

Assessment of the Biological Nitrogen Fixation in Soybean Sown in Different Types of Moroccan Soils

F. Z. Aliyat, B. Ben Messaoud, L. Nassiri, E. Bouiamrine, J. Ibijbjen

Abstract—The present study aims to assess the biological nitrogen fixation in the soybean tested in different Moroccan soils combined with the rhizobial inoculation. These effects were evaluated by the plant growth mainly by the aerial biomass production, total nitrogen content and the proportion of the nitrogen fixed. This assessment clearly shows that the inoculation with bacteria increases the growth of soybean. Five different soils and a control (peat) were used. The rhizobial inoculation was performed by applying the peat that contained a mixture of 2 strains *Sinorhizobium fredii* HH103 and *Bradyrhizobium*. The biomass, the total nitrogen content and the proportion of nitrogen fixed were evaluated under different treatments. The essay was realized at the greenhouse the Faculty of Sciences, Moulay Ismail University. The soybean has shown a great response for the parameters assessed. Moreover, the best response was reported by the inoculated plants compared to non-inoculated and to the absolute control. Finally, good production and the best biological nitrogen fixation present an important ecological technology to improve the sustainable production of soybean and to ensure the increase of the fertility of soils.

Keywords—Biological nitrogen fixation, inoculation, rhizobium, soybean.

I. INTRODUCTION

SOYBEAN (*Glycine max*) is the most frequently grown oilseed in the world since it is known for its richness on protein, which is essential for human consumption [1]. Soy is one of the most important crops in the world, it represents 27% of the grain produced worldwide [2]. Actually, in many countries oilseed production is progressively evolving. Morocco is the only North African country that has developed industrial logs and significant production of oilseeds [3]. However, low production requires imports of soybean seeds, with a cost of US \$ 16.9 million. However, this culture is still very vulnerable in Morocco as many other countries that have an important deficit on the oils [3]. In order to deal with this deficit, our study aims to assess the nitrogen biological

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fixation in soybean in different Moroccan soils.

II. MATERIALS AND METHODS

A. Soils

Five different soils were used: Biada, Tirs, Hamri, Ramli, Hamri Brun and the vermiculite as control soil. The chemical characteristics of these soils before the fertilization are represented in Table I.

TABLE I
PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE DIFFERENT SOILS USED

Soils	pH (H ₂ O)	cmol _c /dm ³			Mg/dm ³		%N
		Ca+Mg	Ca	Mg	P	K	
Hmari (S1)	7.9	31.6	28.7	2.9	10	88	0.101
Ramli (S2)	8.3	3.9	3	0.9	72	50	0.015
Tirs (S3)	7.9	33.3	31.0	2.3	18	200	0.151
Biada (S4)	8.1	23.7	21.7	2	4	61	0.163
Hamri Brun (S5)	7.6	40.4	36.9	1.5	100	198	0.175
Vermiculite (S6)	7.7	34.2	8.6	25.6	108	1210	0.013

The study was conducted in the greenhouse of the Faculty of Sciences. The plastic pots contained 3 kg of sieved soil (dry weight) (<2 mm). The fertilizer marked with the 15 N was applied as ammonium sulfate solution with 10% 15N at the rate of 10 mg of N per kg of soil.

B. Bacterial Material

Two species of rhizobia were used: *Sinorhizobium fredii* HH103 and *Bradyrhizobium japonicum*.

C. Plant Material

Surface disinfection of the soybean seeds with 95% ethanol for 30 seconds, then with a solution of HgCl₂ at 500 µg / ml. followed by 10 washes with sterile distilled water.

Disinfected seeds were inoculated with 2 g.pot⁻¹ of peat containing rhizobia (10⁹ cells.g⁻¹).

Four soybean (*Glycine max*) seeds were sowed by pot. After germination, just two plants by pot were conserved.

D. Plant Analysis

63 days after the emergence, the aerial part of the plants were harvested, oven-dried at 65 °C for 72 h and finely ground.

Different parameters were determined:

- Dry weight,
- Estimation of the total nitrogen using Kjeldhal's method,
- Calculation of the proportion of the nitrogen fixed (PNF):

$$\%PNF = \left[1 - \frac{\% \text{atom excess } 15N \text{ in soybean}}{\% \text{atom excess } 15N \text{ in the control}} \right] \times 100$$

E. Statistical Analysis

Data are expressed as means for each treatment. Comparison of means was performed by Tukey's test ($P \leq 0.05$). The statistical analysis was performed using MSTATC software.

III. RESULTS

A. Effect of the Inoculation on the Aerial Dry Weight

The results of the inoculation of the soybean with the two species have shown a great increase on the aerial dry weight (Fig. 1), this result has been shown mainly for the soils 2, 3, 4 and 6. However, for soils 1 and 5 the difference between the inoculated and the non-inoculated plants was not significant.

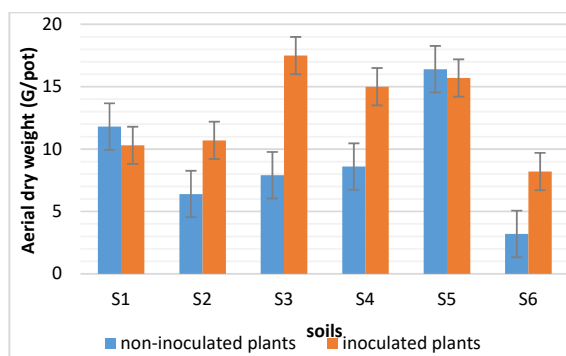


Fig. 1 Effect of the inoculation on the aerial dry weight of plant

The aerial dry weight was twice high for the inoculated plant compared to the non-inoculated plants. Our results confirm those of Samba et al. [4], Okito et al. [5], Ben Messaoud et al. [6], [7]. Similar experiment that has been done by Antoun et al. [8], on radish, showed significant difference on the dry weight of inoculated plants than non-inoculated. Moreover, our results confirm those Meghvansi et al. [9], which explained that inoculation with bacteria increase the growth and nodulation of soybean mainly if these bacteria are Rhizobium strains. The beneficial effect of rhizobia strain on the increase in plant growth, nodulation and yield soybean have been verified in Brazil [10], South Africa [11], Canada [12] and Pakistan [13].

B. Effect of the Inoculation on the Total Nitrogen

The results have shown an importance difference of the total nitrogen contained in the inoculated plants and the non-inoculated ones.

The total nitrogen contained in the inoculated plants sown on the soils S2, S3, S4 and S6 was twice more important than that of the inoculated plants sown on the soils S1 and S5 (Fig. 2).

The present results confirm those of Freitas [14], Ben Messaoud et al. [7] which have shown that the rhizobial inoculation improves the mineral nutrition of plants that is verified by their content on nitrogen and phosphorus.

C. Effect of the Inoculation on the PNF

The proportion of fixed nitrogen in the inoculated plants

was higher than those in non-inoculated.

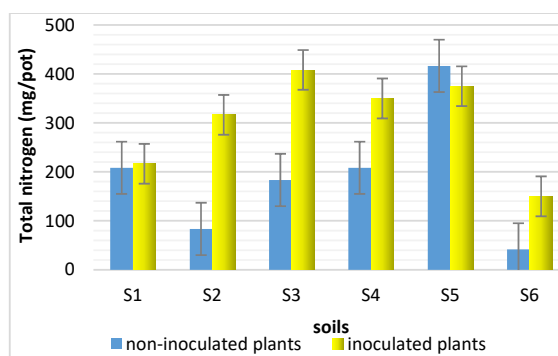


Fig. 2 Effect of the inoculation on the total nitrogen content

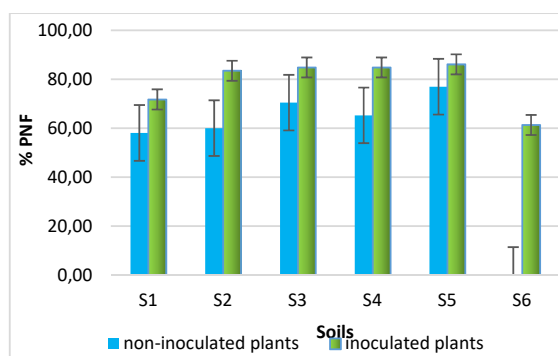


Fig. 3 Effect of the inoculation on the PNF

The PNF of the non-inoculated plant in soil 6 showed 0% that may be due to nature of the soil (control). However, the PNF of all treatment showed a very important result which varied from 61% to 87% (Fig. 3). This result showed that the plants tested used a large portion of their nitrogen requirement from the atmosphere.

The inoculation had shown a great effect on the PNF for all soils tested. The lower PNF (61%) for the inoculated plants was that of the soil 6. Nevertheless, the most important PNF was observed for the inoculated plants in soil 5 with 87%.

The %PNF is influenced by various factors mainly by both type of soil (pH, salt, mineral nutrient) and inoculation.

D. Effect of the Phosphorus Content on the Biomass Production

The soils used in the present study showed different values on the phosphorus content. There are soils rich on phosphorus like the soil 5 with 100 mg/dm^3 . However, the other soils are relatively poor on this element (Fig. 4).

The results showed that the phosphorus contained in soils affect non-inoculated plants more. Nevertheless, there is no effect of the phosphorus content in soils on the biomass production of the inoculated plants, which had shown a great variability on the biomass production.

Our result is in concordance with those of Tairo et al. [15], which showed that the effect of the inoculation increase, significantly according to the phosphorus content (0 to 20

mg/dm³).

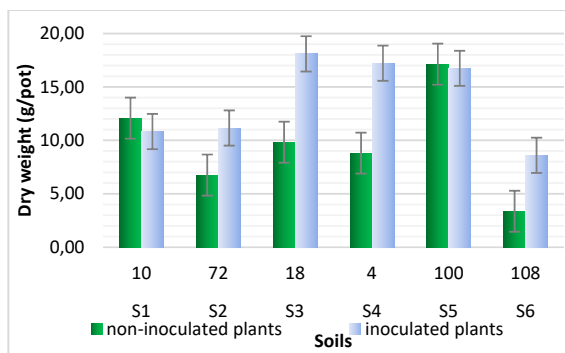


Fig. 4 Effect of the phosphorus content in soil on the aerial dry biomass production

E. Effect of the Phosphorus Content on the PNF

The effect of the phosphorus contained in the soils on the PNF is significant for the inoculated plants (Fig. 5).

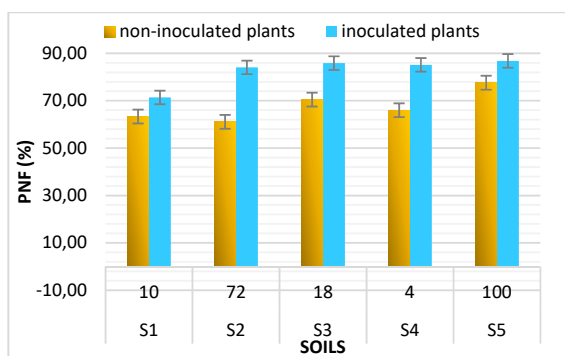


Fig. 5 Effect of the phosphorus content in soil on the PNF

Phosphorus is vital to plant growth and is found in every living plant cell. This nutrient is very important in biological nitrogen fixation, as it is a key element in the energy process [16].

For all the soils tested, the inoculated plants have shown a significant increase on the PNF compared to the non-inoculated. However, there is no correlation between the contents of the phosphorus and the PNF. Because, an important result of PNF was recorded even at 4 mg/dm³ as well as at 100 mg/dm³. Our results confirm [15], [17]-[22] which explained the importance of the phosphorus on the biological nitrogen fixation at different levels and which may be a limiting factor for the plant growth.

IV. DISCUSSION

In the present study, we assessed the biological nitrogen fixation in the soybean tested in different Moroccan soils combined with the rhizobial inoculation. The parameter examined was plant growth, mainly through the aerial biomass production, total nitrogen content and fixed nitrogen content. The results show that inoculation with bacteria increases soybean growth.

The plants inoculated had great positive response in aerial dry weight and they were richer in total nitrogen content compared with the non-inoculated plants. These improvements in inoculated plants could be attributed to improved biological nitrogen fixation by rhizobial inoculation, which increased the amount of nitrogen supply to the plant and therefore improved the plant growth. This result is in well concordance with [23]-[26], [15]. Moreover, the great biomass production of soybean may also be explained by the amount of phosphorus available in soils.

Many previous studies have shown the importance of the phosphorus for the growth of many legumes because it serves many functions like the cell division, photosynthesis etc. [7], [15]. This interpretation is also valid for the soybean production [27], [28].

The inoculation had also shown a great effect on the nitrogen fixation for all inoculated plants and in the different soils. The difference in the PNF can be due to the rhizobial species and soil characteristics (pH, salt, P...). It is real fact that phosphorus content affect the biological nitrogen fixation directly or indirectly [15]-[22], but in our case there was no correlation between these two parameters. However, other researches have reported that not only the phosphorus is necessary for plant growth, but also its availability might be very prominent in influencing the performance of the biological nitrogen fixation [29]. Thus, the present results are in well concordance with those reported by [27] and [30].

In this study, inoculation had an important influence on the plant growth, total nitrogen content and the PNF. In view of increasing the soybean production locally, research efforts should be directed to assess the optimum combinations between inoculation and phosphorus content considering the climatic conditions that will offer immediate ecological and economic solution.

V. CONCLUSION

The improvement of the BNF by soybean-rhizobial associations combined with the availability of the phosphorus can be used to further expand global crop production and reduce the need for synthetic fertilizers. Soybean had a greater effect on variation in PNF. This study indicates the possibility to improve the soybean production through selection of adequate soil. Future studies are needed to investigate additional soils under different climatic conditions.

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