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**Response of soybean (*Glycine max* (L) Merr) growth, nodulation and yield to inoculation with Bradyrhizobium strains in the Lubumbashi region, DR Congo**

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**Abstract**

The interaction between two symbiotic components, legumes and rhizobium is very specific and is determined by signal changes between the plant and the bacteria at different growthstages. The main objective of this article was to evaluate the influence of three strains of Bradyrhizobium (GraphEx™, Bradyrhizobium sp and Sojapak® 50) on the growth, nodulation, and yield of four soybean varieties (TGX 1740-7F: V1, PAN 1867: V2, TGX 1880-3E: V3 and LUKANGA: V4) and identify the most determining parameters of performance. An experiment was set up using a split plot design. Three strains of bradyrhizobium were inoculated to four soybean varieties. A total of 16 treatments were obtained and evaluated. The results showed that the best performance in terms of germination rate is obtained with the following treatments: T3, T7, T9, T11, T13 and T14. The addition of the inoculum contributed to stimulate and improve the germination of variety V1. Plant height, number of pods, weight of 100 seeds and yield were highly dependent on strain, variety and their interaction. Higher plant heights were obtained with T16 while the number of pods is similar between all the treatments except T2 which gave the lowest value. The best response regarding the weight of 100 seeds was obtained with T2, T6, T9, T10, T13, T14 while T11 gave the highest yield. No inoculum effect was observed on collar diameter, dry biomass and number of nodules. This study showed that soybean grain yield is mostly influenced by the germination rate, collar diameter, plant heights, fresh biomass, number of pods, number of nodules, and weight of 100 seeds. Different soybean varieties have differing response to applied bradyrhizobium strains.

Keywords: Haut-Katanga, acidic soils, legume, and biological fertilizers



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## **Introduction**

Soybean (*Glycine max* (L.) Merrill.) is one of the most important and widely cultivated crops accounting for 30% of the processed vegetable oil in the world (Graham & Vance, 2003). Under most working conditions, the average yields of legumes are very low. These low yields are associated with reduced soil fertility due to continuous cultivation without soil replenishment, which reduces nitrogen fixation due to various biological and environmental factors (Ferguson et al. 2006).

One of the most important ways to take advantage of the useful interaction of microorganisms and maintain the diversity of agricultural ecosystems is to use soil microorganisms capable of improving plant nutrition and soil fertility through biological fixation of nitrogen and the solubilization of phosphorus (Akram, 2014). Indeed, the biological fixation of nitrogen plays an essential role in the growth of crops and their yield. Soybeans have the ability to fix nitrogen from the air during the symbiosis with rhizobia (Ferguson et al. 2006). Under optimal conditions, soybeans can fix up to 450 kg N ha<sup>-1</sup>, thus limiting the need for chemical nitrogen fertilizer in the agroecosystem (Keyser and Li, 1992; Giller, 2001). However, soybean varieties differ in their ability to nodulate and fix nitrogen with rhizobia strains. The symbiotic interaction between legumes and rhizobium is very specific and is determined by signal changes between the plant and the bacteria at different stages. In some areas, native nodulation is more often insufficient because of certain constraints, notably edaphic constraints and the absence of specific strains in the soil. In these areas the use of inoculum (rhizobium) in legume cropping systems is widely spread (Bruno et al., 2003). Thus, the need is to introduce for each agroecological zone, suitable soybean varieties and tested bacteria strains to obtain a better variety-strain combination because generally, the number of root nodules and the root structure of soybeans depend on the varieties.

The main objective of this article is to evaluate the influence of three strains of Bradyrhizobium on the behavior of four soybean varieties and to identify the most determining parameters of yield.



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## Materials and methods

### Study site

An experiment was installed in Lubumbashi, DR Congo at 11 ° 60869 'S and 027 ° 47692 E. The climatic type is Cw according to the Köppen classification (tropical climate). There is a dry season lasting an average of 185 days, which begins around April 15 and ends between October 20 and 25. The mean annual precipitation is around 1200-1500 mm, with a maximum in December. The mean annual temperature varies around 20 ° C. The mean annual humidity rate is 68%-86 % (Mpundu, 2010). The soils of Lubumbashi and its surroundings belong to the group of desaturated, yellow-red lateritic soils. These soils are characterized by an abundance of clay minerals with low cation exchange capacity (kaolinite) (less than 16 C moles. Kg<sup>-1</sup>), iron and aluminum oxides and hydroxides (Kasongo et al., 2013) and a pH-water around 5.2. Prior to the installation of the test, the soil samples revealed showed a pH = 5.4, soil organic carbon 2.6%, total nitrogen 0,2 %, C/N 16,3, total phosphorous 509.2 mg.kg<sup>-1</sup>, and available phosphorous 1,6 mg.kg<sup>-1</sup>.

### Experiment setup

The experiment was installed on a plot where maize was previously cultivated. After mechanical plowing and harrowing, the experiment was set up in a split plot arrangement with three replicates. In the main plot three strains of bradyrhizobium plus the uninoculated control and four varieties of soybean in the secondary plot. The combination of the different strains and varieties resulted in 16 treatments. Two varieties (i.e. TGX 1740-7F and 1880-3E) were obtained at INERA KIPOPO (National Institute for Agricultural Research and Study), the other two varieties were obtained in Zambia (Lukangavariety from ZAMSEED and PAN 1867 from PANNAR). The three bradyrhizobium strains (Sojapak®50, GraphEx™ and Bradyrhizobium sp) were obtained on the local market.

Before sowing, the inoculations were made in the shadow in order to maintain the viability of the bacterial cells. The GraphEx™ and Bradyrhizobium sp strains were used at a rate of 1 kg for 50 kg of seed while 1000 ml for 50 kg of seed were used for the Sojapak® 50 strain (Schiffers and Frassel, 1982). The follow-up to the trial consisted of three weeding; the first to the second week after sowing, the second two weeks after the first and the third two weeks after the second.



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## Statistics

The analysis of variance was performed to highlight the differences between the means of the different treatments on all the parameters. When the analysis of variance detected a significant effect, a post-hoc Tukey HSD test was performed to highlight the different classes (Dagnelie, 2012). Pearson correlation matrix was performed to understand the links between of soybean growth and yield parameters. These statistical analyzes were performed using the Munitab 17 software.

## Results

### Influence of strain and variety on soybean growth

The influence of bradyrhizobium on soybean behavior is presented in Table 1. As a result, there is a highly significant difference between the different treatments ( $P = 0.000$ ) with regard to the germination rate. Indeed, the germination rate depends on the strain, the variety and their interaction. The highest and similar values are obtained with T3 (uninoculated), T7, T9, T11, T13 and T14. The addition of bradyrhizobium seems to induce the germination of soybeans (V1). Regarding the collar diameter, table 1 shows that there is no significant difference between the treatments ( $P > 0.05$ ). The heights of plants show a significant difference between treatments with a significant effect of both bradyrhizobium strain and variety as well as their interaction. The fresh biomass is influenced by the strain and strain-variety interaction, but not by the variety itself. The dry biomass is similar between treatments while fresh biomass is influenced by bradyrhizobium strain and the interaction strain-variety.



Table 1. Influence of the strain, variety, and their interaction on soybean growth. GR: germination rate, CD: collar diameter, PH: plant height, FB: fresh biomass, DB: dry biomass.

Treatments			Growth parameters				
	Strains	Varieties	GR (%)	CD (cm)	PH	FB(g)	DB (g)
T1	S0	V1	0.00 ± 0.00d	-	-	-	-
T2		V2	65.43 ± 17.22abc	7.1111 ± 1.1706	32.778±3.272ab	28.26±12.95ab	14.410±7.959
T3		V3	83.11 ± 4.73a	5.2222 ± 1.3472	31.222 ± 7.987ab	31.97±27.32ab	22.840±19.323
T4		V4	42.00 ± 15.10c	6.1111 ± 0.8389	26.889 ± 3.368ab	17.93±3.72ab	14.540±5.349
T5	S1	V1	78.22 ± 16.95abc	6.8889 ± 0.5092	26.367 ± 8.215ab	33.49±10.21a	13.408±6.518
T6		V2	74.89 ± 9.48abc	6.2222 ± 0.3849	26.778 ± 1.925ab	24.94±4.77ab	11.031±7.646
T7		V3	86.44 ± 12.62a	4.8889 ± 0.1925	23.889 ± 2.502ab	18.57±2.38ab	11.279±6.028
T8		V4	57.33 ± 24.48abc	6.1111 ± 0.8389	32.444 ± 3.849ab	32.97±1.18a	12.242±6.309
T9	S2	V1	90.11 ± 9.35a	6.6222 ± 0.6194	18.511± 10.755ab	26.93±8.34ab	12.218±5.390
T10		V2	75.78 ± 13.10abc	5.8889 ± 0.5092	29.444 ± 1.058ab	26.81±5.41ab	8.403±6.736
T11		V3	89.33 ± 9.82a	5.5556 ± 1.2620	27.667 ± 2.309ab	27.09±12.26ab	9.341±9.679
T12		V4	41.78 ± 13.04c	4.6667 ± 1.4530	29.111 ± 1.925ab	45.1 ± 3.41a	16.298±14.198
T13	S3	V1	84.67 ± 2.91a	6.3333 ± 0.8819	27.389 ± 0.977ab	29.18± 9.59ab	8.410±5.636
T14		V2	81.11 ± 7.19a	6.1111 ± 0.5092	30.000 ± 3.464ab	26.57±9.51ab	7.651±3.388
T15		V3	80.89 ± 7.73ab	5.1111 ± 1.3472	24.667 ± 5.196ab	26.85±12.19ab	6.657±4.226
T16		V4	44.22 ± 6.58bc	5.3333 ± 1.4530	33.333 ± 5.485a	26.81±13.85ab	11.800±8.758
		<b>P</b>	<b>0.000</b>	<b>0.21</b>	<b>0.000</b>	<b>0.024</b>	<b>0.457</b>
		<i>Strain effect</i>	<b>0.000</b>	<b>0.124</b>	<b>0.027</b>	<b>0.069</b>	<b>0.633</b>
		<i>Variety effect</i>	<b>0.000</b>	<b>0.217</b>	<b>0.000</b>	<b>0.325</b>	<b>0.456</b>
		<i>Interaction</i>	<b>0.000</b>	<b>0.321</b>	<b>0.000</b>	<b>0.025</b>	<b>0.319</b>

The average number of pods is shown in Table 2. Based on ANOVA results, the number of pods varies depending on the treatment. The treatment T2 has the lowest number of pod while the other treatments are similar. As for the weight of 100 seeds, the results reveal an influence of the strain, the variety and as well as their interaction. However, the highest values with the supply of inoculum are similar to those obtained with uninoculated V2. Regarding the yield of soybean seeds, it is observable that the different



variety-strain combinations show markedly different results. Furthermore, the yield obtained strongly depends on the variety, the strain and their interaction. This implies that the strains of bradyrhizobium exhibit various aptitudes which influence the yield on the one hand and on the other hand, the soybean varieties have different genetic potential ( $P = 0.000$ ). However, some strain-variety combinations show similar results with treatments that did not receive the inoculum.

Table 2. Influence of strain and variety and their interaction on soybean yield and nodulation. NP: number of pods, W100 G: weight of 100 grains, YLD: grain yield, NN: number of nodules.

Treatments						
	Strains	Varieties	NP	W100 G (g)	YLD (t. ha <sup>-1</sup> )	NN
T1	S0	V1	-	-	-	-
T2		V2	8.00± 0.00b	11.167 ± 0.764a	2.2340 ± 0.1206ab	39.00 ± 10.87
T3		V3	58.78±15.15a	8.000± 0.000bc	2.1677 ± 0.5031ab	50.78 ± 15.15
T4		V4	42.33± 6.67a	10.667± 0.289ab	2.0337± 0.1734ab	34.33 ± 6.67
T5	S1	V1	45.78±11.53a	10.500 ± 1.000abc	1.9720± 0.2560ab	37.78 ± 11.53
T6		V2	50.00±8.57a	11.167 ± 0.764a	2.0523 ± 0.2389ab	42.00 ± 8.57
T7		V3	59.44±7.67a	7.833 ± 0.289c	2.3727± 0.4672ab	51.44 ± 7.67
T8		V4	48.33±11.67a	10.167 ± 1.258abc	1.9845± 0.2735ab	40.33 ±11.67
T9	S2	V1	39.94±4.87a	10.833 ± 0.577a	1.7097± 0.1007ab	33.22 ± 6.19
T10		V2	46.44±7.17a	11.000 ± 0.500a	2.5377 ± 0.4048b	38.44 ± 7.17
T11		V3	62.78± 6.17a	9.167 ± 1.155abc	3.2717 ± 0.1675a	54.78 ± 6.17
T12		V4	52.22±10.22a	10.500 ± 0.000abc	2.2173 ± 0.1753ab	44.22 ± 10.22
T13	S3	V1	41.56±14.79a	11.500 ± 1.000a	1.9937 ± 0.2207ab	33.56 ± 14.79
T14		V2	45.44±6.80a	11.333 ± 0.764a	2.3400 ± 0.0560ab	34.22 ± 7.90
T15		V3	57.22±21.09a	8.000 ± 0.000bc	2.2773± 0.1250ab	52.44 ± 16.84
T16		V4	42.00± 9.81a	10.333 ± 2.255abc	1.9537± 0.0577ab	34.00 ± 9.81
<b>P</b>			<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.42</b>
<b>Strain effect</b>			<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.27</b>
<b>Variety effect</b>			<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.21</b>
<b>Interaction</b>			<b>0.004</b>	<b>0.000</b>	<b>0.000</b>	<b>0.058</b>



**Correlation between the different parameters of soybean growth and yield**

The correlation matrix presented in Table 4 shows that there are positive correlations between all the parameters observed. This situation implies a strong inter-influence between these parameters. Although positive, some correlations are weak. In addition, certain parameters are correlated with each other at more than 80%, these are the collar diameter and the weight of 100 grains, the heights of the plants and the weight of 100 grains, the heights of the plants and the yield, the number of nodules and pod number, nodule number and yield.

Table 3. Pearson correlation matrix between soybean growth and yield parameters. NP: number of pods, W100 G: weight of 100 grains, YLD: grain yield, NN: number of nodules. ER: germination rate, CD: collar diameter, PH: plant height, FB: fresh biomass, DB: dry biomass. \*significant at P< 0,05

	<i>GR</i>	<i>CD</i>	<i>PH</i>	<i>FB</i>	<i>DB</i>	<i>NP</i>	<i>W100 G</i>	<i>YLD</i>	<i>NN</i>
ER	1,0								
CD	0,7*	1,0							
HP	0,5	0,8*	1,0						
FB	0,4	0,6*	0,8*	1,0					
DB	0,3	0,5	0,6*	0,6*	1,0				
NP	0,6*	0,4	0,5	0,5	0,4	1,0			
W100 G	0,6*	0,9*	0,8*	0,7*	0,5	0,4	1,0		
YLD	0,7*	0,8*	0,9*	0,7*	0,5	0,7*	0,8*	1,0	
NN	0,7*	0,6*	0,7*	0,6*	0,6*	0,8*	0,5	0,9*	1,0



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## Discussion

The contribution of bradyrhizobium strains to different soybean varieties strongly influenced the germination rate of studied varieties. The addition of inoculum allowed the initiation of germination of the variety V1. Without inoculum, the germination rate was 0% while the addition of inoculum resulted in a relatively high germination rate. In addition, some inoculated treatments gave higher germination rate values than the control treatments except for the T3 treatment (V3 without inoculum). The observations made above show that supply of inoculum would stimulate germination of V1. However, the mechanisms by which rhizobia would be involved in the initiation of germination have not yet been elucidated. The differences in germination power may be related to differences in germination energy, physiological maturity, and seed harvesting and storage conditions (Ferguson et al., 2006).

Regarding the collar diameter, the results showed that the supply of inoculum did not induce a significant difference. In addition, neither the variety nor the strain-variety interaction induced a significant difference in collar diameter.

The heights of the plants strongly depended on the varieties and the strains as well as their interaction. This observation would be justified by the genotype of each material used. However, some treatments that received the inoculum have similar plant heights with those that did not. This would be justified by the low efficiency of these strains in inducing higher performance with certain varieties of soybean. It is established that the specificity between strain and variety determines the performance of the latter (Hardarson and Broughton, 2003; Maingi et al., 2006).

The fresh biomass obtained in this study is influenced by the strain, the treatment, the strain-variety interaction and not by the variety. This implies that the biomass potential is similar between all varieties. By providing the inoculum, the fresh biomass of some varieties is improved, while for others, this contribution has no effect compared to control treatments (not inoculated). This observation would be due to the same explanation given above, according to which, certain strain-variety combinations do not prove to be more efficient than the control treatments because of the inefficiency of the strain revealed by the lack of specificity with the variety of soybean (Abaidoo et al., 2007). The best strain-variety combination was obtained with T2, which gave a higher number of pods, while all the other treatments were similar with each other and with the control. This would imply a lower efficiency of the strains (to

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induce the formation of pods), which are higher than those obtained in the absence of an inoculum. In case of low N content when the pH is below 5.5, Ferguson et al. (2006) recommend nitrogenous mineral fertilization at the pod filling stage to maximize this determining parameter of yield. However, in the case of our study site, the pH = 5.4 and an N content of 0.2%, supplementation with mineral nitrogen fertilization would increase crop performance. A large literature abounds on the fact that rhizobia are in general neutrophils but their response to a fluctuation in pH varies from one strain to another (Jordan, 1984; Glenn and Dilworth, 1994). Graham and Parker (1964) showed that a low pH critical for the growth of *Rhizobium japonicum* is between 4 and 6.

Although the weight of 100 seeds depends on the variety, the strain and their interaction, the results obtained with some treatments do not give the inoculated treatments an advantage over a control T2 treatment. The highest values obtained with addition of the inoculum were found to be similar to that obtained with variety V2 when it did not receive the inoculum. This could be an indicator of a high potential of this variety (V2).

Regarding the grain yield of soybean, it is observable that the different variety-strain combinations show markedly different results. Furthermore, the yield obtained strongly depends on the variety, the strain and their interaction. This implies that the strains of bradyrhizobium exhibit various aptitudes which influence the yield on the one hand and on the other hand, the soybean varieties have different genetic potential. However, some strain-variety combinations show similar results with treatments that did not receive the inoculum. Among the treatments evaluated, the highest yield is obtained with T11. This yield (3.3 t. ha<sup>-1</sup>) is similar to that obtained by Kasongo et al. (2013) by providing 10,143 kg of *Tithonia diversifolia* biomass per hectare as mulch, a yield greater than the dose of chemical fertilizer applied. In addition, neither the varieties, nor the strains and even their interaction did not influence the nodulation of soybeans. Nodulation and symbiotic nitrogen fixation are strongly linked to several factors (some of which were not controlled in this study): the nutritional and physiological state of the host plant. Nutrient toxicity, mineral toxicity, salinity, plant diseases, weed competition, extreme temperature and humidity are factors that affect legume host vigor, receptive physiology, nodulation and / or symbiotic nitrogen fixation in soil (Taylor et al., 1991; Giller, 2001). According to Mulaji (2011), in soils with pH <5.5; nitrifying and nitrogen-fixing bacteria are affected by soil acidity. Nodulation would also be linked to the quality of the strain which itself depends on the number, the efficiency as well as the genetic stability of the rhizobial strains contained in these inocula (Hiltbold et al., 1980; Brockwell and



Bottomley, 1995; Stephens and Rask, 2000; Amarger, 2001), but also their level of competition with native rhizobia and their persistence in the soil (Graham and Vance, 2003). The conservation of inocula is also an important criterion for their quality. Boonkerd (1991) has shown that storage of inocula at temperatures above 10 ° C considerably reduces their longevity, while under local conditions the average temperature is usually above 10 ° C.

Furthermore, the various growth parameters observed are all positively correlated with each other. This observation would imply a strong dependence between the parameters of soybean growth and yield. However, the degrees of significance of these correlations are different between the parameters. The most determining parameters of seed soybeans are: the germination rate, the collar diameter, the heights of the plants, the fresh biomass, the number of pods, the number of nodules and the weight of 100 seeds. Indeed, the germination rate determines the density of plants upon emergence. The collar diameter determines the vigor of the plant and its performance. Plant height is strongly related to the biomass of the plant and alongside its photosynthetic capacity, which directly influences soybean grain yield. In addition, the number of pods and the weight of 100 grains are directly linked to the yield of soybean seeds while the number of nodules (infected) defines the N binding capacity and consequently the best performance of the plant (Amarger, 2001, Tshibuyi et al. 2019ab).

## **Conclusion**

The main objective of this article was to evaluate the influence of three strains of bradyrhizobium on the behavior of four soybean varieties and to identify the parameters most determining the yield. The results obtained showed an influence of inoculation on the germination of soybeans. The best performance in terms of germination rate is obtained with the following treatments: T3, T7, T9, T11, T13 and T14. The addition of the inoculum helped to stimulate the emergence of variety V1. This study also showed the inoculum inefficiency on parameters such as collar diameter, dry biomass and number of nodules.

On the other hand, plant height, number of pods, weight of 100 seeds and yield are highly dependent on strain, variety and their interaction. Larger size plants are obtained with T16 while the number of pods is similar between all the treatments except T2 which gave the lowest value. The best answer regarding the weight of 100 seeds is obtained with T2, T6, T9, T10, T13, T14 while T11 gave the highest yield.



Overall, some strain-variety combinations gave similar performance compared to controls (without inoculum), showing poor efficiency. This study showed that there is a specificity between soybean varieties and bradyrhizobium strains. Indeed, some varieties are influenced by specific strains on specific parameters.

The general trend observed during this study showed a close link between the different parameters through the positive correlations. The most determining parameters of the soybean grain yield are: the germination rate, the collar diameter, the plant heights, the fresh biomass, the number of pods, the number of nodules and the weight of 100 seeds.

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