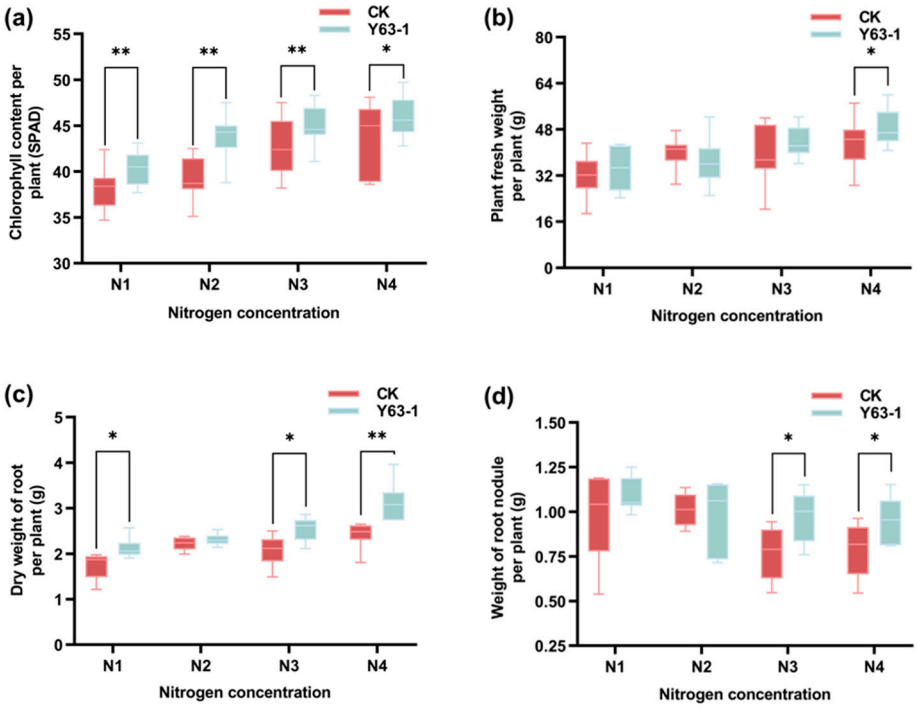


Figure 5. Symbiotic phenotypes of soybean ZD 63 inoculated or uninoculated with Y63-1 at four nitrogen concentrations. (**a**) Chlorophyll content per plant. The numbers of plants measured in the two groups at N1-N4 were both 15. (**b**) Fresh weight per plant. The numbers of plants measured in the control groups in N1-N4 were 14, 14, 13, and 13, respectively, and in the Y63-1 inoculation groups were 15, 14, 13, and 14, respectively. (**c**) Dry weight of root per plant. The numbers of plants measured in the two groups in N1-N4 were both 6, 6, 6, and 6, respectively. (**d**) Dry weight of root nodules per plant. The numbers of plants measured in the two groups in N1-N4 were 6, 6, 6, and 6, respectively. Pairwise *t*-tests (equal variance assumed) were used to determine significant differences between treatments. Boxes represent the interquartile range (middle 50% of the data), horizontal lines within boxes indicate medians, and whiskers show the data range. Significant differences are indicated by asterisks (*, $p \leq 0.05$; **, $p \leq 0.01$).

Yield enhancement serves as a key indicator for evaluating the practical application potential of rhizobia. To evaluate the effect of Y63-1 inoculation on the yield traits of soybean ZD63, several parameters, including the number of fruiting branches, stem nodes, grain number per plant, and grain weight per plant were measured and compared between the inoculated and un-inoculated groups. The number of grains in soybean ZD63 in the inoculated group increased by 4.5%, 7.6%, 14.8%, and 3.3% compared to the un-inoculated group at N1, N2, N3, and N4, respectively, and reached its highest value at N3 (Figure 6a). The grain weight of soybean ZD63 in the inoculated group showed increases of 7.3%, 2.0%,

7.5%, and 5.1% compared to the un-inoculated group at N1, N2, N3, and N4, respectively, and also reached its highest value at N3 (Figure 6b). As shown in Figure 6c, the inoculated plants exhibited significantly higher numbers of fruiting branches compared to the un-inoculated groups, although this parameter showed no consistent relationship with nitrogen levels. Similarly to symbiotic effects, the yield-related traits of soybean ZD63 were improved by inoculation with Y63-1, especially at N3 (Figure 6). Collectively, the nodulation effect of soybean ZD63 inoculated with Y63-1 at N3 was superior to the other three nitrogen concentrations.

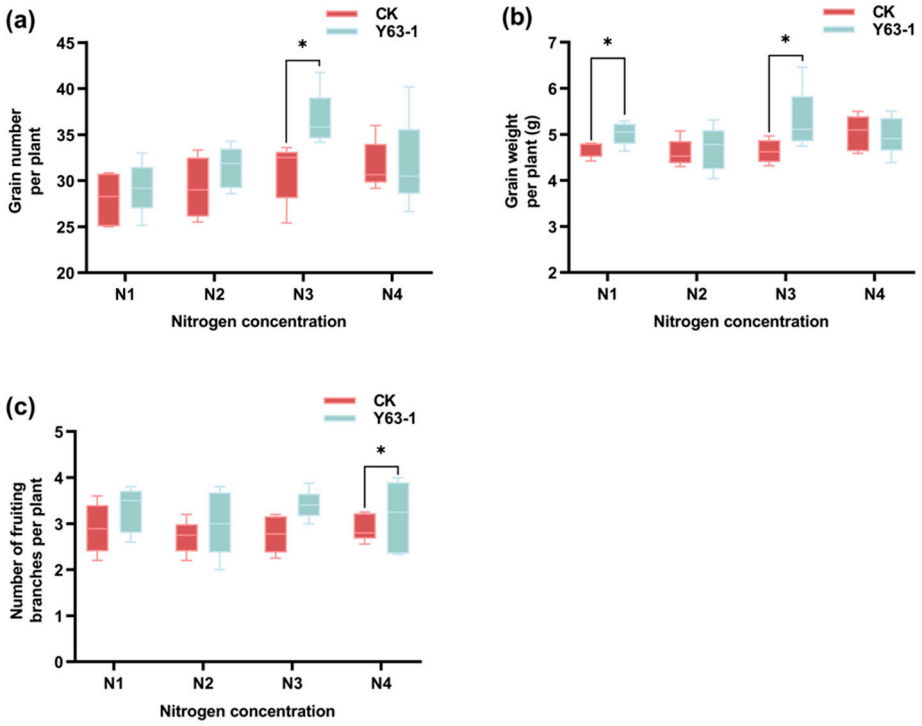


Figure 6. Yield-related traits of soybean ZD 63 inoculated or uninoculated with Y63-1 at four nitrogen concentrations. Both two treatments included 5 biological replicates (n = 5), with 8 plants per replicate; the data of each replicate represents the average value of 8 plants. (**a**) Grain number per replicate. (**b**) Grain weight per replicate. (**c**) Number of fruiting branches per replicate. Pairwise t-tests (equal variance assumed) were used to determine significant differences between treatments. Boxes represent the interquartile range (middle 50% of the data), horizontal lines within boxes indicate medians, and whiskers show the data range. Significant differences are indicated by asterisks (*, $p \leq 0.05$).

4. Discussion

China acts as the center of soybean production and has a long history of soybean cultivation, possessing abundant soybean germplasm resources as well as diverse rhizobial strains. However, the application of rhizobial inoculants is far behind other major soybean-producing countries, mainly due to poor compatibility between the rhizobial strains and soybean varieties, inadequate inoculation techniques, and incompatibility with mechanical sowing, as well as the inhibitory effects of chemical nitrogen fertilizer on nodule formation and nitrogen fixation [13]. The presence of elite rhizobial strains, which are characterized by their broad compatibility with various soybean cultivars and strong adaptability to diverse environmental conditions, is a prerequisite for the effective substitution of chemical nitrogen with symbiotic nitrogen fixation [25]. In our previous study, we identified a new *B. elkanii* Y63-1, which is a highly efficient broad-spectrum soybean rhizobium strain [26], and in this report, very good symbiotic effects were exhibited by Y63-1 inoculated on

soybean ZD63 under high nitrogen conditions and in variable environments. Our findings provide a theoretical foundation for the practical application of the Y63-1 strain.

The inoculation effects of exogenous rhizobia largely depend on the competitive nodulation capacity of the strain with indigenous rhizobia [27], which possess high nodulation competitiveness bases on their adaptation to the local soil and climate conditions [28]. Some advantageous rhizobia isolated from local soils exhibit stronger competitive nodulation capacity [29]; for example, Jovino reported that strain ESA 123, isolated from native soil, exhibited superior nodulation performance in its local environment compared to the commercial strain SEMIA 6144 [30]. Similarly, Wu found that inoculation with selected indigenous rhizobia significantly enhanced both nodulation and yield [31]. To investigate the competitive nodulation capacity of Y63-1, we firstly conducted a pot nodulation experiment of Y63-1 inoculation on ZD63 using soil from three soybean planting areas (Figures 1–3) and then performed field trials in three different areas (Figure 4). The results demonstrated that the Y63-1 strain is more competitive than indigenous rhizobia in both the tested soil and native environments; thus, Y63-1 inoculation on soybean ZD63 displays excellent nodulation phenotypes and yield-enhancing performance both in greenhouse and native field environments. In addition, we also investigated the application potential of Y63-1 in other soybean varieties (Zhongdou 41, Zhongdou 55, Zhudou 19, and so on), and the symbiotic effects of Y63-1 inoculation on these soybean varieties will be exposed in our future articles. These results provide a theoretical basis for the application of the Y63-1 strain in soybean production.

The compatibility between this rhizobia and soybean crops is not only influenced by genotype, but also by environment [32]. For example, rhizobial strains isolated from saline–alkali soils often exhibit superior nodulation performance under saline conditions but demonstrate reduced nodulation efficiency in normal soils [33]. Wu also reported that screened rhizobial strains generally outperformed wild strains; however, the optimal rhizobium–soybean combinations varied across different regions [34]. To test the adaptability of the Y63-1 strain to different environmental conditions, we performed field trials to detect the symbiotic effect of soybean ZD63 inoculation with Y63-1 in three regions (Figure 4). The results demonstrated that Y63-1 consistently promoted nodule formation and enhanced both the biomass and yield of ZD63 across all tested environments, which aligns with the findings from previous studies [29,35]. Our findings suggest that Y63-1-ZD63 symbiosis is suitable for the heterogeneous soil environments of multiple soybean-producing areas in China.

Previous studies have shown that soil nitrogen content significantly affects soybean nodulation traits [36]. Generally, the yield-enhancing effect of rhizobial inoculation is more pronounced under low-nitrogen conditions than in high-nitrogen environments [37]. For example, rhizobial inoculation without additional nitrogen application led to an 11% yield increase and the soil nitrogen content was classified as poor [11]. Similarly in this study, the yield-increasing effect of Y63-1 inoculation on soybean ZD63 in Hanchuan, with the lowest soil nitrogen content, was better than in the other two areas (Figure 4), and a stronger nitrogen-fixation capacity of Y63-1 inoculation on soybean ZD63 was observed in low-nitrogen environments (Figure 5). These results are consistent with previous findings, where nitrogen deficiency during early growth stages promoted nodule formation due to nutrient limitations [38]. Conversely, high nitrogen levels were found to inhibit rhizobial infection, nodulation, and nodule development. The similar phenomenon was found in the un-inoculated group, while the inhibitory effect of Y63-1 inoculation on soybean ZD63 was minimal (Figure 5), indicating that Y63-1 maintains a high nodulation capacity even under nitrogen-sufficient conditions, highlighting its potential application in nitrogen-over-fertilized agricultural systems. These findings suggest that the Y63-1 strain possesses

the potential to effective fix nitrogen across a wide range of soil nitrogen levels, which is different from the other rhizobial strains previously studied [33].

5. Conclusions

In summary, the Y63-1 strain was more competitive than indigenous rhizobia in the tested soil and displayed excellent nodulation phenotypes and yield-enhancing performance in both greenhouse and native field environments. The pot nodulation experiment with different nitrogen levels in greenhouse showed that the Y63-1 strain possesses the potential to effectively fix nitrogen across a wide range of soil nitrogen levels. Our current findings provide theoretical support for the field application of the Y63-1 strain, as well as a theoretical basis for increasing the yield potential of soybean ZD63 through inoculation with highly efficient rhizobia in production.

Author Contributions: L.L.: Data curation, Writing—original draft, Investigation, Validation, and Visualization; P.L.: Validation, Investigation, Data curation, and Visualization; F.J., J.L. and Q.H.: Validation, Investigation, and Visualization; W.L.: Writing—review and editing and Funding acquisition; Y.H., C.Z., C.L., Z.X., Z.Y. and S.C.: Formal analysis and Resources; S.Y.: Conceptualization, Supervision, Project administration, Data curation, Visualization, Writing—original draft, Writing—review and editing, and Funding acquisition; H.C.: Conceptualization, Supervision, Project administration, and Resources. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by funds from the National Key Research and Development Program of China (2024YFD1201400) to Songli Yuan, the Central Public-interest Scientific Institution Basal Research Fund (CAAS-ZDRW202416) to Songli Yuan, and the OCRI-CAAS Outstanding Young Talents Cultivation Program (110217160011519) to Wanwan Liang. The funding body played no role in the study design, sample collection, data analysis, data interpretation, and manuscript drafting.

Data Availability Statement: All data supporting the findings of this study are available within the paper, published online.

Conflicts of Interest: The authors declare that they have no competing interests.

Abbreviations

The following abbreviations are used in this manuscript:

ZD63	Zhongdou 63
YMA	Yeast Extract Mannitol Agar
SNF	Symbiotic nitrogen fixation
Car	Carbenicillin
Cep	Cephalosporin
Amp	Ampicillin
Rif	Rifampicin
HB	Hubei
HN	Henan
SD	Shandong
ZMD	Zhumadian
HC	Hanchuan
EZ	Ezhou

References

1. Tian, Y.; Wang, M.J.; Wang, Z.Q.; Li, S.N.; Zhao, W.X.; Jia, Y.P. Effects of *Rhizobial* Inoculation on Growth and Physiology of Gorage Soybean at Different Nitrogen Levels. *Acta Agrestia Sin.* **2025**, 1–11. Available online: https://link.cnki.net/urlid/11.3362.S.20250508.1712.002 (accessed on 15 October 2025).
2. La Menza, N.C.; Monzon, J.P.; Lindquist, J.L.; Arkebauer, T.J.; Knops, J.M.H.; Unkovich, M.; Specht, J.E.; Grassini, P. Insufficient nitrogen supply from symbiotic fixation reduces seasonal crop growth and nitrogen mobilization to seed in highly productive soybean crops. *Plant Cell Environ.* **2020**, *43*, 1958–1972. [CrossRef]
3. Leng, P. Isolation, Identification and Application of *Rhizobium* y63. Master's Thesis, Chinese Academy of Agricultural Sciences, Beijing, China, 2024. Available online: https://link.cnki.net/doi/10.27630/d.cnki.gznky.2024.000598 (accessed on 15 October 2025).
4. Ju, X.T.; Zhang, F.S. Thinking about nitrogen recovery rate. *Ecol. Environ. Sci.* **2003**, 192–197. [CrossRef]
5. Ciampitti, I.A.; de Borja Reis, A.F.; Córdova, S.C.; Castellano, M.J.; Archontoulis, S.V.; Correndo, A.A.; Antunes De Almeida, L.F.; Moro Rosso, L.H. Revisiting biological nitrogen fixation dynamics in soybeans. *Front. Plant Sci.* **2021**, *12*, 727021. [CrossRef]
6. Zhang, F.S.; Chen, X.P.; Vitousek, P. An experiment for the world. Nature. *Nature* **2013**, *497*, 33–35. [CrossRef]
7. Zhang, Q.L.; Lin, M.; Ping, S.Z. Biological nitrogen fixation and its application in sustainable agriculture. *Biotechnol. Bull.* **2008**, *18*, 1–4.
8. Santi, C.; Bogusz, D.; Franche, C. Biological nitrogen fixation in non-legume plants. *Ann. Bot.* **2013**, *111*, 743–767. [CrossRef]
9. Wang, Q.Q.; Sheng, J.D.; Pan, L.Y.; Cao, H.Y.; Li, C.C.; Lambers, H.; Wang, X.R. Soil property determines the ability of rhizobial inoculation to enhance nitrogen fixation and phosphorus acquisition in soybean. *Appl. Soil Ecol.* **2022**, *171*, 104346. [CrossRef]
10. Alves, B.J.R.; Boddey, R.M.; Urquiaga, S. The success of BNF in soybean in Brazil. *Plant Soil* **2003**, *252*, 1–9. [CrossRef]
11. Tian, Y.X.; Gao, J.F.; Cao, P.P.; Gao, Q.; Hua, F.J.; Wang, C.Y.; Zhu, G.X. Effects of nitrogen reducing and inoculation with rhizobium agents on the growth, yield and economic benefit of high protein summer soybean in huang-huai-hai region. *J. Hebei Agric. Sci.* **2022**, *26*, 72–77+82.
12. Sun, S.; Leng, P.; Yang, S.Q.; Zhou, S.X.; Zhang, C.J.; Huang, Y.; Shan, Z.H.; Yang, Z.L.; Hao, Q.N.; Chen, S.L.; et al. Isolation and identification of soybean rhizobium Y63 strain. *Chin. J. Oil Crop Sci.* **2024**, *46*, 920–929. [CrossRef]
13. Guan, D.W.; Li, J.; Cao, F.M.; Shen, D.L.; Jiang, X. Application of Soybean *Rhizobial* Inoculants in China: Existing Problems and Countermeasures. In Proceedings of the 11th National Symposium on Soil Microbiology, the 6th National Symposium on Soil Biology and Biochemistry, and the 4th National Symposium on Microbial Fertilizer Production Technology, Changsha, China, 18 October 2010. Available online: https://kns.cnki.net/KCMS/detail/detail.aspx?dbcode=CPFD&filename=TRXH201010001062 (accessed on 15 October 2025).
14. Subramanian, P.; Kim, K.; Krishnamoorthy, R.; Sundaram, S.; Sa, T. Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. *Plant Growth Regul.* **2015**, *76*, 327–332. [CrossRef]
15. He, C.h.; Gao, H.; Wang, H.; Guo, Y.; He, M.; Peng, Y.; Wang, X.L. GSK3-mediated stress signaling inhibits legume-rhizobium symbiosis by phosphorylating GmNSP1 in soybean. *Mol. Plant* **2021**, *14*, 488–502. [CrossRef]
16. Alam, F.; Bhuiyan, M.A.H.; Alam, S.S.; Waghmode, T.R.; Kim, P.J.; Lee, Y.B. Effect of *Rhizobium* sp. *BARIRGm901* inoculation on nodulation, nitrogen fixation and yield of soybean (*Glycine max*) genotypes in gray terrace soil. *Biosci. Biotechnol. Biochem.* **2015**, *79*, 1660–1668. [CrossRef]
17. Fiori, A.K.; de Oliveira Gutuzzo, G.; dos Santos Sanzovo, A.W.; de Souza Andrade, D.; de Oliveira, A.L.M.; Rodrigues, E.P. Effects of rhizobium tropici azide-resistant mutants on growth, nitrogen nutrition and nodulation of common bean (*Phaseolus vulgaris* L.). *Rhizosphere* **2021**, *18*, 100355. [CrossRef]
18. Yang, F.Q.; Xu, X.P.; Wang, W.; Ma, J.C.; Wei, D.; He, P.; Pampolino, M.F.; Johnston, A.M. Estimating nutrient uptake requirements for soybean using QUEFTS model in China. *PLoS ONE* **2017**, *12*, e0177509. [CrossRef]
19. Zhang, Y.; Liu, J.; Zhang, J.; Liu, H.; Liu, S.; Zhai, L.; Wang, H.; Lei, Q.; Ren, T.; Yin, C. Row ratios of intercropping maize and soybean can affect agronomic efficiency of the system and subsequent wheat. *PLoS ONE* **2015**, *10*, e0129245. [CrossRef] [PubMed]
20. Bogino, P.; Banchio, E.; Bonfiglio, C.; Giordano, W. Competitiveness of a *Bradyrhizobium* sp. strain in soils containing indigenous rhizobia. *Curr. Microbiol.* **2008**, *56*, 66–72. [CrossRef] [PubMed]
21. Hu, F.X.; Shen, H.L.; Zheng, B.; Yang, L.; Dong, Z.Y.; Wan, C.X. Biodiversity of indigenous rhizobia from soybean nodules in Xinjiang and its matching relationship with different soybean varieties. *Xinjiang Agric. Sci.* **2025**, 1–23. Available online: https://link.cnki.net/urlid/65.1097.S.20250224.1248.002 (accessed on 15 October 2025).
22. Guo, J.; Yang, F.; Han, J.C.; Wei, S.J.; Zhang, X.X. Isolation, identification, evaluation of soybean rhizobia from saline-alkali soil in Yellow River Delta. *Soil Fertil. Sci. China* **2025**, 231–238. [CrossRef]
23. Leng, P.; Jin, F.X.; Li, S.; Huang, Y.; Zhang, C.J.; Shan, Z.H.; Yang, Z.L.; Chen, L.M.; Cao, D.; Hao, Q.N.; et al. High efficient broad-spectrum *Bradyrhizobium elkanii* Y63-1. *Oil Crop Sci.* **2023**, *8*, 228–235. [CrossRef]
24. Li, Q.; Yang, J.; Guan, W.; Liu, Z.; He, G.; Zhang, D.; Liu, X. Soil fertility evaluation and spatial distribution of grasslands in Qilian Mountains Nature Reserve of eastern Qinghai-Tibetan Plateau. *PeerJ* **2021**, *9*, e10986. [CrossRef] [PubMed] [PubMed Central]
25. Helios, W.; Serafin-Andrzejewska, M.; Kozak, M.; Lewandowska, S. Impact of nitrogen fertilisation and inoculation on soybean nodulation, nitrogen status, and yield in a central European climate. *Agriculture* **2025**, *15*, 1654. [CrossRef]

26. Leng, P.; Jin, F.X.; Huang, Y.; Zhang, C.J.; Chan, Z.H.; Yang, Z.L.; Yuan, S.L.; Chen, H.F. Screening and optimization of culture medium for *Bradyrhizobium elkanii* Y63-1. *Soybean Sci.* **2024**, *43*, 641–647.
27. Unay, J.; Perret, X. Synthetic plasmids to challenge symbiotic nitrogen fixation between rhizobia and legumes. In *Methods in Rhizosphere Biology Research*; Reinhardt, D., Sharma, A.K., Eds.; Springer: Singapore, 2019; pp. 3–18. [CrossRef]
28. Wang, H.J.; Zeng, Q.F.; Wei, X.D.; Wang, J.C.H.; Wei, X.; Chen, C. The rhizobia of wild legume forage in guizhou tailings areas and determination of resistance. *Acta Agrestia Sin.* **2022**, *30*, 10.
29. Wang, Q.L.; Huo, X.X.; Zhang, H.; Huang, Y.H.; Hao, Y.R.; Li, Z.; Zheng, Z.H.; Guo, K. Isolation, identification and field application of soybean rhizobia in saline-alkali land of the Yellow River Delta. *Shandong Agric. Sci.* **2023**, *55*, 143–151. [CrossRef]
30. Jovino, R.S.; da Silva, T.R.; Rodrigues, R.T.; de Sá Carvalho, J.R.; Cunha, J.B.A.; de Lima, L.M.; Dos Santos, R.C.; Santos, C.E.; Ribeiro, P.R.; de Freitas, A.D. Elite Bradyrhizobium strains boost biological nitrogen fixation and peanut yield in tropical drylands. *Braz. J. Microbiol.* **2022**, *53*, 1623–1632. [CrossRef] [PubMed]
31. Wu, H.; Zhong, Z.D.; Fan, W.; Peng, Y.Q.; Du, S.; Chen, D.S.; Wang, X.L.; Li, Y.G. Symbiotic compatibility among eight elite soybean rhizobia strains and twenty-seven soybean cultivars from different planting regions. *Soybean Sci.* **2017**, *36*, 405–418.
32. Lan, X.J.; Jin, Y.L.; Yao, T.; Zhou, H.; Zhang, J.G.; Li, C.N.; Yang, X.M.; Chen, J.G. Isolation, identification and screening of rhizobium strains from white clover in Lanzhou. *Grassl. Turf.* **2022**, *42*, 132–138.
33. Xiang, Y.Z.; Zhang, W.; Tang, Z.J.; Fu, J.Y.; Li, Z.J.; Zhang, F.C. Effects of nitrogen fertilizer reduction and rhizobia inoculation on physiological growth, nitrogen use efficiency and yield of soybean. *Trans. Chin. Soc. Agric. Mach.* **2024**, *55*, 340–351.
34. Liu, X.J.; Zhang, Y.; Han, W.X.; Tang, A.H.; Shen, J.L.; Cui, Z.L.; Vitousek, P.; Erisman, J.W.; Goulding, K.; Christie, P.; et al. Enhanced nitrogen deposition over China. *Nature* **2013**, *494*, 459–462. [CrossRef]
35. Tian, J.X.; Liu, S.Y.; Wang, W.F.; Zheng, F.; Han, L.L.; Zhang, L.M. Diversity of soybean rhizobia in Northeast China and their application. *Chin. J. Appl. Ecol.* **2024**, *35*, 1850–1858. [CrossRef]
36. Xu, Y.X.; Xu, Y.; Li, L.; Huang, X.J.; Wang, D.S.; Wu, Q.Z.; Cheng, G.D.; Zhai, Y.L. Effect of Nitrogen Fertilizer and Rhizobium Inoculation on Nodulation and Growth of Southern Xinjiang Spring Soybean. *Soybean Sci.* **2021**, *40*, 241–248.
37. Basile, L.A.; Lepek, V.C. Legume–rhizobium dance: An agricultural tool that could be improved? *Microb. Biotechnol.* **2021**, *14*, 1897–1917. [CrossRef] [PubMed]
38. Mishra, P.K.; Bisht, S.C.; Mishra, S.; Selvakumar, G.; Bisht, J.K.; Gupta, H.S. Coinoculation of rhizobium leguminosarum-pr1 with a cold tolerant *Pseudomonas* sp. improves iron acquisition, nutrient uptake and growth of field pea (*Pisum sativum* L.). *J. Plant Nutr.* **2012**, *35*, 243–256. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.