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Effects of *Bradyrhizobium Japonicum* on Nitrogen Concentration in Soybean Leaves and Seeds Cultivated on Acidic Soils

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Abstract: The legume-rhizobium symbiosis plays an important role in Nitrogen (N) uptake by plants, more particularly in the humid tropical region where soils are deeply weathered and have poor electrochemical properties. However, the influence of *Bradyrhizobium* on N allocation in soybean organs is not clearly understood. Here, we assessed the effect of *Bradyrhizobium japonicum* on the N concentration of soybean leaves and seeds cultivated on acidic soils. We conducted two experiments using a randomized full device with three replicates in two sites (i.e., Kasapa and Kanyameshi). The main plots included three strains of *Bradyrhizobium* and the uninoculated control while the subplots included soybean variety. We found that different strains of *Bradyrhizobium* did not induce significant effects on total N concentration in soybean leaves and seeds in the Kasapa site. In contrast, *Bradyrhizobium* strains affected significantly N concentration in soybean leaves in the Kanyameshi site. We demonstrated that soil N concentration, which varies between investigated sites, positively influences yield and nodulation. We conclude that the efficiency of the soybean-*Bradyrhizobium* symbiosis and its influence on the allocation of N through soybean organs strongly depend on the chemical characteristics of soil and particularly on the initial N concentrations in soils.

Keywords: Ferralsol, Lubumbashi, *Rhizobium*, Symbiosis

Introduction

Constituting one of the major processes of transformation of atmospheric nitrogen (N) into a form that can be assimilated by plants, the biological fixation of N is an important pathway for N to enter the biogeochemical cycle (Graham and Vance, 2003). Nitrogen fixation is a process occurring via a symbiotic association between bacteria of the Rhizobium genus and leguminous plants (Graham and Vance, 2003; Vieira *et al*., 2010). This symbiosis can store up to 65% of N used in the world per year (Sahgal and Johri, 2003). Given its agronomic, economic, and ecological interests, the legume-rhizobium symbiosis is an important component in the humid tropical region (Vieira *et al*., 2010). Indeed, it is reported by Faghire *et al*. (2011) that when this symbiosis works well, it can provide adequate N nutrition to plants and ensure proper production, thus saving farmers the cost of chemical fertilizers and relieving environmental pollution. However, this process is subject to several edaphic factors such as temperature variation, salinity, lack of nutrients, metal toxicity, or even soil acidity that can affect the physiological state of the host plant or impact the microbial community and thus influence the rate of N fixation (Dita *et al*., 2006; Borucki and Sujkowska, 2008).

In the humid tropical zone in general and the city of Lubumbashi and its surroundings in particular, soil acidity remains one of the most limiting factors of agricultural productivity and particularly when N nutrition is provided by the symbiotic fixation of N. Indeed, this acidity affects most soil parameters such as the solubility of metallic elements, the assimilation of mineral elements by plants, the structure, and activity of soil microorganisms. It is reported that soybeans adapt less well in acidic soils which may require limestone amendments to optimize their yield. Recently, in a study conducted in Germany and Poland by Griebsch *et al.* (2020), it was proved that pH has no significant effect on the mass or number of nodules, but only on the relative abundance of *Bradyrhizobium sp.* and this is attested by Albareda *et al.* (2009) and Gazdag *et al.* (2015) who report that *Bradyrhizobium sp* prefer soil with neutral or slightly acidic pH. All of these studies, like many others, evaluated the influence of soil acidity on the nodulation and abundance of *Bradyrhizobium sp.*

However, how *Bradyrhizobium* could affect the allocation of N throughout soybean plants is still unclear. Therefore, the objective of this study is to assess the effect of *Bradyrhizobium sp*. on the N concentration of soybean leaves and seeds grown on acidic soils.

Materials and Methods

Study Sites

This study was carried out in two experiments during the 2015-2016 cropping season in two agroecological zones. The first experiment was carried out in the experimental field of the Faculty of Agronomic Sciences of Lubumbashi University (Kasapa site), located at an altitude of 1250 m, 11° 60869 'South latitude and 027° 47692' East longitude. The second experiment was conducted in Kipushi territory, precisely in Kanyameshi, and located at 11° 45'25 "South and 27° 16" 59 "East and 1320 m altitude. The annual rainfall in these agro-ecological zones is around 1270 mm with a rainy season of 118 days, while the average annual temperature is around 20°C with great interannual stability. Soils of the study are characterized as Ferralsols a pH-water around 5.2 (Kasongo *et al*., 2013).

The biological material used consisted of four varieties of soybean (*Glycine max* (L.) Merr) and three strains of *Bradyrhizobium*. Within these four soybean varieties, two (TGX 1740-7F and 1880-3E) were obtained at the National Institute for Agronomic Study and Research (INERA), Kipopo station, and the other two varieties were obtained from Zambia (LUKANGA variety from ZAMSEED and PAN 1867 from PANNAR). Three commercial inoculum strains of the *Bradyrhizobium* genus were used, namely: The *Bradyrhizobium sp* strain of pasty formulation, the Sojapak ® 50 strain of liquid formulation, and the GraphEx™ strain of powder formulation. The *Bradyrhizobium sp* strain was supplied by the National Fertilizer and Input Service (SENAFIC) and the other two strains (Sojapak ® 50 and GraphEx™) were obtained on the local market.

Experiment Setup and Follow-Up

At each site, the experiment was installed following a Split-Plot setup with three replicates. In the main plot, three strains of *Bradyrhizobium* plus the uninoculated control and four varieties of soybean were in the subplot. The combination of the different strains and varieties resulted in 16 treatments. The seedlings were made at spacings of 0.40 × 0.20 m at the rate of 2 seeds per pocket, i.e., a density of 250,000 plants per hectare. A detailed description is provided by Tshibuyi *et al.* (2019). For this study, data from the combination of the ZAMSEED variety and the three *Bradyrhizobium* strains were used. For symbiotic inoculation, a single procedure was used for all formulations to ensure that all seeds received a thin layer of the inoculant for the enhancement of biological N fixation (Gemell *et al*., 2005). All inoculations were made in the shade just before sowing to maintain the viability of the bacterial cells. The time between inoculation and seedling was 30-45 min. The different formulations were used for 1 kg per 50 kg of seed for the GraphEx™ and *Bradyrhizobium sp*. strains and 1000 mL for 50 kg of seed for the Sojapak ® 50 strain in liquid form.

Sample Collection and Laboratory Analysis

Topsoil (0-20 cm) samples were taken before, during (at flowering), and at the end of the experiment (i.e., after harvesting). To measure N concentration in the soybean leaf and seed, samples were taken following the treatments.

Chemical analyses of soil samples, leaves, and seeds were carried out at the Agro-pedological laboratory of the Faculty of Agronomic Sciences of the University of Lubumbashi. The pHKCl was determined using an electronic pH meter in a soil solution with a ratio of soil/KCl 1N of 1/2.5. The organic carbon concentration was determined by the Walkley and Black method. Organic matter concentration was obtained by multiplying the carbon concentration by 1.724. The available P was determined by spectrophotometry after extraction with 0.03 M NH_4F and 0.1 M HCl. The N concentration in soils and aerial organs (leaves and seeds) was determined by the Kjeldahl method.

Statistical Data Treatments

The comparison of the means between the two sites was made using a one-way analysis of variance by considering 0.05 as the significance level using the R 4.0.3 software. Figures were produced using Prism GraphPad 9 software.

Results

Chemical Parameters

The pH_{KCl} is higher ($P<0.05$) in site 2 than in site 1 (Fig. 1). However, both sites have a pH_{KCl} below 5.5. Regarding organic C (OC), Organic Matter (OM) and C/N ratio, a comparison of means show that there is no statistically significant difference ($P>0.05$) between the two sites.

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The comparison of total N between the two sites shows that the total N concentration is higher in site 2 compared to site 1 (respectively 0.16% against 0.11% N) ($P<0.05$). For the available P, the concentration is higher in site 2 than in site 1 ($P<0.05$). In contrast, K concentration was highest in site 1 and lowest in site 2 ($P<0.05$).

Effects of Bradyrhizobium Strains on N Concentration s in Soybean Leaves and Seeds

It emerges from Fig. 2 that the different strains of *Bradyrhizobium* did not induce significant effects on the total N concentration in the leaves ($P = 0.444$) and in the seeds ($P = 0.476$) at Site 1. Regarding the N in the leaves, the result shows that the plots without inoculum show slightly low N concentration in the leaves although the statistical test did not show any significant differences. At Site 2, the different strains of *Bradyrhizobium* influenced the N concentration in soybean leaves ($P = 0.01$). The control treatments (non-inoculated) have the lowest N concentration data compared to treatments inoculated with *Bradyrhizobium*. However, in the seeds, the result shows similarities between the strains used ($P = 0.925$).

Correlation Among N Concentration in Soil, Leaves, and Seeds as Well as Soybean Yields

Figure 3 shows the results of the correlation between N contained in soils (Site 1 soil and Site 2 soil), leaves, and seeds. It appears that in Site 1, the N concentration of leaves is highly and positively correlated with the N concentration of seeds and soil at both sites ($P<0.05$). Nitrogen is also highly and positively correlated with the yield and the number of nodules ($P<0.05$). Nitrogen in seeds correlates positively with yield and very positively correlates with soil N at Site 1 ($P<0.05$). Furthermore, soil N is positively correlated with yield and number of nodules ($P<0.05$).

At Site 2, N concentration of leaves is highly and negatively correlated with N concentration of seeds ($P<0.05$), but positively correlated with soil N at Site 1 and highly and positively correlated with yield, nodule number, and soil N at Site 2 ($P<0.05$). Nitrogen concentration of seeds is negatively correlated with yield, the number of nodules, and soil N at Site 2 ($P<0.05$). Finally, soil N concentration at Site 2 is positively correlated with yield and the number of nodules.

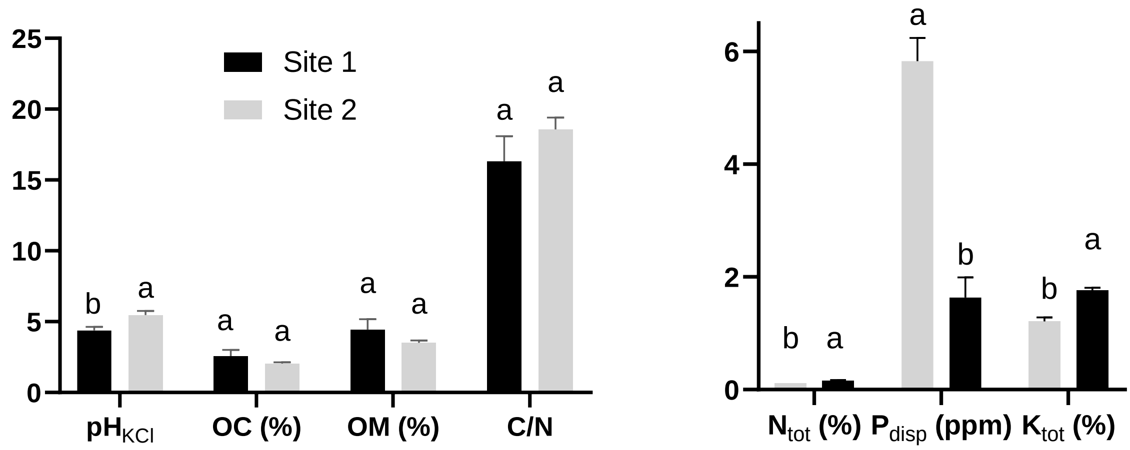

Fig. 1: Some chemical parameters in the study sites. Site 1: Kasapa, Site 2: Kanyameshi

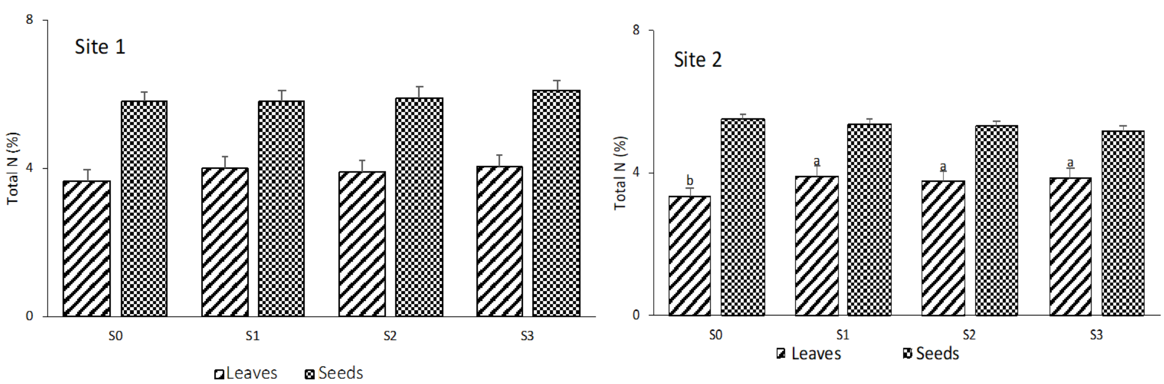

Fig. 2: Effects of Brady rhizobium strains on N concentrations in soybean leaves and seeds. Site 1: Kasapa, Site 2: Kanyameshi

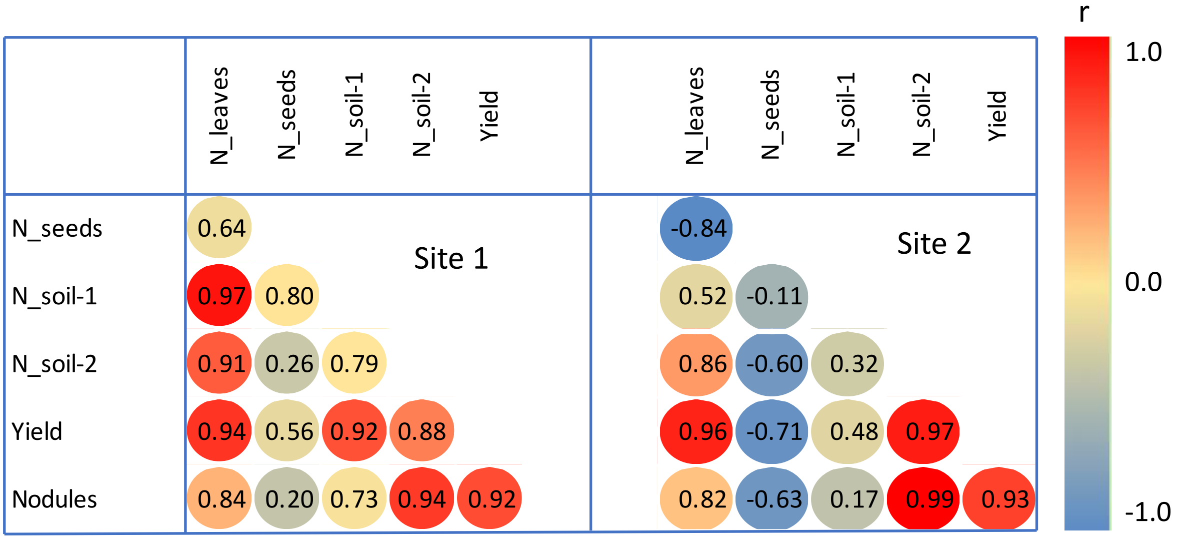

Fig. 3: Correlation among N concentration in soil, leaves, and seeds as well as soybean yields at sites 1 and 2. N_soil-1: Nitrogen concentration in soils at flowering, N_soil-2: Nitrogen concentration in soils at harvest. Site 1: Kasapa, Site 2: Kanyameshi

Discussion

Chemical Properties of the Soils

The obtained pH_{KCl} in both sites was <5.5 with the highest values at Kanyameshi compared to Kasapa, respectively 5.5±0.3 and 4.4±0.26. These results corroborate those found by several authors for soils in the Lubumbashi region and its surroundings. There were no statistically significant differences in OC concentration between the studied sites. Mean OC concentration in both sites was <3% (i.e., 2±0.0.1 for site 2 versus 2.6±0.4 for site 1). This low OC concentration corroborates the results found by Six *et al*. (2002), (and Kemmitt *et al*., 2006) and for soils in the tropics. Apart from the fact that tropical soils are generally low in OC, values observed in our case could be explained on the one hand by a low entry of the litter characterizing agroecosystems and on the other hand, the loss of surface layer rich in OM by erosion.

Available P concentration was higher at site 2 than at site 1 (respectively 5.8±0.4 and 1.6±0.4) (P<0.05). However, the available P concentration observed at both sites is considered to be low. This corroborates the results of the literature, in particular as regards the low value of phosphorus available in tropical soils, which would be due, in large part, to retention by precipitation with free aluminum, which at a pH <5, is extensively mobilized (Hue *et al*., 2001; Crawford *et al*., 2008). For, the fixation of P compounds by iron and aluminum oxides and by OM, as well as their degree of insolubility, are responsible for the low availability of forms that can be assimilated by plants. Total N was highest at site 2 compared to site 1 (respectively 0.16% against 0.11% N) (P<0, 05). Landon (1991) considers N concentrations higher than 0.13% as satisfactory in tropical soils. At site 2, N concentration is therefore acceptable according to Landon (1991) than in site 1.

Effects of Bradyrhizobium Strains on N Concentration in Soybean Leaves and Seeds

The results obtained showed that soybeans contained significantly more total N in both study sites compared to leaves. This is explained by the fact that the soybean contains between 30 and 45% of proteins, which are composed of 16% N. Regarding N in the leaves, the results showed an influence of different strains of *Bradyrhizobium* (P = 0.01). The control plots (non-inoculated) showed the lowest N concentration compared to plots with *Bradyrhizobium* strain. Statistical analysis also revealed high levels of N in the leaves of plants inoculated with different strains compared to the leaves of the uninoculated control. Danso *et al.* (1988) report that, in practice, *Bradyrhizobium* strains introduced into the soil are exposed to the effects of numerous ecological factors which subsequently determine their survival and effectiveness. This would explain the differences in the results obtained in this study between the strains. In addition, the success of inoculation depends not only on the strain used but also on its ability to withstand adverse environmental conditions (Thies *et al*., 1991; Nazih *et al*., 1993) on the one hand and the other hand, be able to survive in the soil as saprophytic microorganisms beyond the first month following inoculation (Gibson *et al*., 1982). Nutritional constraints can also limit symbiotic N fixation by affecting the survival and multiplication of rhizobia, initiation, development, and nodular function as well as the growth of the host plant (Delgado *et al*., 1994).

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Correlation between N Concentration in Soil, Leaves, and Seeds as well as Soybean Yields

The results of correlation in Fig. 3, showed that in Site 1, the N concentration of leaves is highly and positively correlated with the N concentration of seeds and soil ($P<0.05$). Nitrogen was also highly and positively correlated with the yield and the number of nodules ($P<0.05$). Nitrogen in seeds correlates positively with yield and positively correlated with soil N at Site 1 ($P<0.05$). For soil N at sites 1 and 2, it correlated positively with yield and number of nodules ($P<0.05$). These results seem to be in contradiction with those found in Europe by Griebsch *et al*. (2020) who found no relationships between N supplied alone as inorganic fertilizer on nodulation and yield except when supplied in combination with phosphorus. Furthermore, Voisin *et al*. (2013) found that N supplied as mineral fertilizer slowed down nodulation and a lower yield than the control. However, the results found by Zamukulu *et al*. (2018) in South Kivu in Ahmed *et al*. (2006) in Pakistan found that yield and modulation increased with increasing doses of mineral N fertilizer indicating a positive relationship between soil N and nodulation. The difference observed between our results and those obtained by certain authors such as Voisin *et al*. (2013) and Griebsch *et al*. (2020), could be explained by the fact that these different studies were described under different conditions. Indeed, Mendes *et al*. (2003) report that the soybean response to N depends on several factors such as the efficiency of the *Bradyrhizobium* strain, the soybean variety, and the initial soil N in the event of N supply in the form of mineral fertilizer.

Conclusion

This study aimed at assessing the effect of *Bradyrhizobium sp*. on the N concentration of soybean leaves and seeds cultivated on acidic soils. This study showed that the studied soils are acidic and generally poor in nutrients. The different strains of *Brady rhizobium* did not induce significant effects on the total N concentration in the leaves and the soybeans in site 1 (Kasapa) on the other hand in site 2 (Kanyameshi) only the N concentration in the leaves was influenced by the different strains of rhizobium. However, in both sites, it was found that the plots without inoculum had lower mean N concentrations compared to plots with inoculum. The N concentration in soil, which varies from one site to another, positively influences yield and nodulation. Thus, the effectiveness of the soybean-rhizobium symbiosis and its influence on the allocation of N through plants strongly depend on the chemical characteristics of the soil and particularly on the initial levels of N in the soils.

Author's Contributions

Ben Tshibuyi Kasu-Bandi: Design, acquisition of data, analysis and interpretation of data, drafting.

Fabien Kitengie Kitengie, Aristote Bwende Nkolomoni, and Placide Kamanda Kamanda: Analysis and interpretation of data and reviews.

Jonas Lwamuguma Bagaluza: Analysis and interpretation of data, drafting and reviewing.

Antoine Kanyenga Lubobo: Design, data interpretation, and reviewing.

Emery Kasongo Lenge: Design, data interpretation, and reviewing.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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