



## Growth analysis, photosynthate partition and nodulation in bean and soybean

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**ABSTRACT:** Photoassimilate partition and allocation among plant organs varies throughout their development and is also influenced by factors inherent to the genotype and the environment. Nodulation in the soybean-diazotrophic bacteria interaction is more effective than in the bean-diazotrophic bacteria interaction. This investigation studied growth and photoassimilate partitioning throughout the bean and soybean cycles and inferred how much it could affect the nodulation of the roots. For this purpose, an experiment with two treatments was carried out, soybean (cultivar BRS GO – 7760 – RR) and bean (cultivar BRS Estilo), with four replications, conducted in pots and entirely randomized. The seeds were inoculated with commercial rhizobia specific for bean and soybean LeguMax<sup>®</sup> (Novozymes–Turfa). Plants were analyzed throughout their cycles based on leaf area and dry mass of all organs, including nodules. Mathematical models were fitted to the data and based on them, the instantaneous physiological indicators of growth were estimated, and the percentages of photoassimilate partition among organs were evaluated. Crop growth rate, relative growth rates, net assimilation rate as well as net photosynthesis rate had higher values in soybean compared to bean, following the pattern of leaf area and total dry mass. For both species, the highest rates occurred at the beginning of the cycle, decreasing with age. Unlike the bean, soybean has a high capacity to supply photosynthates to all of its organs throughout its entire cycle, favoring the maintenance of nodule growth and explaining its greater capacity for nitrogen assimilation.

**Key words:** *Phaseolus vulgaris*, *Glycine max*, biomass, nodule, photosynthesis.

### Análise de crescimento, partição de fotoassimilados e nodulação em feijão e soja

**RESUMO:** Durante o desenvolvimento das plantas a partição e alocação de fotoassimilados variam entre os seus órgãos, e dependem de fatores inerentes ao genótipo e ao meio ambiente. A nodulação é mais efetiva na interação soja-bactéria diazotrófica do que na interação feijão-bactéria diazotrófica. Esta pesquisa tem como objetivo estudar o crescimento e a partição de fotoassimilados ao longo dos ciclos do feijão e da soja e inferir o quanto isso pode afetar a nodulação das raízes. Para tanto, foi instalado um experimento com dois tratamentos, soja (cultivar BRS GO - 7760 - RR) e feijão comum (cultivar BRS Estilo), com quatro repetições, conduzido em vasos e inteiramente ao acaso. As sementes foram inoculadas com inoculantes comerciais específicos para feijão e soja LeguMax<sup>®</sup> (Novozymes–Turfa). Plantas foram coletadas ao longo de seus ciclos e medidas as áreas foliares e as massas secas de todos os órgãos, incluindo nódulos. Modelos matemáticos foram ajustados aos dados e, com base neles, foram estimados os indicadores fisiológicos instantâneos do crescimento e, também, foram avaliadas as porcentagens de partição de fotoassimilados entre os órgãos. A taxa de crescimento das culturas (CGR), as taxas de crescimento relativo (RGR), a taxa líquida de assimilação (NAR) e a taxa líquida de fotossíntese (Pn) apresentaram maiores valores na soja em relação ao feijoeiro. Para ambas as culturas as maiores taxas ocorreram no início do ciclo, decrescendo ao longo do ciclo cultural. Diferentemente do feijoeiro a soja tem alta capacidade de suprir de fotoassimilados a todos os seus órgãos ao longo de todo o seu ciclo, o que favorece o maior crescimento dos seus nódulos, justificando a sua maior capacidade na captura do N<sub>2</sub> atmosférico.

**Palavras-chave:** *Phaseolus vulgaris*, *Glycine max*, biomassa, nódulo, fotossíntese.

## INTRODUCTION

Allocation and partition of photosynthates occur throughout the entire plant life cycle and are important because both vegetative and reproductive production and productivity depend on them. Allocation is the flow of photosynthates between the

photosynthate-producing organs, called source, and the consuming organs, called sink. Partition is the proportion of photosynthates (mass) in each organ in relation to the total dry mass (TDM) of the plant (HELDT & PIECHULLA, 2011; TAIZ et al., 2014).

Allocation and partition are intrinsic characteristics to each species and even to

varieties within the same species. They are genetically controlled, but they change according to the environment in which the plants are reported (VEGA, et al., 2001; WANG et al., 2018; STEIN & GRANOT, 2019). Variations of the environment such as photoperiod, light intensity, temperature, soil moisture, availability of nutrients, and atmospheric CO<sub>2</sub> concentrations, substantially alter the flow of photosynthates in plants and, therefore, the intensity of allocation and partition (TAIZ et al., 2014; CHEVARRIA et al., 2015; HAGEMAN et al., 2019). Hormones have a great effect on the development of photosynthate sources and sinks, but studies involving the effects of hormones on the allocation and partition of photosynthates are scarce.

The main photosynthate transported between producing organs and consumers is sucrose. This carbohydrate is a source of organic carbon that supplies the energy needed by all the developing organs (LEMOINE, 2000; KERBAUY, 2008; HELDT & PIECHULLA, 2011; STEIN & GRANOT, 2019).

The photosynthates produced in the leaves can be retained in the chloroplasts of the leaves themselves or be transported to other organs where they can enter the formation of structural tissues or be accumulated as reserve substances such as sucrose or starch in the amyloplast of the cell (LEMOINE, 2000; STEIN & GRANOT, 2019). These substances are temporarily immobilized until another organ demands the products and they are relocated to that organ (WARDLAW, 1990; STITT & ZEEMAN, 2012).

The allocation and partition of photosynthates in plants can be better understood by using the technique of growth analysis of plants throughout their entire cycle or even part of it. Growth analysis is a laborious technique, but relatively simple and easy to perform using a few pieces of equipment. It basically consists of determining the leaf area of plants and the dry mass of each organ of the entire plant, normally every week (PORTES & CARVALHO, 2009).

With the obtention of the leaf area and the dry mass of the different plant organs, the instantaneous or average physiological indicators of growth such as crop growth rate, relative growth rate, net assimilation rate, specific leaf area, leaf area ratio and leaf area duration can be determined (PORTES & CASTRO, 1991). To determine the instantaneous physiological indicators of growth, it is necessary to fit a mathematical equation to the leaf area and total dry mass values (and, if it is of interest, fit equations

to the dry masses of the different organs). The ideal equation is one with minimal deviations between the calculated values and the observed ones, resulting in high correlation coefficients and a statistically significant fit (ZULLO & ARRUDA, 1987; PORTES & CARVALHO, 2009).

Bean and soybean are leguminous species capable of symbiotically associating with the diazotrophic bacteria of the genus *Rhizobium*. These bacteria, inside nodules in the roots, are able to fix N<sub>2</sub> from atmosphere and assimilate it, transforming it from inorganic to organic and transferring part of this element to the plant. Through that process, legumes obtain all or part of the nitrogen they need to satisfy their needs throughout their cycles, in return, plants supply the bacteria with photosynthates from their leaves. There are many studies reporting soybean as highly efficient in this process, unlike the bean which in most cases are not so successful. For both species, carbohydrate deficiency will result in the death of the bacteria or ineffective infection (NDAKIDEMI et al., 2006; HUNGRIA, M. et al., 2013; FANKEM et al., 2015).

The percentage of photosynthates partitioned from the aerial part to the roots depends on the presence and efficiency of nodules associated with the roots (PAUSCH et al., 1996; BOUCHO et al., 2019) and there is a fine regulation between the aerial organs and the roots in the partition and allocation of photosynthates. In addition, the root can regulate the allocation of photoassimilates itself, depending on the presence or efficiency of nodules (KOSSLAK & BOHLOOL, 1984; BOUCHO et al., 2019).

Many studies have sought *Rhizobium* strains that are more efficient in nitrogen fixation (HUNGRIA et al., 2000), but few studies have compared the ability of plants of the same species or different species to efficiently supply the rhizobia with photosynthates, as well as evaluating the efficiency of these bacteria in fixing and assimilating N as a function of the capacity of the plant to adequately supply them with carbohydrates

The objectives of this research were to A) measure the growth and photosynthate partitioning among the organs of bean and soybean plants B) Using a new mathematical equation to estimate average net photosynthesis rates (P<sub>n</sub>) C) evaluate whether the differential partition of photosynthates between the two species studied is related to the increase in the number of nodules and their permanence in the roots during the cycles of the two crops, thus trying to explain the greater efficiency of soybean in fixing and assimilating N in relation to bean.

## MATERIALS AND METHODS

The experiment was carried out in a greenhouse in Goiânia, Brazil, Latitude: 16°40'43"S, Longitude: 49°15'14"W, altitude of 730 m. The soil used in the experiment was an Acric Red Latosol (Brazilian soil classification system - *Sistema Brasileiro de Classificação de Solos* - Embrapa), whose chemical characteristics before the experiment were as follows: pH (CaCl<sub>2</sub>) - 5.2, available P (Mehlich I) - 2.3 mg kg<sup>-1</sup>, K - 50 mg kg<sup>-1</sup>, Ca - 5.7 cmolc kg<sup>-1</sup>, Mg - 0.5 cmolc kg<sup>-1</sup>; cation exchange capacity (CEC) - 9.4 cmolc kg<sup>-1</sup> and organic matter (OM) - 30 g kg<sup>-1</sup>. Fertilization to correct the soil was performed based on a chemical analysis of the soil and recommendation of Embrapa Arroz e Feijão.

The experiment was conducted in plastic pots, 44 for bean and 48 for soybean respectively. Six seeds per pot of bean and soybean were sown. After thinning, two plants were kept in each pot arranged at 50 cm from each other. The pots in which the bean and soybean seeds were sown contained 16 kg of soil and each pot was fertilized with 1.225 g of mixture 4-30-10 NPK for bean and 5.25 g of 2-20-10 NPK for soybean. The seeds were previously inoculated with LeguMax® (Novozymes-Turfal), a commercial product containing diazotrophic bacteria specifically indicated for bean or soybean.

The experimental design was completely randomized, consisting of one cultivar of soybean (BRS GO - 7760 - RR) and one cultivar of bean (BRS Estilo) with four repetitions. The experiment was carried out in a greenhouse with maximum PAR radiation of 1600 μmol photons m<sup>-2</sup> s<sup>-1</sup>. Plants were watered twice a day at 6:00 am and 6:00 pm and the weed control was done manually.

Four pots containing bean and four pots containing soybean, with two plants per pot, were used every week for evaluation.

Evaluations occurred at 10, 17, 24, 31, 38, 45, 51, 58, 65, 72 and 79 days after emergence (DAE), with one additional soybean evaluation taken at 87 DAE due to its longer cycle, totaling 44 pots for bean and 48 for soybean, respectively. The evaluation dates correspond to phases V1, V2, V3, V4, R1, R1, R3, R4, R5, R6, R6 for soybean (NLEYA et al., 2013) and V3, V4, V4, R5, R6, R7, R7, R7, R8 for bean (OLIVEIRA et al., 2018).

A total of eight soybean plants and eight bean plants were harvested on each evaluation day. The plants were cut at ground level and taken to the laboratory, where the leaves were detached to determine the leaf area (LA) with an LI-COR

electronic leaf area meter (LI 3100 model). The leaves and all the other organs (roots - after being properly washed using a coarse mesh sieve), stems, lateral branches, petioles and pods were separated, placed in paper bags and oven dried at 70°C for 48 hours (when they attained constant mass). Subsequently, the plant material was weighed, and mathematical models were fitted to the obtained data for leaf area (LA) and dry mass (DM) of separated organs (dry mass of leaves, stems, petioles, roots, lateral branches, pods and total dry mass) as a function of the evaluated plant age (DAE). Total dry matter (TDM) is the result of the sum of the dry masses of all plant organs.

From the equations fitted to the total dry mass (TDM) and leaf area (LA), the instantaneous physiological indicators of growth: crop growth rates (CGR), relative crop growth rate (RGR), leaf area rate (LAR) and net assimilation rate (NAR) were calculated using the following equations  $CGR = dTDM / dt$ ,  $RGR = CGR / TDM$ ,  $LAR = LA/TDM$ ,  $NAR = dTDM / dt / LA$ , where  $d$  is the derivative of the equation and  $t$  (time) = DAE (PORTES & CASTRO JR, 1991; PORTES & CARVALHO, 2009). Due to the very small values of the nodule variables, they were disregarded here.

In order to estimate the average net photosynthesis rate (P<sub>n</sub>) in μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> from net assimilate rate (g of dry mass m<sup>-2</sup> of leaf day<sup>-1</sup>) the following equation was used (PORTES et al., 2017).

$$P_n = NAR(g \text{ of dry matter } m^{-2} \text{ day}^{-1}) * \left(\frac{C}{100}\right) * \left(\frac{44}{12}\right) * \left(\frac{1}{44}\right) * \left(\frac{1}{86400}\right) * (10^6) = \mu\text{mol } CO_2 m^{-2} s^{-1}$$

Where: C = % of carbon (C) in the dry mass; 44/12 transforms C into CO<sub>2</sub>; 1/44 transforms g of CO<sub>2</sub> into mols of CO<sub>2</sub> (that is, one mol of CO<sub>2</sub> equals 44 g); 1/86400 transforms hours into seconds and 10<sup>6</sup> transforms mols of CO<sub>2</sub> into μmols. The percentage of carbon (C) in the dry mass is approximately 45% (C = 45) (WALKER, 1992). The final equation is:

$$P_n = NAR(g \text{ of dry matter } m^{-2} \text{ day}^{-1}) * \left(\frac{C}{100}\right) * k = \mu\text{mol } CO_2 m^{-2} s^{-1}$$

Where: k = 0,964506173

For C = 45%: k = (45/100) \* (44/12) \* (1/44) \* (1/86400) \* (106) = 0,434027778

If NAR is given in cm<sup>2</sup> of leaves, multiplying the numerical value by 10,000 gives P<sub>n</sub> in μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>.

To carry out the evaluations of the nodules, they were manually detached from the roots after properly washed and then their fresh and dry masses, volumes and densities were quantified.

To determine the volume of fresh nodules, they were immersed in a graduated cylinder containing water. The volume of nodules is equal to the difference between the initial and final volumes of displaced water. After that, they were placed in paper bags and dried in an oven at 65 °C for 48 h, and finally, weighed in a precision scale to obtain the dry mass. The density of the nodules was obtained by dividing the fresh mass by the volume ( $d = \text{mass} / \text{volume}$ ). The variables evaluated were presented per plant. Mathematical equations were fitted to the data obtained for the nodules.

Statistical analysis was performed with Assisat software (SILVA & AZEVEDO, 2016). Growth analyzes were performed according to PORTES & CASTRO (1991).

## RESULTS

The equations fitted to the data of leaf area and dry mass of plant organs are displayed in table 1. The equation that best fit the observed data was the cubic exponential, except for branches and pods, for which the equation that gave the best fit was the quadratic exponential. The quality of the adjustments

is observed by the high correlation coefficients as well as by the high levels of significance.

The soybean leaf area (LA) grew exponentially until it reached 556.50 cm<sup>2</sup> plant<sup>-1</sup> at 38 days after emergence (DAE), the point of curve inflection. From then on it started to grow slower until reaching to the maximum growth area of 881.50 cm<sup>2</sup> plant<sup>-1</sup> at 58 DAE. From that date on, growth rate was negative, that is, the leaf area gradually lowered over the cycles of the plants (Figure 1A).

For bean, the growth kinetics was similar to soybean; the curve inflection point also occurred at 38 DAE and, on that date, the leaf area was 243.10 cm<sup>2</sup> plant<sup>-1</sup>. The maximum growth of 314.90 cm<sup>2</sup> plant<sup>-1</sup> occurred at 51 DAE, with negative growth thereafter. The maximum leaf area attained by the soybean was 2.8 times greater than that of the bean.

The ratio between LA and total dry mass (TDM) corresponds to the leaf area ratio (LAR). For both soybean and bean, the LAR gradually lowered over the growth cycles (Figure 1B). For soybean, the maximum LAR was 123.05 cm<sup>2</sup> g<sup>-1</sup> at 10 DAE and it decreased until 33.09 cm<sup>2</sup> g<sup>-1</sup> at 87 DAE. For bean, the maximum value was 95.2 cm<sup>2</sup> g<sup>-1</sup> at 10 DAE, decreasing until 12 cm<sup>2</sup> g<sup>-1</sup> at 79 DAE.

Table 1 - Equations fitted to the leaf area (LA) per plant (cm<sup>2</sup>) and dry mass (DM) of leaves, stems, petioles, roots, lateral branches, pods and total dry mass (TDM) of bean and soybean in function of age of the plants (x = DAE), in g plant<sup>-1</sup>. Exp = e. DAE = days after emergence. Due to their very small masses, the nodules were disregarded.

Variable	-----Bean-----	r	p
LA	$y = 44.8014 * \text{Exp}^{(3.2913 * 10^{-2}x + 8.9172 * 10^{-4}x^2 - 1.5439 * 10^{-5}x^3)}$	0.9864	≤ 0.01
DMleaves	$y = 0.1984 * \text{Exp}^{(2.920 * 10^{-2}x + 1.105 * 10^{-3}x^2 - 1.693 * 10^{-5}x^3)}$	0.9977	≤ 0.01
DMstems	$y = 2.5512 * 10^{-2} * \text{Exp}^{(0.1007x - 4.8948 * 10^{-4}x^2 - 3.0540 * 10^{-6}x^3)}$	0.9901	≤ 0.01
DMpetioles	$y = 4.8241 * 10^{-3} * \text{Exp}^{(0.1674x - 1.450 * 10^{-3}x^2 + 4.1603 * 10^{-8}x^3)}$	0.9929	≤ 0.01
DMroots	$y = 7.4424 * 10^{-2} * \text{Exp}^{(0.1706x - 2.756 * 10^{-3}x^2 + 1.491 * 10^{-5}x^3)}$	0.9956	≤ 0.01
DMbranches	$y = 6.1712 * 10^{-7} * \text{Exp}^{(0.399x - 3.180 * 10^{-3}x^2)}$	0.8877	≤ 0.05
DMpods	$y = -15.390 + 0.456x - 3.00 * 10^{-3}x^2$	0.9947	≤ 0.05
TDM	$y = 0.2640 * \text{Exp}^{(0.109x - 1.047 * 10^{-3}x^2 + 2.357 * 10^{-6}x^3)}$	0.9980	≤ 0.01
	-----Soybean-----		
LA	$y = 4.9225 * \text{Exp}^{(0.2157x - 2.8319 * 10^{-3}x^2 + 1.1283 * 10^{-5}x^3)}$	0.9961	≤ 0.01
DMleaves	$y = 1.6333 * 10^{-2} * \text{Exp}^{(0.2139x - 2.5472 * 10^{-3}x^2 + 9.040 * 10^{-6}x^3)}$	0.9976	≤ 0.01
DMstems	$y = 2.0225 * 10^{-2} * \text{Exp}^{(0.1420x - 9.9317 * 10^{-4}x^2 + 6.4343 * 10^{-7}x^3)}$	0.9968	≤ 0.01
DMpetioles	$y = 1.7469 * 10^{-4} * \text{Exp}^{(0.371x - 4.830 * 10^{-3}x^2 + 2.01 * 10^{-5}x^3)}$	0.9982	≤ 0.01
DMroots	$y = 0.4310 * \text{Exp}^{(-2.360 * 10^{-2}x + 2.4332 * 10^{-3}x^2 - 2.136 * 10^{-5}x^3)}$	0.9530	≤ 0.01
DMbranches	$y = 3.414 * 10^{-4} * \text{Exp}^{(0.228x - 1.548 * 10^{-3}x^2)}$	0.9828	≤ 0.01
DMpods	$y = 4.230 * 10^{-9} * \text{Exp}^{(0.494x - 3.016 * 10^{-3}x^2)}$	0.9998	≤ 0.01
TDM	$y = 0.424 * 10^{-2} * \text{Exp}^{(0.203x - 2.09 * 10^{-3}x^2 + 6.35 * 10^{-6}x^3)}$	0.9981	≤ 0.01



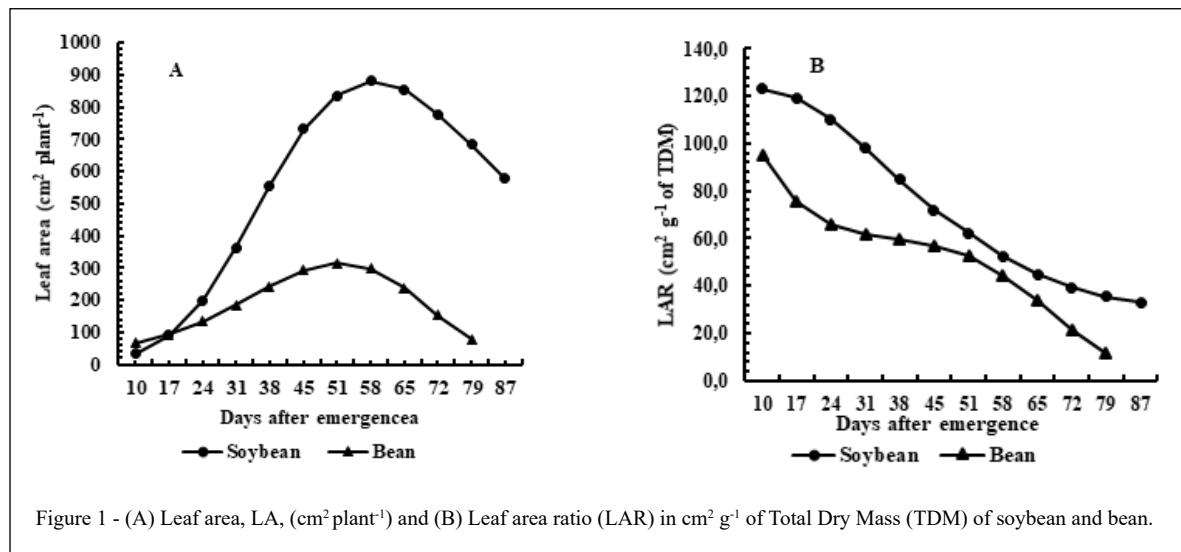


Figure 1 - (A) Leaf area, LA, (cm<sup>2</sup> plant<sup>-1</sup>) and (B) Leaf area ratio (LAR) in cm<sup>2</sup> g<sup>-1</sup> of Total Dry Mass (TDM) of soybean and bean.

The growth kinetics of total dry mass (TDM) as a function of days after emergence of plant (DAE), for both soybean and bean, followed cubic exponential curves, with a high correlation coefficient and significance at the level of 1% ( $P \leq 0.01$ ) (Table 1 and Figures 2A and 2B).

The maximum total dry mass of 19.84 g plant<sup>-1</sup> for soybean was reached at 72 DAE, thereafter there was a reduction due to the loss of dry mass of roots (DMroots) and leaves (DMleaves). For bean, the maximum total dry mass of 7.0 g plant<sup>-1</sup> occurred at 65 DAE, decreasing from that date onwards as a function of the reduction in leaf dry mass. From 51 DAE, for both genotypes, the allocation of photosynthates predominated towards the growth of pods (DMpods) with a consequent increase in their masses (Figure 2).

Throughout the crop cycle, the allocation of photoassimilates was predominantly to the roots and leaves, with the roots prevailing as the sink with greater partition of mass (Figure 3 A and B). For the other organs, stems, petioles and branches, a small variation was observed in the partition of mass over their cycles.

For soybean (Figure 3 A) the proportion of the mass allocated to the roots was 69.24 %, at 10 days after emergence (DAE), higher than that of all other organs, it quickly decreased to 30.07 % at 38 days. From 38 DAE onwards, the allocation of photosynthates to the roots increased again, surpassing the observed values for leaves at 51 days.

It continued to grow until reaching a maximum total dry mass (TDM) value of 38.29% at 65 days, then decreasing again and presenting 26.72% at 87 DAE. The proportion of TDM retained in the leaves was 17.68% at 10 DAE, with a rapid increase to 39.17 % at 31 days, exceeding the values allocated to the roots. From that date onwards it was gradually reduced to 19.91 % at 87 days. The lateral branches of the soybean appeared at 31 DAE presenting a proportion of 2.56 % of the TDM in relation to the other organs. It increased to 6.31 % at 58 days, remaining stable until the end of the culture cycle. The same was true for petioles. Stem allocation increased up to 24 DAE, reaching 18.17 % of the TDM, then remaining practically constant until the end of the cycle. The participation of pods in the TDM of the plants, from 51 DAE on, grew gradually, reaching 14.40 % at 87 DAE, a smaller proportion than that of bean.

For bean (Figure 3 B) there was a rapid allocation of photoassimilates to the roots, with the proportion of the TDM increasing from 45.36 to 54.89 % from 10 to 24 DAE. From 24 days onwards, there was a gradual reduction to 36.07 % at 65 DAE. From that date on, the proportion of the TDM increased again, reaching 44.09 % in relation to the other organs, at 79 days. The leaves presented 41.92 % of the TDM in relation to the other organs at 10 DAE, approximately the same proportion presented by the roots, decreasing their participation from then on, until presenting a minimum value of 7.33 % at 79 days, with the values always lower than the roots. The participation of pods

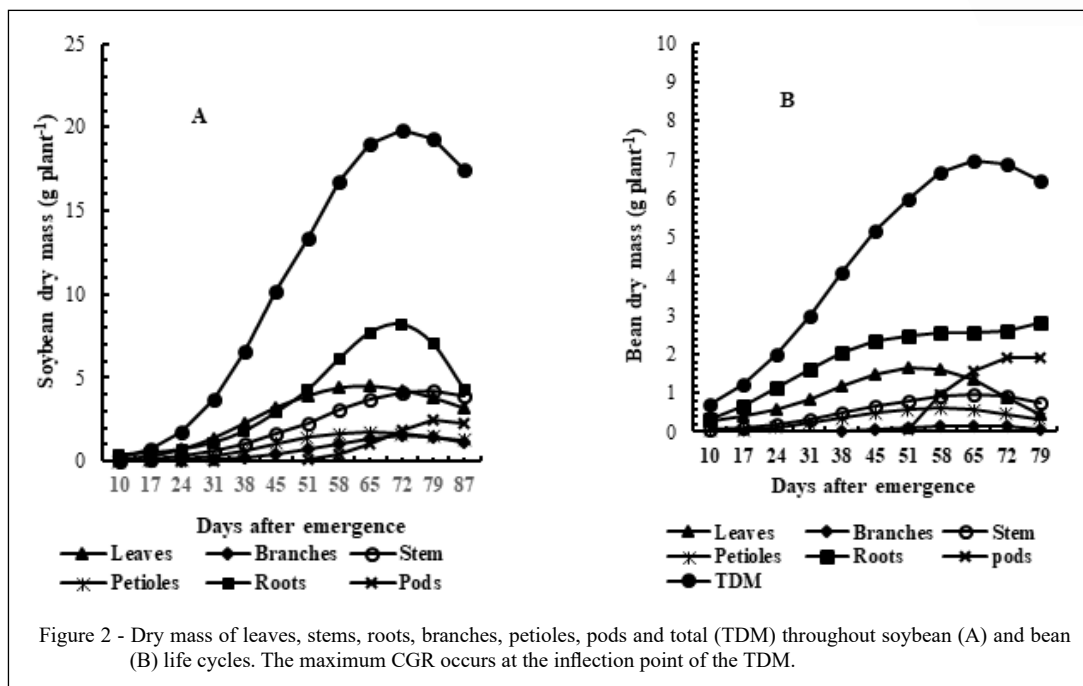


Figure 2 - Dry mass of leaves, stems, roots, branches, petioles, pods and total (TDM) throughout soybean (A) and bean (B) life cycles. The maximum CGR occurs at the inflection point of the TDM.

in the total dry mass of plants, from 51 DAE, increased rapidly, reaching 30.35 % at 79 DAE.

It is important to note that the percentages of photoassimilate partition in the pods in relation to the TDM of the plants, from 51 DAE onwards, were always higher in bean at the expense of leaves, compared to soybean (Figure 3).

The instantaneous crop growth rates (CGR), figure 4A, of both cultures were close at the beginning of their cycles (10 DAE),  $6.22 \times 10^{-2} \text{ g plant day}^{-1}$  for bean and  $4.30 \times 10^{-2} \text{ g plant day}^{-1}$  for soybean, but soybean grew abruptly reaching a maximum value of  $5.43 \times 10^{-1} \text{ g plant day}^{-1}$  at 45 DAE, while a maximum value of  $1.60 \times 10^{-1} \text{ g plant day}^{-1}$  for bean occurred at 38 DAE. The maximum CGR occurred at the inflection point of the TDM as a function of the age of the plants (Figures 4A, 2A, and 2B). They became negative after 65 DAE for bean and 72 DAE for soybean.

The instantaneous relative growth rates (RGR), figure 4B, were significantly different at the beginning of the cycle (10 DAE),  $16.30 \times 10^{-2} \text{ g g}^{-1} \text{ day}^{-1}$  for soybean and  $8.83 \times 10^{-2} \text{ g g}^{-1} \text{ day}^{-1}$  for bean, gradually reducing in both cultures over the cycles of the plants. As for CGR, they became negative from 65 DAE for bean and 72 DAE for soybean.

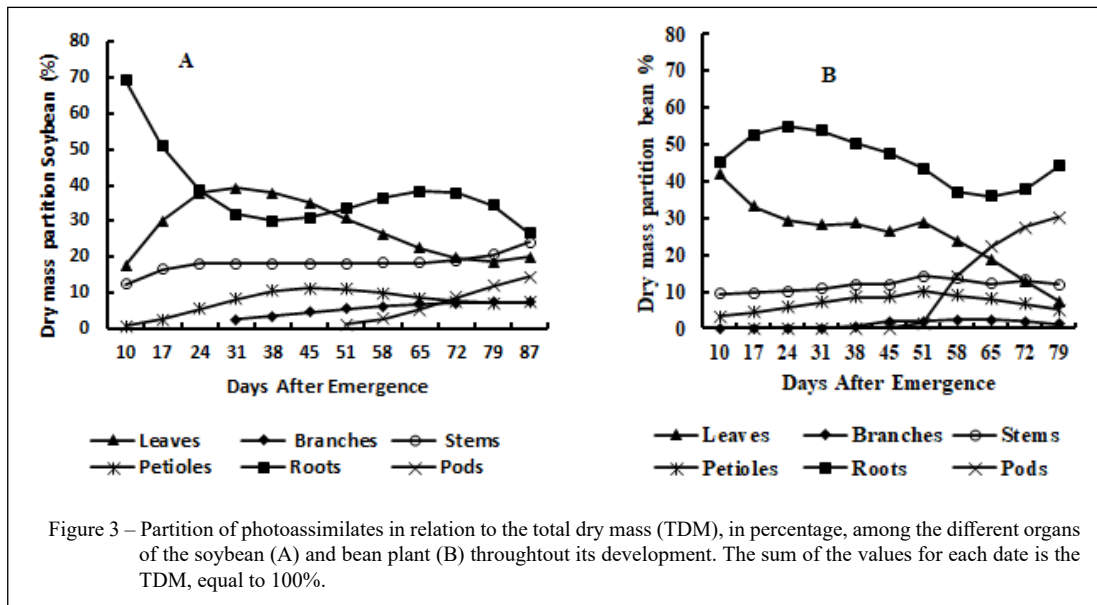
The instantaneous net assimilates rates (NAR) of the two genotypes, figure 4C, decreased over the crop cycles; however, soybean always

showed higher values than bean. The bean showed a slight increase in the rates between 10 and 17 DAE, but from that date on the rates were decreasing, in the same proportion as the rates observed for the soybean. At 65 and 72 DAE the NAR for soybean and bean, respectively, became negative.

As expected, the average net photosynthesis rates for both crops ( $P_n$  in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Figure 4D) estimated from the NAR (PORTES et al., 2017), showed the same behavior as the NAR.

Considering the organs that had great variation in their photoassimilates throughout their cycles, figure 5A shows the Dry Mass of leaves (DMleaves) and Dry Mass of roots (DMroots) of soybean and bean. For soybean, the leaves grew up to 65 days after emergence (DAE), presenting on that date  $4.52 \text{ g plant}^{-1}$ . The root growth attained to  $8.19 \text{ g plant}^{-1}$  at 72 DAE, almost double the mass contained in the leaves at 65 DAE, so throughout the soybean cycle there was a great allocation of photoassimilates to the roots, but there was a drastic reduction in growth after the 72 DAE. Similarly, the leaves of bean grew less than the root presenting  $1.65 \text{ g plant}^{-1}$  at 51 DAE and the roots  $2.49 \text{ g plant}^{-1}$  at 51 DAE, but unlike soybean, there was no drop in root growth, it remained almost constant until 72 DAE.

Figure 5B shows the shoot/root relationships for soybean and bean. For soybean, there was a fast growth of the aerial part at the expense of



the roots until close to 40 DAE (Figure 5A). From that date onwards there was greater photoassimilate allocation to the roots until approximately 65 DAE. However, the allocation to the aerial part started to grow again from then onwards. For bean, there was a high allocation to the roots until 20 DAE, but after that date the allocation increased to aerial part until close to 65 DAE. Unlike soybean, the greater allocation was towards the roots.

The data of fresh and dry mass, volume and density of nodules per plant are shown in figure 6. For all variables, there was the same trend of the curves, showing that they are related. Up to 45 DAE, bean showed slightly higher values than soybean, but thereafter soybean surpassed bean for all evaluated variables. The values for soybean grew consistently until 79 DAE, having dropped only at 87 DAE.

The correlations between the dry masses of nodules ( $n$ ) and the dry masses of the aerial part (canopy =  $c$ ), leaves ( $l$ ) and roots ( $r$ ) of bean and soybean plants are shown in table 2. There was a significant correlation between dry mass of nodules and dry mass of aerial parts and roots of plants for both legumes, except for dry mass of nodules and dry mass of leaves.

## DISCUSSION

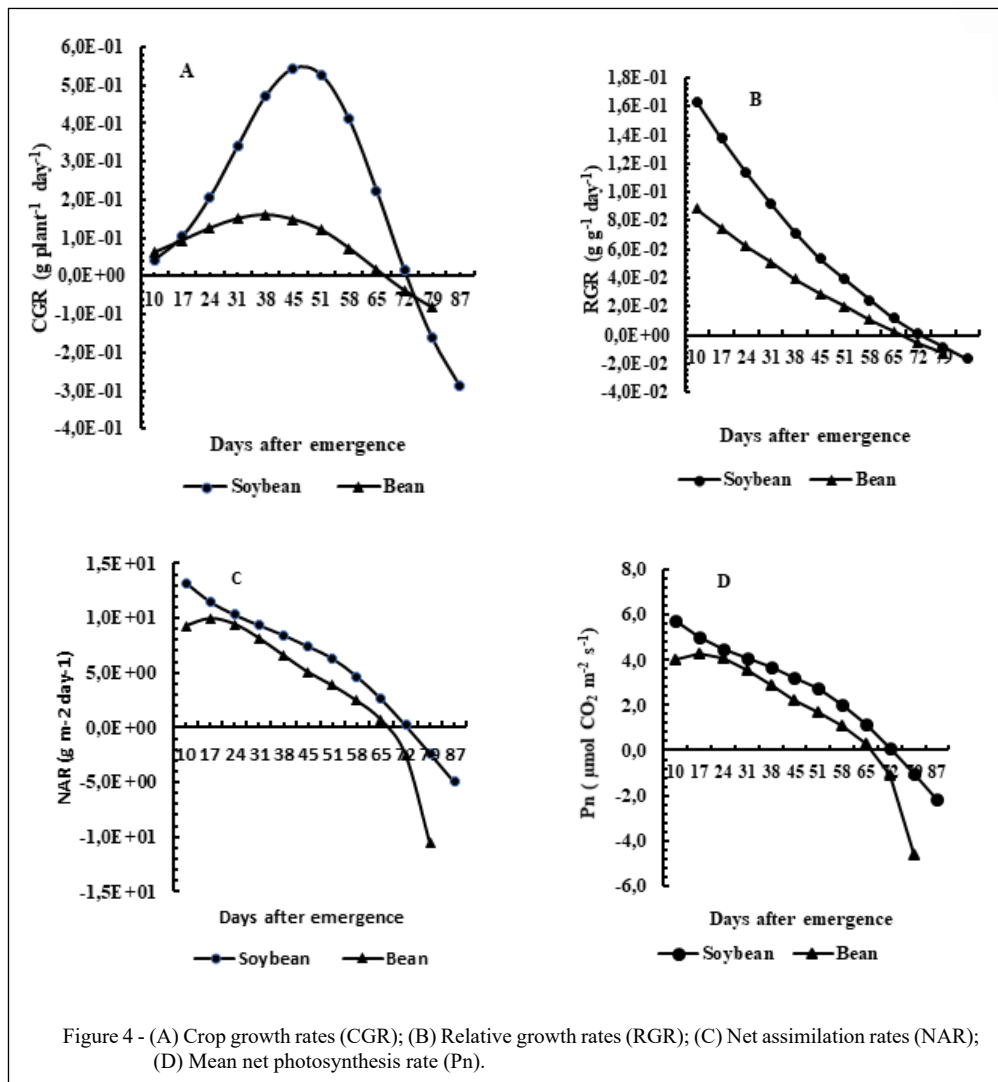
Growth analysis is a classic technique for analyzing the growth of plants among species, as well as within the same plant species, especially when they are

subjected to different environments. Analyzing growth during part of the plant cycle is not enough, as errors can occur due to the variability among plants even within the same community. Ideally, the analysis should take place during the entire plant cycle, as in the present research and others (BUSO et al., 2021). Through equations fitted to the experimental data, excellent mathematical models are developed, with high levels of significance and excellent correlations among the variables analyzed.

In the present research, the models fitted to the data of leaf area, total dry mass and dry mass of the different organs of the plants, as a function of age, presented high correlation coefficients and the majority, were significant to a 1% probability level, both for soybeans and for beans (Table 1).

Due to the high quality of the fits, from the mathematical models obtained, the instantaneous physiological growth indicators for the two legumes were estimated, with a high level of confidence due to being able to compare them throughout their cycles.

The leaf area (LA) is a central factor for plant growth studies because it represents the surface that receives radiation, triggering the photosynthetic process on which the production of plant biomass depends and consequently, agricultural production (SRINIVASAN et al., 2017; REIS et al., 2019). This is visible in this study, in which, at 58 DAE for soybean (R4 phase) and 51 DAE for bean (R7 phase), registered maximum areas of  $881.50 \text{ cm}^2 \text{ plant}^{-1}$  and  $314.88 \text{ cm}^2 \text{ plant}^{-1}$ , respectively, resulting in total dry masses (TDM) of  $19.84 \text{ g plant}^{-1}$  at 72 DAE and



7.0 g plant<sup>-1</sup> at 65 days, respectively. At 58 DAE for soybean and 51 DAE for bean, LA decreased in both cultures and occurred due to leaf senescence and abscission, which justifies the cubic exponential model as the most adequate for the growth of this variable (Table 1).

However, the peak of dry mass accumulation occurred at 72 DAE and 65 DAE for soybean and bean respectively, since the leaf area guaranteed, until then, positive net photosynthesis (Figure 4D). After those dates, leaf senescence caused organic carbon losses through respiration, overcoming the gain, with a decline in TDM (STODDART & THOMAS 1982; WOO et al., 2018), which also justifies the cubic exponential mathematical model as the most suitable for the growth of dry mass of leaves, stems, roots, petiole and TDM (Table 1).

The leaf area ratio (LAR) is the relationship between the leaf area (LA) and the total dry mass (TDM) of plant, that is, it is the ratio between the assimilatory surface and the amount of assimilated mass ( $RAF = LA / TDM$ ). Therefore, the higher the TDM in relation to LA, the lower the LAR will be, and this occurred throughout all the cycle of the two crops under study (Figure 1B), with a reduction of approximately 73 and 87% of the initial LAR for soybean and bean, respectively. Apparently, bean are more efficient than soybean, because according to the results, more assimilated is synthesized per unit of leaf area. However, the average photosynthetic rates become negative from 72 and 65 DAE for bean and soybean, respectively (Figure 4), but some plant organs continue to grow, especially the pods (fruits) and the



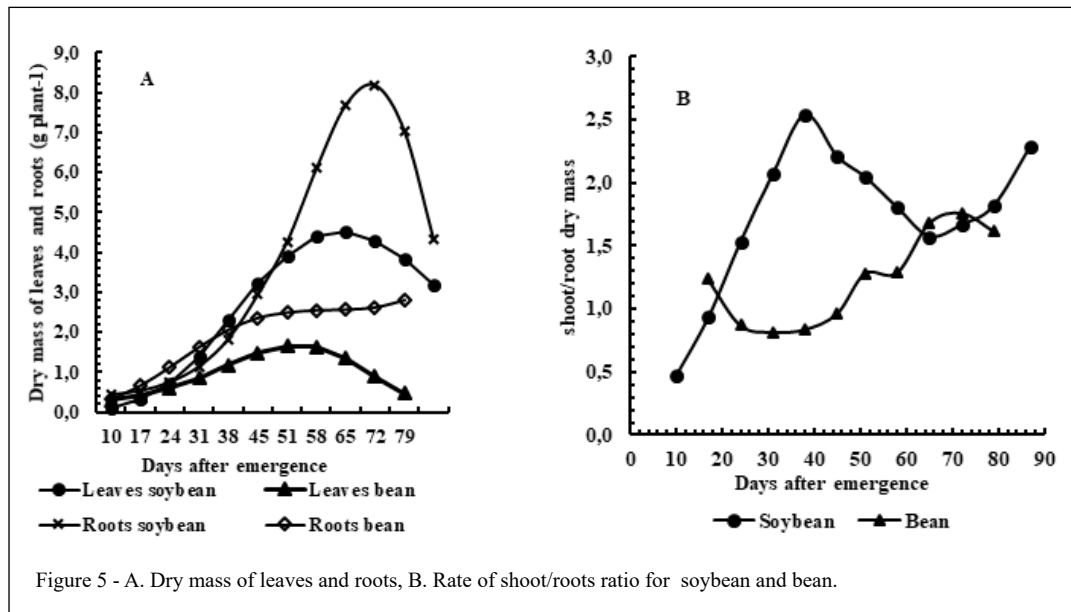


Figure 5 - A. Dry mass of leaves and roots, B. Rate of shoot/roots ratio for soybean and bean.

others are maintained. This is only possible because plants have stored photosynthates (WARDLAW, 1990; LEMOINE, 2000; STITT & ZEEMAN, 2012).

The gain of Dry Mass of pods (DMpods) after 51 DAE, until the end of the cycle, 87 DAE for soybean and 79 DAE for bean, is important for grain production. The allocation of photosynthates to the sink was to the detriment of their retention by the source, especially for bean (Figure 3 A and B). For soybean pods, the increase in the photosynthates partition was from 1.12% at 51 DAE to 14.40% at 87 days, while for bean the increase was from 1.44% at 51 days to 29.76 % at 79 DAE.

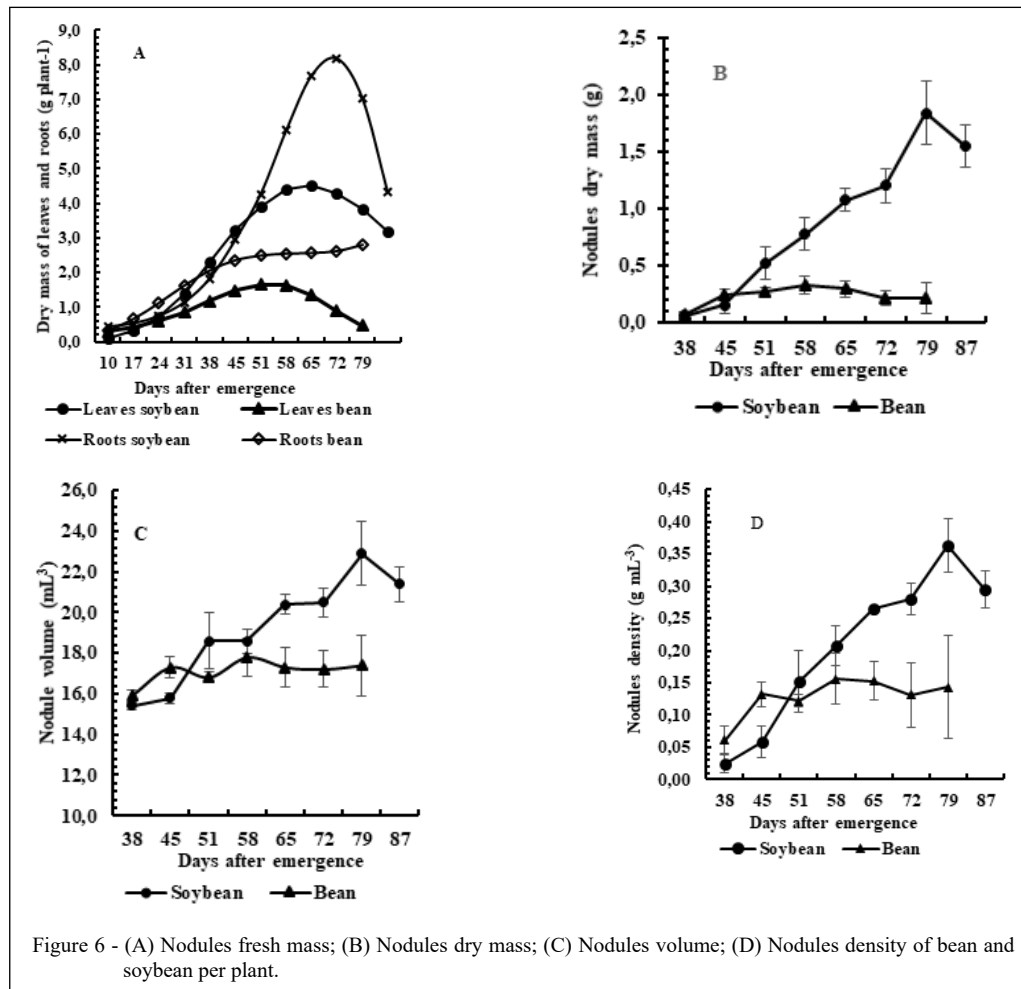
For soybean, apparently, the reduction in the retention of photosynthates by the leaves did not jeopardize their supply to the other organs. For bean, contrary to what was reported by PORTES & ARAÚJO (2012) it was somewhat atypical, because even at the end of the cycle, photosynthates were allocated to the roots reaching 44% of the total dry mass (TDM), higher than that observed for soybean, which presented 26.72% of the TDM.

The gain of Dry Mass pods in soybean may have been partly attributable to reallocation of photoassimilates from the roots and leaves to the pods, since in soybean there was a decrease of dry matter of leaves (DMleaves) from 65 DAE and dry matter roots (DMroots) from 72 DAE. In bean, the decrease in DMleaves occurred after 58 DAE, but, unlike soybean, there was no decrease in DMroots. For both

crops, the gain of dry matter pods (DMpods) did not exceed the loss of TDM from 72 DAE for soybean and 65 DAE for bean, which was to be expected, since the crop growth rate (CGR) and relative growth rates (RGR), as well as the net assimilation rate (NAR) and net photosynthesis rate (Pn) became negative from 65 DAE for bean and 72 DAE for soybean, because the loss of CO<sub>2</sub> by respiration exceeded the amount assimilated for photosynthesis.

The gain in dry mass by pods (DMpods), even with negative average photosynthesis rates, is the result of relocation of photoassimilates from roots and leaves to the pods, since there was low carbon gain by photosynthesis.

At 72 and 65 DAE the leaf areas of soybean and bean measured 778.92 and 237.27 cm<sup>2</sup> plant<sup>-1</sup>, respectively (Figures 1A and B). Although, they are expressive values of leaf areas, crop growth rate (CGR), relative crop growth rate (RGR), net assimilation rate (NAR) and net photosynthesis rate Pn, on the respective dates, became negative. It means that the amount of CO<sub>2</sub> lost by respiration, which occurs uninterruptedly in all metabolically active tissues, was greater than that assimilated by photosynthesis which occurs only in cells with chlorophyll when exposed to light. Respiratory rates vary little by unit of leaf area (μmol of CO<sub>2</sub> cm<sup>-2</sup> or dm<sup>-2</sup> or m<sup>-2</sup>) and even by unit of metabolically active organic mass (μmol of CO<sub>2</sub> g<sup>-1</sup> or kg<sup>-1</sup>). Respiratory rates are around 2.0 - 3.0 μmol of CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (BYRD



et al., 1992), varying only slightly according to the temperature and age of the organ where it is occurring.

Photosynthesis rates vary a lot per unit of leaf area or per unit of metabolically active organic mass, usually as a function of Photosynthetic Photon Flux Density (PPFD) and temperature. Photosynthesis rates for C<sub>3</sub> plants, such as soybean and bean, reach maximum values between 20 and 25  $\mu\text{moles of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (BYRD et al., 1992; RIBEIRO et al., 2004; CHAVARRIA et al., 2015). In intense light conditions, photosynthesis rates are around 10 times higher than respiratory rates (BYRD et al., 1992). For that reason, over a day, in good light conditions, the amount of CO<sub>2</sub> assimilated in photosynthesis is greater than that lost in respiration.

From these considerations it can be deduced that in the metabolically active vegetal mass, practically the entire mass of the plant, the metabolism is intense, resulting in a high amount

of CO<sub>2</sub> as a by-product (HELD & PIETCHULLA, 2011; LEMOINE, 2000; TAIZ et al., 2014). The photosynthetic rates, even though they are high, are not enough to supply the entire plant (Figure 4). To supply the high demand for photosynthates, since photosynthesis is not enough, plants use previously stored reserves, both of sucrose and starch (BYRD et al., 1992; LEMOINE, 2000). Therefore, under those conditions, the amount of CO<sub>2</sub> produced exceeds that of CO<sub>2</sub> assimilated by photosynthesis, resulting in negative values (Figure 4).

The RGR (Figure 4 B) gives us the real growth of the plants because it takes into account the mass previously produced by the plant. For that reason, it is a very important variable for comparing the actual growth of two different plant species, especially when they have different sizes. For example, the maximum TCC for soybean and bean were  $4.30 \times 10^{-2} \text{ g plant}^{-1} \text{ day}^{-1}$  and  $6.22 \times 10^{-2} \text{ g plant}^{-1}$

Table 2 - Regression equations that best correlate the fit to dry mass of nodules (n), with the dry mass of the canopy (c), leaves (l) and roots (r) of the bean (b) and soybean (s) plants. vs (versus); ns (not significant); \* and \*\* and significant at 5% and 1% of probability respectively.

Equations		
Interaction	Bean (b)	Correlation
n vs c (yb)	$yb = 1.343 * 10^{-3} Exp^{(2.690X - 0.338X^2)}$	r = 0.93*
n vs l (zb)	$zb = 0.397 - 0.504X + 0.270 X^2$	r = 0.65 (ns)
n vs r (jb)	$jb = 1.203 * 10^{-17} Exp^{(29.45X - 5.753X^2)}$	r = 0.97**
-----Soybean (s)-----		
n vs c (ys)	$ys = 2.70 * 10^{-4} Exp^{(1.320X - 5.10 * 10^{-2}X^2)}$	r = 0.97**
n vs l (zs)	$zs = 8.780 * 10^{-7} Exp^{(6.643 X - 0.791 X^2)}$	r = 0.78 (ns)
n vs r (js)	$js = 2.575 * 10^{-3} Exp^{(1.904 X - 0.143 X^2)}$	r = 0.94**

day<sup>-1</sup>, respectively at 10 DAE, while the maximum RGR was  $16.3 * 10^{-2} \text{ g g}^{-1} \text{ day}^{-1}$  and  $8.83 * 10^{-2} \text{ g g}^{-1} \text{ day}^{-1}$  on the same date.

Analyzing the variables related to the root nodules (Figure 6), it can be seen that before 51 DAE, when there was no pod sink as yet in the plants, the volume, mass and density of nodules were higher in bean. However, after 51 DAE there was a progressive increase in volume, mass and density of nodules in soybean and those high values were maintained until the end of its cycle. This behavior is equivalent to that observed in the photosynthate partition among the organs (Figure 3), in which the bean had small variations in percentage values between 58 and 72 DAE to the root-nodule set (root + nodule), at the same time that the soybean had a progressive increase in the allocation of photosynthate into the root-nodule set from 51 to 72 DAE, reducing from then on. The nodule dry mass assessment is considered the best method for evaluating the nodulation factor (SOUZA et al., 2008); although, it is not necessarily related to the efficiency of biological fixation; however, that is question outside the scope of this study.

Throughout the reproductive phase of the plants, the mass gain of nodule and the root-nodule set in soybean, unlike bean, denotes a shoot-root regulation modulating the photoassimilates partition. The allocation of photosynthates to inefficient nodules and their associated roots is less compared to the allocation to efficient nodules and their associated roots (SINGLETON & VAN KESSEL, 1987), which shows that the strength of the sink depends on the efficiency of the organ (PAUSCH et al., 1996; MINCHIN & WITTY, 2005). It is well known that soybean is much more efficient in obtaining nitrogen

from biological fixation than bean (NDAKIDEMI et al., 2006; FANKEM et al., 2015).

The availability of nitrogen, especially in the leaves and roots, is essential for the full development of reproductive organs. Amino acids are the main substances assimilated in plants, which are allocated to the reproductive organs in order to supply them with nitrogen. The assimilation of N depends directly on the availability of sucrose in the plant for the supply of energy and carbon (ZHANG et al., 2015).

After 72 DAE, when NAR and Pn assumed negative values, there was a decrease in photoassimilates of the soybean root-nodule set, but not in the mass of isolated nodules, which corroborates previous studies reporting that in addition to shoot-root/nodule, there may also be a root self-regulation in photoassimilate partition between root and nodule (KOSSLAK & BOHLOOL, 1984). The nodules depend more on organic carbon from current positive net photosynthesis than roots (KOUCHI et al., 1986); it is supposed that the root self-regulation in soybean may explain the fact that nodules did not lose mass even though there was a loss of organic carbon in the root-nodules set. Unlike the regulatory plasticity of soybean, we saw in bean that even with negative NAR and Pn after 65 DAE there was no percentage decrease in photoassimilates partition in the root-nodule set or isolated nodules.

In the regulation of photoassimilates partition between aerial parts and roots, there is presumed to be a compensatory balance between the quantum of photoassimilates allocated for the purpose of biological nitrogen fixation as a function of the nitrogen quantum available from roots to the aerial part, and it is also necessary for both the photochemical and biochemical apparatus

of photosynthesis (HIKOSAKA & TERASHIMA, 1995; KASCHUK et al., 2010). In that sense, the greater plasticity of photoassimilates reallocation evident in the soybean root-nodule set, whose fluctuation in the percentage of photoassimilates partition was 42% over the cycle whereas in the bean it was only 19%, shows the greater ease the soybean root system has in meeting the needs of the aerial part. It is compatible with the higher nodule mass values from 51 DAE in soybean, compared to bean, and it occurs exactly in the period of greatest demand for photoassimilates for pod formation. That behavior observed in soybean could explain the better performance of this species regarding its nodulation when compared to bean.

In addition to the plasticity of relocating photoassimilates, it must be considered that the higher net photosynthesis rates usually occur according to how much larger the leaf area is as well as how much more durable the photosynthetically active area is (KASCHUK et al., 2010). In that sense, when comparing soybean with bean, the highest values of NAR and Pn for soybean throughout the life cycle are in line with their larger leaf area and greater TDM. Soybean also has seven more days of positive NAR and Pn values than bean. Those characteristics could favor better soybean nodulation.

## CONCLUSION

Due to the leaf abscission of both species, in addition to negative net carbon assimilation rate, from 65 DAE (R7 phase) for bean and 72 DAE (R6 phase) for soybean, the mathematical model that best fit the growth of the leaf area (LA), dry matter of leaves (DMleaves), dry matter of stems (DMstems), dry matter of petioles (DMpetioles), dry matter of roots (DMroots) and total dry matter (TDM) was the cubic exponential model.

Soybean outperformed bean in all variables analyzed, such as leaf area (LA) and total dry mass (TDM), as well as the physiological indicators of growth: crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), and average net photosynthesis rate (Pn). The leaf area of soybean remains greater than that of bean until the end of their cycles.

The physiological indicators CGR, RGR, NAR, as well as the Pn, became negative from 72 DAE for soybean and 65 DAE for bean, from these dates the plants began to be supplied by their own reserves of carbohydrates

The superiority of soybean in relation to bean for all analyzed variables explains its superiority in maintaining the growth of its nodules until the end of its cycle. This is due to their ability to supply the rhizobia with carbohydrates, making them active, with the ability to fix N, unlike bean, whose nodules cease to grow after flowering, indicating inefficiency in their supply of carbohydrates and therefore, inefficiency in fixing the N.

## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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