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Effect of Competitiveness on Nodulation and Nitrogen Fixation in Common Bean (*Phaseolus vulgaris L.*)

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Abstract

The competition on the nodules formation on roots of a determinate growth bean (*Phaseolus vulgaris* L. variety: white Coco), between inefficient BIAT strain, and efficient foreign F_{11} strain of Polish origin, was studied in Culture chamber on sterilized sand.The nodules from the mixed inoculum of equal concentrations were identified using streptomycin antibiotic. Based on the number of nodules formed by each of the two strains inoculated separately; no relationship between efficacy and infectivity.In mixed inoculum, competitiveness for nodulation was influenced by strain efficiency, since strain F_{11} formed more nodules, producing 67.7% of flowering nodules.According to nodulation kinetics, this competitiveness in favor of the most efficient strain definitively, as of the twentieth days after the inoculation. The results of the dry matter yield and the fixed nitrogen amounts of the aerial organs of the three treatments clearly showed that the fixation efficiency of the F_{11} strain; was severely affected by the presence of the inefficient strain.The competition capacity of efficient strains to colonize the nodulation sites and atmospheric nitrogen fixation capacity in the presence of another inefficient strain; are two independent parameters.

1. Introduction

Compared to other legumes, beans have low atmospheric nitrogen fixation capacity [1; 2].One of the factors contributing to this situation is the absence of efficient indigenous strains of *Rhizobium phaseoli*. However, the use of artificial inoculation with highly efficient foreign strains did not improve yield [3; 4]. Native *rhizobiums* are more competitive by decreasing the number and binding activity of nodules formed by introduced efficient strains [5; 6]. Similar results have been demonstrated in the field by Weaver and Frederick [7] and Roughley et al [8]. The success of artificial inoculation depends on the competitive capacity of the newly introduced strain to colonize nodulation sites in the presence of native strains of the same host spectrum of the legume [9]. The work of George and Robert [10] using six efficient strains of *Rhizobium leguminosarum* bv. *Phaseoli*, demonstrated the existence of this competitiveness in all possible combinations of pairs of strains for the formation of nodules on the roots of the bean (*Phaseolus vulgaris* L.). Theoretically, the competitive power of a *Rhizobium* strain can be influenced by two parameters: its intensity in the rhizosphere [11] and / or its nitrogen-fixation efficiency. For the first parameter, Moawad et al. [12], revealed the associations *Phaseolus vulgaris–Rhizobium leguminosarum bv. Phaseoli* and *Glycine max – Bradyrhizobium japonicum* that competitiveness is independent of the number of rhizobial cells.

On the other hand, Denton et al. [13], showed a relationship between the number of nodules formed and the number of rhizobia present in the rhizosphere in other associations such *as M. truncatula- Sinorhizobium sp*. For efficiency, Positive relationship with competitiveness found in soybean associations *Bradyrhizobium japonicum* and bean - *R. leguminosarum bv. Phaseoli* [14], and *Phaseolus vulgaris* - *R. gallicum* [15]. However, this positive relationship has not been demonstrated in chickpea associations *Mesorhizobium loti* [14] or red clover -

Rhizobium leguminosarum biovartrifolii [16]. The partecularity of the present work is: identification of all the nodules formed by the mixed inoculum in the Common Coco bean variety (*Phaseolus vulgaris* L.). And to evaluate the effect of efficiency on the competitiveness of a native strain BIAT, and another efficient strain F_{11}, of Polish origin, by comparing biomass production, total nitrogen yield of the aerial organs and number of Nodules formed when each of strains BIAT and F_{11} treated separately and when the two strains are inoculated together.

2. Materials and Methods:

The competitiveness study aimed to estimate the proportion of the nodules formed by each of the strains constituting a mixed inoculum. In this regard; previous studies in the laboratory have shown using antibiotic disk resistance technique [17], that the two strains selected for our study showed different reactions with respect to streptomycin disc antibiotic at one Concentration of 10 µg: F_{11} strain is very sensible, with an inhibition diameter of 23.5 mm. In contrast, the local strain (BIAT) is resistant with a diameter of 6 mm.

2.1. Competition for nodule formation: culture chamber:

The experiment was carried out in a culture chamber with a photoperiod of 14 hours / day, a luminous intensity of 4700 lux and a temperature of 25°C ± 2°C during the day and 20°C ± 2°C at night.

The cultivation was carried out in clay pots each containing 1.2 Kg of sand and a layer of gravel at the bottom of the pot to ensure drainage; both were sterilized beforehand at 120°C**with a pressure of one bar** for 30 minutes in an autoclave.

The seeds of the same size disinfected with a 0.1% concentration of mercury chloride, washed by sterile distilled water baths for five times to remove any traces of $HgCl_2$; then were sown at a rate of four seeds per pot. Upon emergence, the crop was homogenized, to keep only one plant per pot.

2.2. Inoculum concentration and inoculated treatments:

The inocula were prepared by growing the different strains in liquid medium Vincent [18] on a rotary shaker **with 85 rpm** at 26°C for three days. The concentrations of inoculums has been evaluated by successive dilution technique [18].

The three inoculated treatments are one with the local strain (BIAT), the second with the introduced strain (F_{11}) and the third with the mixture of the two strains. Inoculation was carried out one day after homogenization with 6 ml of inoculums at a concentration of 2×10^9 bacteria / ml for the first two treatments, while the plants of the third treatment received 12 ml of inoculum at the rate of 6 ml of each strain with the same concentration.

2.3. Media and Plant culture:

Plants were feeded once a week by 10 ml of sterilized nutrient solution without azote. The composition of the nutrient solution quoted from Vincent [18], slightly modified by Hamza [19], considering the sensitivity of the bean to sodium chloride. On the other hand, irrigation with sterile distilled water was carried out each day, and the volume supplied is sufficient to cause a slight flow.

2.4. Identification of bacteria from the nodules:

Root was cutted on either side of the nodules and then washed with sterilized distilled water. The surface of a nodule was sterilized by immersion in ethanol 95 % for 10 seconds, and then in 0.1% mercuric chloride ($HgCl_2$) solution, for 3 minutes, followed by rinsing in eight successive baths of distilled water to remove any traces of $HgCl_2$. The nodule thus treated; crushed with a glass rod in 3 ml in sterile physiological water, in order to release the bacteroids, which it contains. Two Petteri dishes containing a yeast extract mannitol (YEM) agar [18] were cultured by 1 ml of this bacterial suspension to study the resistance to streptomycin by antibiotic disk resistance technique [17].

2.5. Measurement parameters:

Every ten days, after inoculation, three pots of each treatment were randomly selected to perform the appropriate measures. The last sampling coincides with the formation of the first pods.

The measurements concerned the number of nodules formed by each strain and / or mixed strains and the yield of dry matter and nitrogen only of the aerial organs. The dry matter of these components was measured after 48 hours of drying in oven at 70°C by electronic precision balance. Produced Nitrogen was determined by the Kjeldhal method on the plant powder of the aerial organs. The efficiency of the different inocula was estimated by measuring the dry biomass and the nitrogen yield of the aerial organs.

3. Results and Discussion:

3.1. Results

3.1.1. Production of Aerial Biomass:

The Dry Matter yield of the aerial parts per plant increased Instead of time (Fig.1). Reason of this progressive evolution of aerial organs yield was elevated numbers of functional nodules. At the flowering stage, the yield of aerial organs yield was 2.095 g, 1.075 g and 0.95 g respectively in the presence of strain F_{11}, mixed inoculum and BIAT (Fig. 1). This result showed that the production of aerial biomass was reduced by 51.31% in the presence of the inefficient strain.

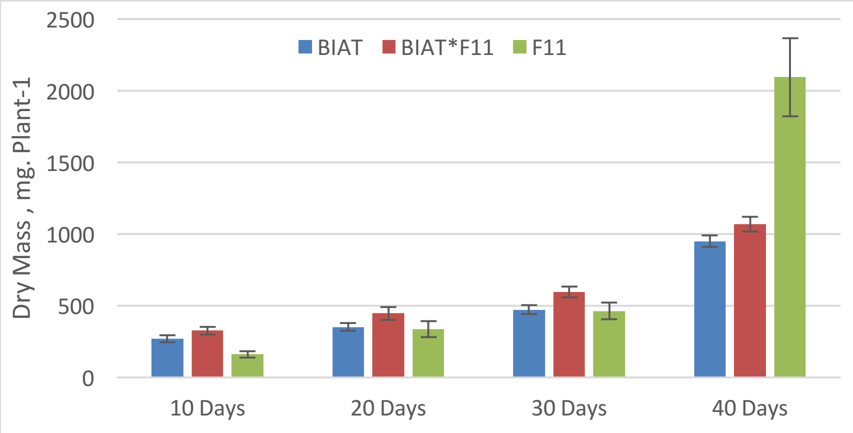

Figure 1: Effect of inoculum type on dry matter yield of the aerial organs.

3.1.2. Nitrogen yield in Aerial Organs:

The total nitrogen yield of the aerial organs followed the same variations over time as their dry weights (Fig. 2). Due to the gradual maturity over time, of all the nodules formed on the root; the nitrogen yield increased gradually. At the flowering stage, the F_{11} strain produced the best nitrogen yield (33.14 mg). This result shows that the nodules of this combination have expressed a better nitrogen-fixation capacity. In contrast, the local strain produced a poor yield (11.75 mg), which is almost 1/3 of that fixed by F_{11}. Thus, this indigenous strain has a low atmospheric nitrogen fixation capacity by using a large proportion of the carbohydrates provided by its plant partner for its own growth. In plants inoculated with the mixture of the two strains "F_{11} + BIAT," the nitrogen yield was almost equal to that observed in plants associated with the BIAT strain alone (13.75 mg). Therefore, the presence of the inefficient BIAT strain reduced the expression of the most efficient strain F_{11}.

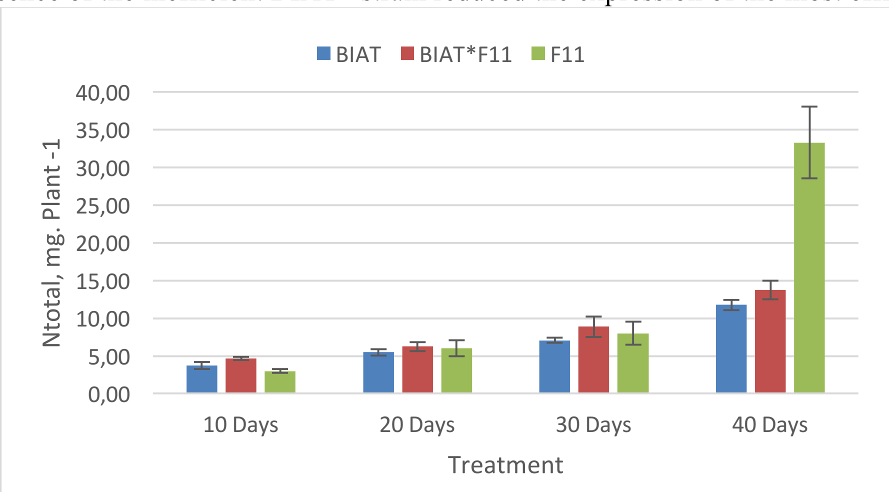

Figure 2: Effect of inoculum type on Nitrogen yield of aerial organs.

3.1.3. Nodulation:

Separately, the two strains produced almost the same number of nodules instead of equal periods.

This shows that the infectivity of the two strains is independent of their atmospheric nitrogen fixation efficiencies (Fig. 3). On the other hand, the root system of the plants inoculated with the mixture of the two strains carries a significant number of nodules compared to that formed by the strains inoculated separately. This refers to the fact that these plants received a double inoculum concentration. This result shows that the number of nodules formed is a function of concentration of the rhizobial cells in the rhizosphere of the host plant. This nodular superiority is countable on the twentieth day after inoculation (Fig. 3).

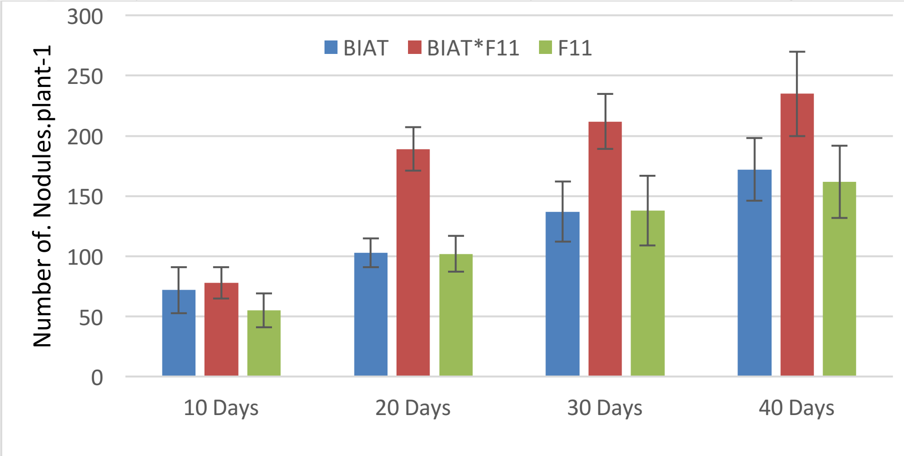

Figure 3: number of nodules Depending on the type of inoculum.

In mixed inoculum, all the nodules formed on the root system of the inoculated plants identified accurately as to their origin by streptomycin antibiotic. The results of the identification show that the host plant formed nodules with the two strains of the mixed inoculum. However, strain F_{11} formed more nodules (Fig. 4).

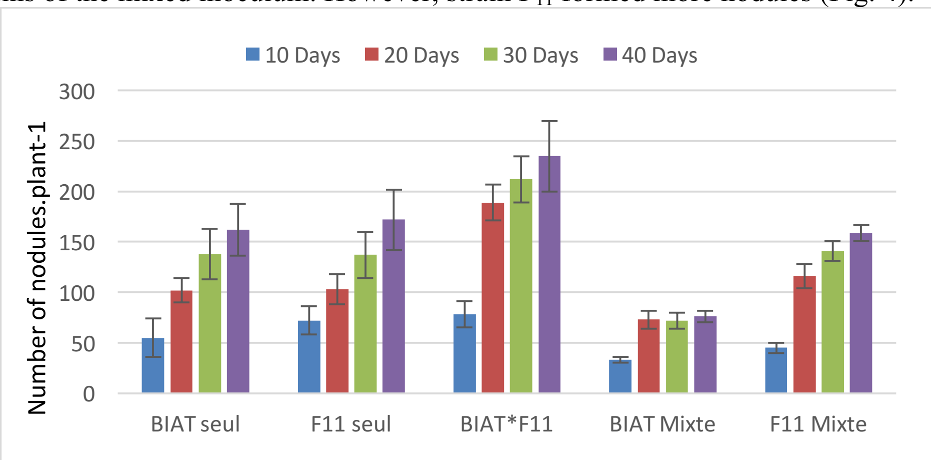

Figure 4: number of nodules Depending on the strains in the pure state and in the mixture.

The nodulation kinetics instead of time shows that the two strains isolated; have almost the same capacity of infection (Fig. 5). Nevertheless, as a mixture, the measured data showed that the host plant had a selection in favor of the efficient strain F_{11}. This selective effect in relation to the efficiency of the strain is permanently installed as of the 20th day after inoculation. In addition, our results show that nodulation is a continuous phenomenon up to the flowering stage.

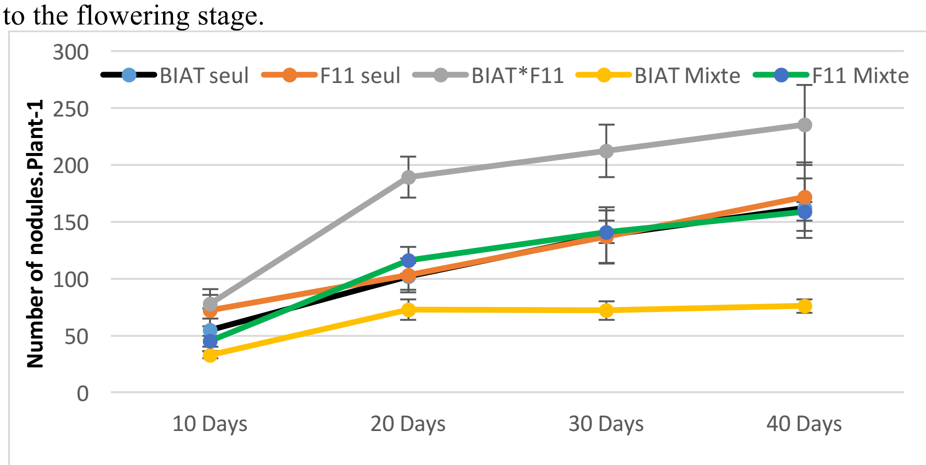

Figure 5: Nodulation kinetics Depending on the type of inoculum.

3.2. Discussion:

The plants inoculated with the F_{11} strain produced the best yield of aerial dry matter compared to the plants inoculated by the BIAT strain. This difference in biomass production was attributed to the nitrogen fixation power of the two strains. The nitrogen yield of plants in symbiosis with the indigenous BIAT strain is only 1/3 that of plants associated with the foreign strain F_{11}.This confirms very well that the local strain is much less efficient than the F_{11} strain; since the two strains produced the same number of nodules separately. However, in mixed inoculum, this biomass yield decreased by 50 %.

Our results agree with those of Rice et al. [4], who demonstrated the weight reduction in alfalfa (*Medicago sativa* L.) by inoculating it with two strains BALSAC and NRG-185 from *Rhizobium meliloti*. Theoretically, this reduction in aerial biomass observed in the presence of mixed inoculum is not related to the availability of reduced nitrogen, since strain F_{11} produced the same number of nodules. It is therefore very probable that this above ground biomass of the plants inoculated with the two strains; is attributed to an additional allocation of carbohydrates to their root systems in comparison with those received by the roots nodulated only by the F_{11} strain. This proportion of carbohydrates, which varies between 8 and 15% [20], is dependent on the number of nodules formed and independent of their efficiencies.

This result shows that in a *Rhizobium* type symbiotic association, the host plant undertakes to ensure all vital sources to its partner even at the expense of its own growth. Thus, the idea of a preferential allocation of carbohydrates to efficient nodules to sanction the ineffective nodules suggested elsewhere [21; 22], not verified in our work. In addition to the "exogenous" competitiveness of the nodulation sites, there appears to be another "endogenous" competitiveness with respect to the energy source between the two types of nodules. However, the identification of the nodules formed by the mixed inoculum shows that the F_{11} strain maintained its infectivity compared to that of BIAT strain which was reduced by about 50%. This result reveal that the host plant has the mechanisms that allow it to promote its association with the most efficient strain of the mixed inoculum; However, without totally inhibiting the inefficient strain of forming nodules [23; 24]. However, our results show that this inhibition was completed twenty day after inoculation. This shows that the selective power exerted by root exudates secreted by the host plant [25], to prevent the BIAT strain from colonizing root infection sites [26] took place only after formation of the two types of functional nodules. This result highlighted a close relationship between the competitive power of a rhizobial strain and its symbiotic efficiency. This positive effect of efficiency in the phenomenon of competitiveness has been indicated previously [27; 28]. While this relationship was not highlighted in other works [29; 31;32]. In addition, the number of nodules formed in the presence of the mixed inoculums; shows the positive effect of the concentration of rhizobial bacteria in the rhizosphere on nodulation [13].

Conclusion:

In our work, the successful competitiveness of the F_{11} strain can be attributed to its rapid mobility [33], to colonize infection sites of root; Which constitute a limiting factor for the nodulation [34;35]. According to literature, data on the relationship between the "exogenous" competitiveness of strains and their efficiencies are controversial. Nevertheless, it remains to be clarified how the presence of ineffective nodules, in small numbers, on the same root system of a legume have reduced the nitrogenase activity of efficiently large numbers of formed nodules.

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