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Response of Rhizobium Inoculation on Different Soybean (*Glycine max* L.) Varieties in Central Vietnam

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ABSTRACT

Background: Soybean (*Glycine max* L.) is an important legume crop in the human diet, which is grown mainly for its edible bean. Rhizobium inoculation can enhance soybean production, but its effectiveness depends on several factors, including crop management, environment, Rhizobium strains and soybean varieties. The study aimed to evaluate how different soybean varieties responded to Rhizobium inoculation in Hue city, Central Vietnam.

Methods: Pot and field experiments were conducted in coastal sandy soil in 2024 in two cropping seasons. Twelve treatments, including two factors of 6 soybean varieties combined with and without inoculation of Rhizobium (control), were laid out in a RCBD and split plot designs with three replications. Data was collected as nodule number, seed yield and nitrogen fixation efficiency.

Result: The results obtained showed significant differences ($p<0.05$) in nodules mean score, seed yields and N fixation between inoculated and no inoculated treatments. The variety DT90 responded to the Rhizobium inoculants recorded the best in all parameters under observation. Although further studies need to be conducted to obtain conclusive information on these nitrogen fixers, the study indicates that DT90, in combination with the right Rhizobium inoculants, could be recommended to replace conventional fertilization for coastal sandy soil in Central Vietnam.

Key words: N fixation, Rhizobium, Sandy soil, Soybean variety, Yield.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is known for its high protein content (roughly 40%) among the most cultivated crops (Ishaq *et al*., 2022) and is one of the most important legumes in Central Vietnam (Nguyen *et al*., 2017). It can also improve soil physic-chemical and biological properties by enriching the soil with nitrogen through biomass addition and N_2 fixation (c). In Central Vietnam, where over 90% of the soil is nitrogen-deficient, especially in coastal sandy soils (Hoang *et al*., 2019). Soybean integration into smallholder farming systems would thereby increase soil productivity in addition to improving human nutrition when the crop is incorporated into dietary patterns. However, when effective agronomic techniques, such as integrated soil fertility management, are applied in soybean production systems, these advantages become apparent.

Soybean production is subject to numerous limitations, including biotic, abiotic and socioeconomic factors, which contribute to regional variations in productivity in Central Vietnam. However, among them, low soil fertility remains the most important limiting factor to soybean production in Central Vietnam. The situation is worsened by the cultivation of soils for a long time in many areas with little or no fallows and rotations, combined with a lack of use of fertilizers, which are either not available or very expensive for most smallholder farmers. Seed yields are therefore still poor when compared to other parts of the country and the world (Banerjee *et al.,* 2017). One practical strategy for the sustainable development of smallholder agriculture is integrated soil fertility management. However, the high cost of soil amendment or nutrient replenishment inputs has prevented smallholder farmers with limited resources from adopting them (Gebrehana *et al*., 2024). An economical way to lessen the application of mineral N would be to use soybean cultivars with a high biological nitrogen fixation capacity and Rhizobium inoculants (Mendoza-Suárez *et al*., 2020). Research studies on biological nitrogen fixation conducted in Ethiopia since 2015 demonstrated the benefits of inoculation of legumes with suitable Rhizobium strains, especially for soybeans. Soybean inoculation was estimated to be equivalent to the application of 100 kg of urea per hectare (Mendoza-Suárez *et al.,* 2020). Futhermore, a number of field tests conducted in Vietnam have verified that leguminous crop like soybean and groundnut exhibit a remarkable response in terms of growth and yield to Rhizobium inoculations in various agroecology (Saeki *et al*., 2005; Nguyen *et al.,* 2017). Concerns have been raised, meanwhile, regarding biological nitrogen fixation (BNF's) ability to accommodate higher N requirements and/or maximize grain yields (Muleta *et al*., 2017). One of the main elements that largely regulates the

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amount of N_2 fixation is the amount of applied and accessible soil N, especially in soils that lack nitrogen, inoculation alone would not be enough to supply all the nitrogen needed for the best soybean yields (Mendoza-Suárez *et al*., 2020). However, a little of researches has been found on Rhizobium inoculation on new soybean varieties in Central Vietnam till now. There is not clearly how effect of Rhizobium inoculation on N fixation and crop yield in coastal sandy soil yet. Therefore, the current study was carried out in Hue city, Central Vietnam to evaluate the response of six soybean varieties to Rhizobium inoculation.

MATERIALS AND METHODS

Description of the study area

Pot and field experiments were conducted during the spring and summer cropping seasons of 2024 at two locations, including Faculty of Agronomy, Hue University of Agriculture and Forestry (16.47.75°N, 107.57.29°E) (pot experiment) and Quang Loi commune (16.60.34°N, 107.48.18°E), Quang Dien district, Hue city (field experiment). The soils used in these experiments are classified as *Haplic Arenosols* (Hoang *et al*., 2019) with deep sands, containing >95% with pHKCl 4.50; OC 0.60%; Ntotal 0.045%; Navail 2.50 mg/100 g. The region has a tropical savannah climate with long dry seasons (January to August) and high evaporation rates (about 100 mm $month^{-1}$), monsoonal rainfall (average of 2,300 mm), which is concentrated over a short spell (September to December) and mean temperatures between 27 and 35°C. Weather conditions during two crop growing seasons was with mean temperature (23.3°C and 29.8°C) and rainfall amount (54.6 mm and 111.6 mm), respectively.

Experimental set up

For the pot experiment, six local soybean varieties (CuJut, DT90, PC19, DT96, HLDN29, MTĐ176) and two levels of inoculation (inoculated and uninoculated) were arranged using randomized complete block design (RCBD) with three replications. A composite coastal sandy soil (0-20 cm) was collected from Quang Loi commune, Quang Dien district, Hue city, in January 2024. Soil was made air dry and sieved through a 5 mm sieve. Pot size with 20 cm width and 20 cm height was filled with 2 kg of soil in each pot. Before sowing soybean varieties, disinfect the surface with 70% alcohol, rinse thoroughly with sterile distilled water and pat dry. All processes were performed under sterile conditions to avoid contamination by other microorganisms. Five seeds of soybean per pot were sown in February 2024 and then 2 plants per pot were removed when they had 3 fully developed leaves and kept 3 plants/pot for further observation. After 5 days, when the seeds germinated, 01 ml of bacterial culture with a concentration of 1×10^9 CFU g^{-1} was injected evenly into the soybean roots and then the pot surface was covered with plastic film for about 1-2 days. The experiment was watered with sterile distilled water and nodule formation was observed at 60 days after sowing. Fertilizer application included 8 g of cattle manure + 4 mg N + 6 mg P_2O_5 + 6 mg K_2O $2kg^{-1}$ soil.

The field experiment was replicated with the same treatments as the pot experiment and conducted in Quang Loi commune, Quang Dien district, Hue city. The experiment was designed in a split-plot with three replications and the treatments tested were two inoculation levels (inoculated and non-inoculated) as the main plot and six soybean varieties as the subplot treatments. Lime ($CaCO_3$-56% CaO) was applied two weeks prior to sowing as a broadcast and incorporated to a soil depth of 20 cm. Cattle manure (1.05-1.15% N, 0.67-0,70% P_2O_5, 1.21-1.32% K_2O) and phosphorus (SSP, 16% P_2O_5) were applied as a basal for all plots at 8 t ha^{-1} and 60 kg P_2O_5 ha^{-1}. Nitrogen (N) and potassium (K) were used as urea (46% N) and potassium chloride (60% K_2O). The N and K fertilizers were also top dressed following row applications at two stages of plant growth, *i.e*., 12 days after sowing, one third of total at full expansion of the third leaf and 35 days after sowing, the remaining two third just prior to flowering. Plot sizes were 3 m*5 m (15 m^2) consisted of six rows, two rows for nodulation and shoot dry weight parameters and the other rows excluding borders were harvestable rows. The plots were kept 0.2 m apart with 0.2 m spacing between blocks. Drains were prepared around each plot making the plots beds. Carrier-based inoculant was applied at the rate of 123 g inoculant for 15 kg seed (used to sow quarter ha). To ensure that all the applied inoculum stuck to the seed, the required quantity of inoculant was suspended in a 1:1 ratio in a 10% sugar solution. The thick slurry of the inoculant was mixed gently with dry seed so that all the seeds received a thin coating of the inoculant. All inoculations were done just before planting under shade to maintain the viability of bacterial cells. The inoculated and un-inoculated seeds were then planted at a spacing of 10 cm between plants and 30 cm between rows, making 33 plants m^{-2}. Seeds were covered immediately with soil after sowing to avoid the death of cells due to the sun's radiation.

A number of nodules, fresh and dry weight of nodules was measured from 10 plants $plot^{-1}$ at the harvesting stage. The 100 days after sowing at the harvesting stage, pod yield at moisture <14% and other yield attributes *viz*., shoot dry matter, pod number and shell weight were assessed from a quadrat measuring 4 m^2. Biological nitrogen fixation (BNF) was calculated by formula as follows (Mogale *et al.,* 2023):

$$\% \text{ BNF} = \frac{N_{fixed}}{N_{total}} \times 100$$

$$N_{fixed} = N_{uptake} \text{ (soybean)} - N_{uptake} \text{ (reference)}$$

$$\% N_{dfa} = \frac{N_{fixed}}{N_{uptake} \text{ (soybean)}} \times 100$$

Data analysis

The statistical data was analyzed with mean, ANOVA (2 factors) and LSD0.05 by using the Statistix 10 program.

RESULTS AND DISCUSSION

Nodule and effective nodule numbers per plant

Seed inoculation with Rhizobium was significantly enhanced over uninoculated treatments with number of nodules and effective nodules per plant. Rhizobium inoculation increased the number of nodulations and effective nodulation per plant of soybean, however, their combination of inoculation and variety further increased the nodulation of soybean. There was a little different nodulation parameter in six tested soybean varieties across cropping seasons. The highest number of nodules and effective number of nodules per plant was found in DT90 in both seasons (28.07-28.86 nodules plant^{-1} and 21.00-21.56 nodules plant^{-1}), higher than the uninoculated treatment with 4.6-9.2% (number of nodule) and 7.4-10.6% (number of effective nodule) (Table 1). Amani *et al.* (2020), Kumar and Reddy (2018) reported similar findings while authenticating French bean and soybean Rhizobium isolated under the same conditions. Thus, nodulation differed significantly ($p<0.05$) between tested isolated and soybean varieties. According to Hanwani *et al*. (2021), inoculating soybeans resulted in a significantly higher number of nodules than the control. Mozambique's efforts to increase soybean production also revealed that some local Rhizobium were more productive than five reference strains (Chibeba *et al*., 2017; Amani *et al*., 2020). The greater number of nodules due to inoculation suggested that there is better combining and symbiotic relationship between indigenous isolates and soybean cultivars Doko and Piramama. However, a unique Rj gene that controls the plant's compactivity with particular Rhizobium strains may be present in the soybean cultivar Canarana which has a poor response to inoculation. Furthermore, among the compatible genotypes, native tested Rhizobium might exhibit a preference for specific genotypes (Iturralde *et al*., 2019; Sakka and Heru, 2017).

N fixation of different soybean varieties

The amount of nitrogen fixed by soybean was significantly influenced by varieties as well as Rhizobium inoculation in both pot and field experiments (Table 2). Soybean fixed more N in inoculation than in uninoculation with the means of 188.00 mg plant^{-1} (MTD176) to 380.33 mg plant^{-1} (DT90) in pot experiment and 120.33 mg plant^{-1} (PC19) to 157.00 mg plant^{-1} (DT90) in field experiment. Furthermore, the results indicated that the soybean variety namely DT90 in inoculation was able to fix 58.8% and 35.2% more N in the pot and field experiments, respectively as compared with uninoculation.

Low values of BNF was found with DT90 (35.43%) and PC19 (23.40%) in Rhizobium uninoculation at the pot experiment. Similarly, the same results were recorded in field experiment with these varieties. Treatment of DT90 with inoculation had the highest BNF in the pot and field experiments of 52.36% and 56.17%, respectively, followed by HLDN29 with 45.65% (pot experiment) and CuJut with 51.66% (field experiment).

There was a significant variation between inoculation and uninoculation treatments on $\%N_{dfa}$ during the pot and field experiments (Table 2). The varieties of PC19 (uninoculation) had the lowest $\%N_{dfa}$ (23.67-49.49%) and DT96 (inoculation) (42.01-77.83%) in both pot and field experiments. The $\%N_{dfa}$ values ranged from 23.67 to 85.13% in pot experiment and from 49.49 to 100.36% in field experiment.

Soybean fixed more N in the inoculation treatments, due to high number of effective numbers of nodule and biomass production as reported in the findings of this study.

Table 1: Response of inoculation of Rhizobium on nodule characteristics.

Rhizobium application	Varieties	Pot experiment (Spring season)		Field experiment (Summer season)	
		Total nodules plant^{-1}	Effective nodules plant^{-1}	Total nodules plant^{-1}	Effective nodules plant^{-1}
Uninoculation	HLDN29	24.67^{b}	19.30^{a}	26.75^{ab}	19.62^{abc}
	DT90	26.83^{ab}	20.07^{a}	26.43^{ab}	18.99^{abc}
	CuJut	25.66^{ab}	21.48^{a}	26.55^{ab}	18.32^{bc}
	PC19	26.07^{ab}	21.55^{a}	25.11^{b}	19.18^{abc}
	DT96	24.78^{ab}	18.29^{a}	26.16^{ab}	19.08^{c}
	MTĐ176	26.44^{ab}	18.51^{a}	25.36^{b}	18.32^{bc}
Inoculation	HLDN29	26.04^{ab}	20.07^{a}	26.23^{ab}	19.41^{abc}
	DT90	28.07^{a}	21.56^{a}	28.86^{a}	21.00^{a}
	CuJut	25.66^{ab}	18.88^{a}	27.19^{ab}	19.74^{abc}
	PC19	26.57^{ab}	21.00^{a}	26.14^{ab}	20.56^{ab}
	DT96	25.00^{ab}	18.55^{a}	28.45^{a}	21.00^{a}
	MTĐ176	26.24^{ab}	19.60^{a}	26.88^{ab}	19.59^{abc}
$LSD_{0.05}$		3.40	4.26	2.82	2.36

Values with a common letter in the same column are not significantly different using LSD at 5% level.

According to studies by Gebrehana *et al*. (2024) and Mendoza-Suárez *et al*. (2020), biological nitrogen fixation promotes growth as well as the grain yield of legumes under seasonal variations. This may explain why the N activities reported in this study were different across locations and seasons. The amount of N_2 fixed by soybean in this study was influenced by cropping seasons, which ultimately influenced partition into biomass (Ciampitti *et al*., 2021). We found that Rhizobium inoculation had positive effects on the plant biomass in all the treatment except control which expressed relatively poor growth in all parameters measured. Inoculation with Rhizobium strains significantly increased the soybean growth by enhancing nutrients availability for plant uptake. Similar assertions reported that increase in plant growth following bacteria treatment can be as a result of production of phytohormones and the enhancement of nutrient availability (Ghorbanpour and Hatami, 2013; 2014). The increased plant biomass observed in the study might be due to symbiotic relationship between the inoculated Rhizobium and root nodules of legumes which fix atmospheric nitrogen into a usable form to be utilized by the plant for growth and development. Bioenhancers such as arbuscular mycorrhizal fungi and plant growth promoting bacteria interactions increase supply and access to other nutrients (Osman *et al*., 2021). Improvement of N availability at the early growth stage of soybean may result in more nodule mass formation and adequate N fixation during growing season specifically seed setting. The significant increase in number of seed number and yield

Table 2: Response of inoculation of Rhizobium on N fixation of different soybean varieties.

Rhizobium application	Varieties	Pot experiment (Spring season)			Field experiment (Summer season)		
		BNF (%)	N_2 fixed (mg N plant^{-1})	N_{dfa} (%)	BNF (%)	N_2 fixed (mg N plant^{-1})	N_{dfa} (%)
Uninoculation	HLDN29	30.18^{cd}	149.67^{cde}	33.46^{cde}	42.27^{cd}	86.67^{e}	55.43^{e}
	DT90	25.80^{d}	127.67^{de}	28.53^{de}	41.18^{cd}	82.67^{e}	53.01^{e}
	CuJut	36.09^{bcd}	198.67^{b-e}	44.46^{b-e}	42.60^{cd}	89.00^{de}	56.91^{de}
	PC19	23.40^{d}	104.00^{e}	23.67^{e}	39.51^{d}	77.33^{e}	49.49^{e}
	DT96	40.48^{abc}	234.00^{bcd}	52.37^{bcd}	46.81^{bc}	106.00^{b-e}	67.71^{b-e}
	MTĐ176	34.52^{bcd}	187.67^{b-e}	42.01^{b-e}	43.91^{cd}	92.67^{cde}	59.38^{cde}
Inoculation	HLDN29	45.65^{ab}	289.67^{ab}	64.76^{ab}	51.35^{ab}	125.33^{b}	80.11^{b}
	DT90	52.36^{a}	380.33^{a}	85.13^{a}	56.17^{a}	157.00^{a}	100.36^{a}
	CuJut	42.00^{abc}	245.67^{bcd}	54.94^{bcd}	51.66^{ab}	126.67^{ab}	80.91^{ab}
	PC19	42.67^{abc}	258.67^{abc}	57.86^{abc}	50.35^{ab}	120.33^{bcd}	76.84^{bcd}
	DT96	35.43^{bcd}	251.67^{bc}	42.01^{b-e}	50.68^{ab}	122.00^{bc}	77.83^{bc}
	MTĐ176	41.98^{abc}	188.00^{b-e}	56.33^{bc}	51.08^{ab}	124.00^{bc}	79.15^{bc}
$LSD_{0.05}$		13.87	123.67	27.67	5.97	31.46	20.15

Values with a common letter in the same column are not significantly different using LSD at 5% level.

Table 3: Response of inoculation of Rhizobium on soybean yield.

Inoculation	Varieties	Pot experiment (Spring season)			Field experiment (Summer season)		
		Number of filled pods plant^{-1}	Theoretical yield (t ha^{-1})	Economic yield (t ha^{-1})	Number of filled pods plant^{-1}	Theoretical yield (t ha^{-1})	Economic yield (t ha^{-1})
Uninoculation	HLDN29	14.93^{d}	2.20^{b}	1.67^{bc}	18.17^{d}	2.43d^{ef}	1.65^{bc}
	DT90	16.33b^{cd}	2.13^{b}	1.53^{c}	18.10^{d}	2.27^{ef}	1.63^{bc}
	CuJut	15.77^{cd}	2.07^{b}	1.56^{c}	17.68^{d}	2.23^{ef}	1.49^{c}
	PC19	17.40^{bcd}	2.17^{b}	1.56^{c}	19.17^{cd}	2.10^{f}	1.64^{bc}
	DT96	16.83^{bcd}	2.63^{ab}	2.00^{abc}	20.27^{bcd}	2.83^{cde}	1.70^{abc}
	MTĐ176	15.13^{d}	1.97^{b}	1.60^{bc}	17.27^{d}	2.53d^{ef}	1.54^{bc}
Inoculation	HLDN29	18.10^{abc}	2.63^{ab}	2.03^{abc}	22.68^{ab}	3.46^{ab}	2.00^{ab}
	DT90	18.40^{abc}	3.23^{a}	2.60^{a}	21.30^{abc}	3.63^{a}	2.16^{a}
	CuJut	18.34^{abc}	3.10^{a}	2.23^{a}	22.40^{ab}	2.83^{cde}	2.01^{ab}
	PC19	17.10^{bcd}	3.17^{a}	2.53^{a}	20.30^{bcd}	2.83^{cde}	1.99^{abc}
	DT96	18.97^{ab}	3.06^{a}	2.40^{a}	24.07^{a}	3.36^{abc}	1.94^{abc}
	MTĐ176	20.46^{a}	3.40^{a}	2.56^{a}	22.13^{abc}	2.96^{bcd}	1.82^{abc}
$LSD_{0.05}$		2.74	0.84	0.60	3.05	0.58	0.49

Values with a common letter in the same column are not significantly different using LSD at 5% level.

may be attributed to ability and uptake of nitrogen, phosphorous and potassium by plants in soil which positively has effect on vegetative growth (Abdulkadir *et al*., 2022).

Seed yields of soybean

There was varied the number of filled pods plant^{-1} following treatments and crop seasons. Number of filled pods plant^{-1} had the maximum values in inoculation method with MTD176 in pot experiment (20.46 pods plant^{-1}) and DT96 in field experiment (24.07 pods plant^{-1}), in uninoculation method with PC19 in pot experiment (17.43 pods plant^{-1}) and DT16 in field experiment (20.47 pods plant^{-1}). The highest seed yield of soybean was obtained when inoculation applied with different varieties and fluctuated following varieties and crop seasons, respectively (Table 3). In the pot and field experiments, inoculation together with different varieties increased the highest seed yield by pot and field experiments about 41,2% and 24.5% (DT90) compared to uninoculation in the same varieties, respectively. In both cropping seasons, the inoculated plants had shown a trend that an increase in seed yield with different varieties. The seed yields had significantly ($p<0.05$) affected by the two- way interaction ($p\leq0.05$) (Table 3). The highest seed yield was obtained by DT90 variety in pot experiment (2.60 t ha^{-1}) and in field experiment (2.16 t ha^{-1}) under Rhizobium inoculation and DT96 in both pot and field experiments (2.00 and 1.70 t ha^{-1}) under Rhizobium uninoculation. According to Ahlijah (2017), inoculating the right Rhizobium bacteria can boost grain output by 70-75%. The result of the highest seed yield (2.60 t ha^{-1}) from this study was extremely high when compared to average soybean yield of Vietnam and Central region (2.50 t ha^{-1} and 1.50 t ha^{-1}, respectively) (Nguyen *et al*., 2021). This suggests that Rhizobium inoculation along with suitable variety can greatly increase soybean production (Jarecki and Bobrecka-Jamro, 2019). The findings also agreed with those of Bosera (2019), who noted that Rhizobium inoculation and variety application respectively, resulted in varietal differences in rice and soybean. Similar to results reported by Budiastuti *et al.* (2024), a sufficient amount of nitrogen must be available for the soybean seeds to go through the formation and ripening phases. The effects of Rhizobium inoculation on soybean seed weight were noticeably different. The Rhizobium fixes nitrogen through root nodules, which is essential for seed production. Application of Rhizobium can increase soybean yield by 11% and the number of effective root nodules and seed weight (Borowska and Prusiński, 2021; Ewansiha *et al*., 2022).

CONCLUSION

The present results demonstrated the potential of improving soybean nodulation, growth and seed yield by using Rhizobium inoculant with different soybean varieties in Central Vietnam. In coastal sandy soil, inoculation soybean with appropriate Rhizobium strains improved growth, nodulation, yield and N fixation ability. Among 6 tested varieties, DT90 variety produced the best seed yield and N fixation in both pot and field experiments with Rhizobium inoculation. Farmer in Central, Vietnam could increase soybean productivity by choosing and using Rhizobium strain in DT90 variety. Further study needs to be conducted in different practices on selected soybean variety and considered the soil fertility improvement.

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Conflict of interest

The authors declare no conflict of interest.

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