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Soybean growth, solar energy conversion and seed vigour affected by different nitrogen (N) doses

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Abstract

The objective of this study was to evaluate soybean growth, conversion of solar energy and seed vigour of plants cultivated with different nitrogen doses. We carried out experiments using the cultivar BMX Potência RR belongs to the maturation group 6.7, presenting an indeterminate growth habit. The experimental design was completely randomised with four replications in a 4 x 8 factorial scheme (four nitrogen doses and eight collection periods). In the Vn stage of soybean development, we applied four different nitrogen fertilisation doses, i.e. 0, 25, 50 and 75 Kg N ha^{-1} in the form of urea (45% of N). We evaluated leaf area (A_f), leaf dry mass (W_f), stem (W_c), roots (W_r) and pods (W_v), dry matter production rate (C_t), relative growth rate (R_w), net assimilation (E_a), rate, leaf area ratio (S_a), solar energy conversion factor (ξ), leaf area ratio (F_a) and leaf mass (F_w), dry matter partition between plant structures, seedling emergence (E) and the emergence speed index (IVE) of the seedlings originated from the seeds produced. The experiment was conducted throughout the crop cycle. Nitrogen application positively impacted soybean growth; a nitrogen dose of 50 Kg ha^{-1} resulted in highest values of W_t. However, plants subjected to 75 Kg N ha^{-1} reached higher R_w and L values. Nitrogen fertilisation applied in the stage Vn facilitated plant growth, enhanced the conversion of solar energy and resulted in higher seed vigour.

Keywords: *Glycine max* (L.); relative growth rate; net assimilation rate; emergence; emergence speed index.
Abbreviations: A_f_Leaf area, C_t_Dry matter production rate, E_a_Net assimilation rate, E_Emergence, F_a_ Leaf area ratio, F_w_And leaf mass, IVE_And the emergence speed index, L_Leaf area index, N_Nitrogen, R_w_Relative growth rate, S_a_Leaf area ratio, W_c_Stem, W_f_Leaf dry mass, W_r_Roots, W_v_And pods, ξ_Solar energy conversion factor.

Introduction

Soybean [*Glycine max* (L.) Merrill] is the most important commodity of the Brazilian agribusiness (CONAB, 2015). In the 2013/14 crop season, about 30 million hectares were planted with this species (CONAB, 2015), reaching a production of 86.7 million tons (FIESP, 2015). A large part of the Brazilian gross national product is represented by the production chain of this crop, considering that in 2013, soybean cultivation was responsible for 7.8% of all wealth generated by the country (CNA/CEPEA-USP, 2015).

Nitrogen (N) is the most limiting plant nutrient and a constituent of chlorophyll, amino acids, proteins and nucleic acids (Nobre et al., 2010). Nitrogen deficiency can reduce plant biomass and cause leaf senescence (Sant'ana et al., 2011). Soybean, as a legume, is a nitrogen-fixing plant; however, the population of microorganisms that perform the fixation can be adversely affected by a range of environmental factors (Zilli et al., 2013).

The main N sources are urea and ammonium sulphate (Arf et al., 2011). In addition, application in small doses and at the beginning of the crop cycle can provide optimal initial conditions for plant growth until the onset of roots nodulation (Araújo and Carvalho, 2006). Nitrogen fertilisation causes an increase in the number of pods (Silva et al., 2011), plants height and accelerates the insertion of the first pod, positively influencing seed vigour (Abrantes et al., 2010) and protein levels (Gomes Junior and Sá, 2010).

Plant growth analysis describes the morphological and physiological changes that occur in the plant over time and is therefore the first step in the evaluation of primary production (Concenço et al., 2011). Based on this analysis, we are able to characterise different plant responses to different environmental and management conditions (Pedó et al., 2014). However, seed vigour has been evaluated by the substrate emergency test, used by seed companies to predict seed performance (Grzybowski et al., 2015).

This work tested the hypothesis that nitrogen fertilisation positively impacts soybean growth and seed vigour. We evaluated plant growth, solar energy conversion and the vigour of soybean seeds produced under different nitrogen fertilisation levels.

Results and Discussion

Effect of nitrogen on soybean growth

There was an interaction among the factors for the pods dry matter variable (Table 1). Variance analysis indicated a significant effect of N doses on leaf area, stem dry mass, roots and pods. We also observed a significant effect of the collection periods on leaf area, leaf dry mass, stem, roots and pods.

Total dry matter (W_t) followed a logistic tendency with a high determination coefficient ($R^2 \geq 0.93$), indicating significant differences in the dry matter production as a function of the different treatments (Fig. 1a). The plants submitted to four nitrogen doses showed slow initial growth until 42 DAE (days after emergency), followed by a phase of rapid growth and a maximum value at 112 DAE. At 112 DAE, the highest dry matter allocation in the plants occurred with the dose of 50 Kg N ha^{-1} (1,506.68 g m^2), followed by 25 kg N ha^{-1} (1,388.98 g m^2), 0 kg N (1,290.86 g m^2) and 75 kg N ha^{-1} (1,150.84 g m^2) (Fig. 1a). The application of 50 Kg N ha^{-1} resulted in a 15% increase in total dry matter compared to un-fertilised plants (0 kg N ha^{-1}).

The restricted initial growth is related to the low volume of roots and the reduced leaf area. During plant development, both the volume of roots and the useful leaf area increased, facilitating carbon fixation, production rate and dry matter allocation (Pedó et al., 2014).

Nitrogen application may benefit the initial plant establishment, whereas the biological fixation process starts after the emergence of the seedlings (Uhry, 2010). However, doses higher than 20 kg ha^{-1} negatively affect the root nodule formation in soybean plants (Parente et al., 2015). Thus, the application of nitrogen at the dose of 75 kg ha^{-1} may have reduced the number of plant nodules in this study, affecting W_t.

The dry matter production rate (Ct) was low up to 28 DAE for plants in all treatments (Fig. 1b) and corroborates the low dry matter production in this period (Fig. 1a). Plants not submitted to nitrogen application (26 g m^2 d^{-1}) and those under the influence of doses of 25 (31 g m^2 d^{-1}), 50 (33 g m^2 d^{-1}) and 75 kg ha^{-1} (28 g m^2 d^{-1}) reached the maximum C_t at 80, 74, 74 and 70 DAE, respectively. The maximum value of C_t without N application was 25.12 g m^{-2} d^{-1}, corresponding to a reduction of approximately 6, 18 and 25% in relation to the plants submitted to doses of 75, 25 and 50 kg ha^{-1} of N, respectively. Thus, there was a temporal-quantitative difference in the rates of dry matter production between plants submitted to nitrogen application and un-fertilised plants.

Maximum temperatures were occurred from 32ºC to 42 days after plant emergence (DAE). Solar radiation obtained from 14 to 42 DAE ranged from 550 to 680 cal cm^{-2} day^{-1}, with subsequent decrease thereafter (Fig. 1d).

Dry mass synthesis and allocation are also dependent on solar radiation and temperature throughout the development of plants, among other factors. There was a relation between W_t and Ct values and solar radiation, and the reduction along the development collaborated to decrease the values of this growth attributes. However, it should be noticed that the reduction of C_t is also associated with the natural aging of the plant tissues, which reduces the ability to convert the intercepted solar radiation to dry matter.

The highest relative growth rate (R_w) was reached at 14 DAE, with a subsequent decline of the DA until the end of the crop cultivation cycle; this was observed in all treatments (Fig. 1e). The plants submitted to different doses of nitrogen showed a higher capacity to increase dry mass up to 60 DAE compared to un-fertilised plants, considering that from 60 DAE onward, there was an inversion of R_w values. From 60 DAE until the end of the development, the plants without nitrogen fertilisation maintained higher R_w values compared to those submitted to nitrogen fertilisation.

In young plants, R_w values were related to a high amount of leaves with high photosynthetic activities. However, with increasing plant age, R_w values decreased due to the increase in self-shading and the plants´ inability to produce new biomass (Antoniazzi and Deschamps, 2006; Falqueto et al., 2009).

The net assimilation rate (E_a) showed two peaks during the ontogeny of the soybean plants (Fig. 1f). The first peak occurred at approximately 14 DAE for plants subjected to doses of 25 (163.62 g m^2 d^{-1}), 50 (397.43 g m^2 d^{-1}) and 75 kg N ha^{-1} (112.13 g m^2 d^{-1}). In contrast, un-fertilised plants reached the first E_a peak at 30 DAE (633.12 g m^2 d^{-1}); the peak was notably higher than those of the other treatments. The second E_a peak occurred at about 70 DAE for the plants submitted to the 25 and 50 kg N ha^{-1} doses, whereas for the plants without nitrogen application and those under the influence of the 75 Kg N ha^{-1}, the second peak of E_a occurred at 84 and 63 DAE, respectively.

The highest E_a observed at the beginning of the development cycle was due to the high number of new leaves with high photosynthetic capacity (Pedó et al., 2014). Factors such as the length of the vegetative period, photosynthetic rate, distribution of assimilates, leaf angle and leaf distribution in the canopy can affect E_a (Aumonde et al., 2011).

Regardless of the nitrogen dose used, the leaf area index (L) was adjusted to the quadratic model with a high determination coefficient (R2 ≥ 0.97); in all evaluation periods, the highest L value was verified in the plants receiving the highest nitrogen doses (Fig. 1g). Un-fertilised plants and those subjected to a dose of 25 kg ha^{-1} reached maximum L values at 75 DAE, while those submitted to N doses of 50 and 75 kg ha^{-1} reached maximum L values 84 and 87 DAE, with a subsequent tendency to decline. The regression equations referring to the leaf area index of the plants, produced as a function of the application of the different nitrogen doses, are presented in Table 1.

There was an indication that nitrogen application, especially at higher doses, promoted the temporal-quantitative modification in the leaf area index values, increasing the area of soil covered by leaves. According to Lopes and Lima (2015), the leaf area index determines the net assimilation rate and the total dry matter production rate. Aguiar Netto et al. (2000) stated that the reduction of L values is related to the increase of senescence and foliar abscission.

The solar energy conversion efficiency (ξ) showed similar trends among plants of the different treatments (Fig. 1h).

Table 1. Summary of variance analysis with leaf area mean squares (A_f), leaves dry mass (W_f), stem (W_c), roots (W_r) and pods (W_v). Regression equations for the leaf area index refer to soybean plant growth data during the development, produced under different doses of nitrogen fertilisation

Sources of variation	G.L.	Mean square				
		A_f	W_f	W_c	W_r	W_v
Rates (R)	3	1726494*[1]	22.8^{ns}	138.7*	18.7*	27.5*
Collections (C)	7	2.84×10^{-007}*	708.6*	3384.9*	301.4*	1269.7*
R X C	21	518173.6^{ns}	7.5^{ns}	49.3^{ns}	9.9^{ns}	13.4*
Residue	64	582338	10.9	32.7	6	6
Total	95	-	-	-	-	-
Average		2178.2	11.05	19.42	6.6	5.43
CV (%)		35.03	29.83	29.45	37.17	45.13

Rates (Kg ha^{-1})	Leaf área index equations	R^2	Significance at 5%
0	$y = -2E\text{-}05x^3 + 0.003x^2 - 0.0724x + 0.1908$	0.97	*[2]
25	$y = -2E\text{-}05x^3 + 0.0021x^2 - 0.0159x - 0.1112$	0.99	*
50	$y = -3E\text{-}05x^3 + 0.0036x^2 - 0.0653x + 0.1022$	0.99	*
75	$y = -3E\text{-}05x^3 + 0.0037x^2 - 0.0579x + 0.0954$	0.99	*

[1]Not significant, or *significant at 5% of probability by F test ($p \leq 0.05$). [2]Significant at 5% of probability by Tukey's test.

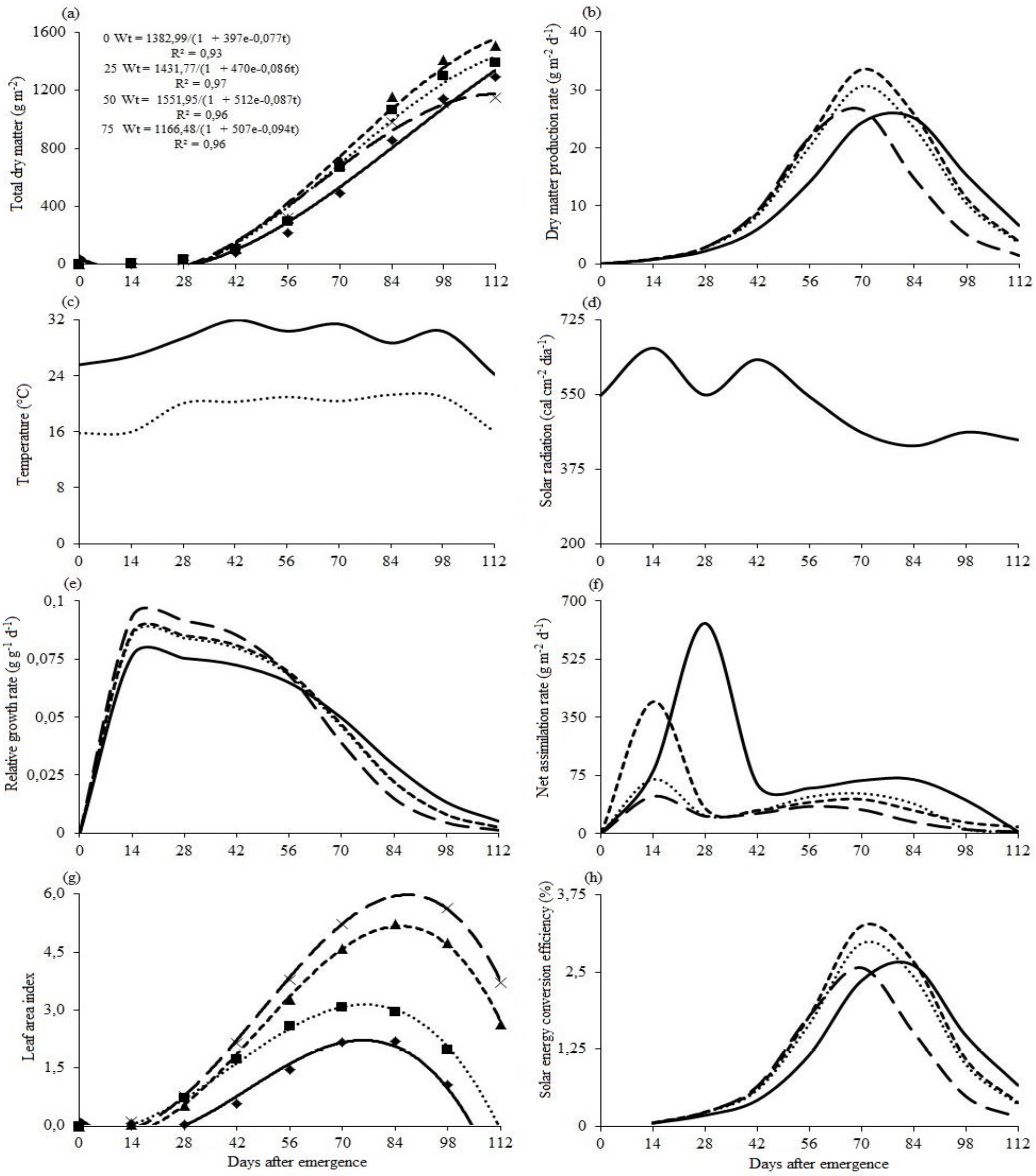

Fig 1. Total dry mass (a), dry mass production rate(b), higher temperature (———) and lower temperature (⋯⋯), solar radiation (d), relative growth rate (e), net assimilatory rate (f), leaf area index (g), solar energy conversion efficiency (h) and soybean plants (G max), considering 0 (———), 25 (⋯⋯), 50 (– – –) and 75 Kg ha^{-1} (—— ——).

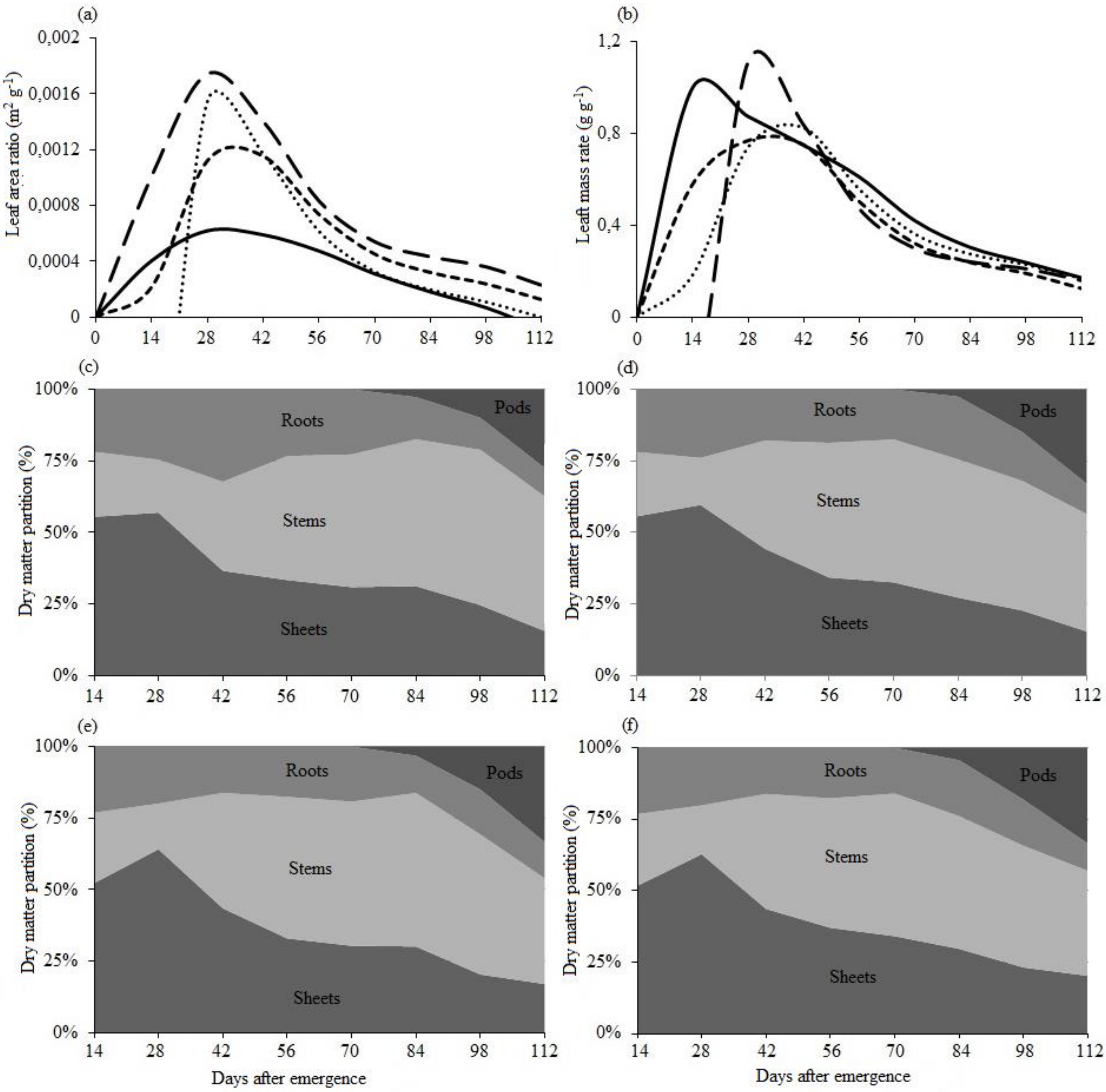

Fig 2. Leaf area (a) and leaf mass ratio (b), considering: 0 (———), 25 (······), 50 (– – –) e 75 Kg ha^{-1} (—— ——). Dry mass partition among the different soybean plant structures (G max), submitted to different nitrogen doses applied, with: 0 (c), (d), 50 (e) and 75 Kg ha^{-1} (f).

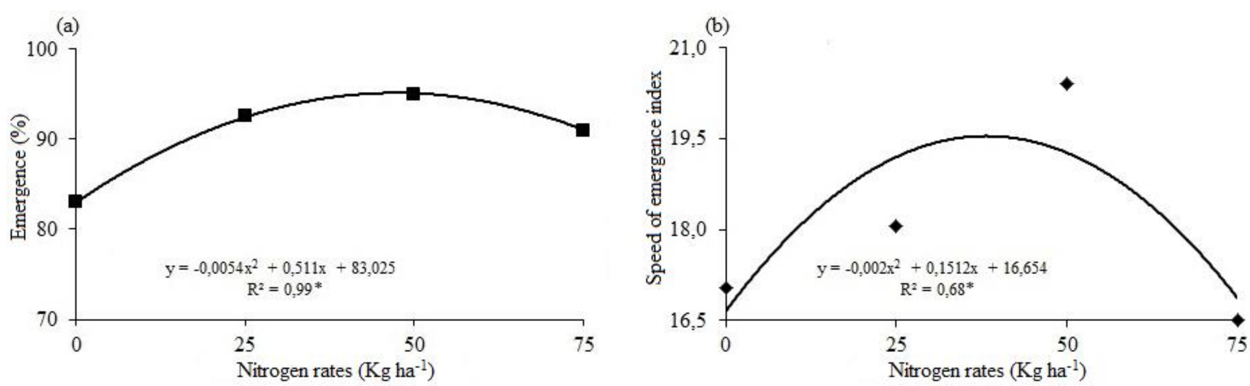

Fig 3. Emergence and emergence speed index of soybean seedlings (Gmax) originated from plants grown under different nitrogen rates. *Significant at 5% probability by Duncan´s test.

The maximum values of ξ for un-fertilised plants occurred at 84 DAE, while plants submitted to nitrogen doses of 25 and 50 kg ha^{-1} reached higher and maximum values of ξ at 77 DAE; plants fertilised with 75 kg N ha^{-1} reached the maximum ξ value at 70 DAE. From 91 DAE until the end of the crop cycle, the un-fertilised plants showed higher ξ values in comparison to the fertilised ones. The reduction of solar energy conversion efficiency correlates with changes in leaf metabolism as degradation rates of compounds begin to increase, reducing the efficiency of the photosynthetic apparatus (Melges et al., 1989). In addition, the reduction of ξ may also be associated with the decrease of solar radiation values during the crop cycle. The maximum values of Fa of un-fertilised plants and those which received doses of 25, 50 and 75 kg N ha^{-1} occurred at 31, 30; 34 and 29 DAE, with subsequent decrease until the end of the development cycle. The Fa reduction tendency along plant ontogeny is related to the gradual decrease of the amount of assimilates translocated to the leaves and demonstrates the reduction of leaf area, which facilitates photosynthetic process (Aumonde et al., 2011). In relation to the leaf mass ratio (F_w), un-fertilised plants reached a maximum F_w value at 14 DAE (Fig. 2b), while plants subjected to doses of 25, 50 and 75 kg N ha^{-1} reached maximum F_w values at 35, 40 and 35 DAE; subsequently, F_w tended to decline. Plants submitted the N fertilisation doses of 75 kg $ha^{-}1$ reached higher F_w values, which indicates that nitrogen application, especially in higher doses, increased the amount of dry matter allocated to the leaves. According to Pedó et al. (2015), F_w represents the estimated fraction of assimilates retained in the leaves. Regardless of the nitrogen dose applied, sequential partitioning of dry matter occurred along the different structures of soybean plants (Fig. 2). At the beginning of the development, plants of all treatments prioritised the allocation of carbon to the leaves, stems and roots; however, with the emergence of the reproductive structures, there was a modification in the preferential metabolic drainage and at 74 DAE, the allocation of dry matter to pods started, definitive drain and strong mobilizing capacity of assimilated. At the end of the crop cycle (112 DAE), un-fertilised plants showed approximately 13, 21 and 22% higher stem carbon allocation compared to the plants subjected to doses of 25, 50 and 75 kg N ha^{-1}, respectively (Fig. 2c, 2d, 2f). For the same period, 0.3, 1.6 and 18% more dry mass was allocated to the pods of plants fertilised with 75 Kg N ha^{-1} of N compared to the other treatments.

Effect of nitrogen on physiological seed quality

Seedling emergence showed a quadratic response with a high determination coefficient (R^2 = 0.99) and a tendency to decrease from the dose of 47.3 kg ha^{-1} (Fig. 3a). Seeds of plants fertilised with 50 kg ha^{-1} of N reached the highest seedling emergence, while seedling emergence of un-fertilised plants was 12, 15 and 10% lower compared to plants fertilised with 25, 50 and 75 kg N ha^{-1}, respectively.

Emergence speed (IVE) increased up to the dose of 37.8 kg ha^{-1}, with a subsequent decrease up to the highest dose (Fig. 3b). Seedlings from seeds under the influence of nitrogen application at a dose of 50 kg ha^{-1} reached IVE values above 16.5, 11.4 and 19.1% compared to those under 0, 25 and 75 Kg ha^{-1} of N, respectively. Nitrogen application resulted in increased soybean seed vigour. Nitrogen applied in the vegetative phase may positively impact seed vigour (Zucareli et al., 2012) as it increases protein contents (Gomes Júnior and Sá, 2010). Some proteins stabilise cell membranes, thereby increasing the physiological performance of the seed (Peske et al., 2012). Gomes Júnior and Sá (2010) evaluated the effect of nitrogen application on the physiological quality of bean seeds and observed an increase in crude protein and total soluble protein contents. The reduction of soluble seed protein values negatively affects physiological quality (Gomes Júnior and Sá, 2010). Thus, in general, favourable temperatures and solar radiation, in association with adequate nitrogen fertilisation, facilitates plant growth and improves the physiological quality of the seeds.

Materials and Methods

Study site

The experiment was conducted in the experimental area of the Department of Plant Science, Federal University of Pelotas, Capão do Leão, RS. Temperature and solar radiation data were obtained from the bulletin of the Agroclimatological Station of Pelotas, a few hundred meters from the cultivation place.

Plant material soil classification

We used "BMX Potência RR" soybean seeds, arranged to germinate in black polyethylene pots containing soil substrate from the A1 horizon of a Solubic Eucalyptus Haplic Planosol with the following chemical and physical characteristics: of pH (H_2O), 5.4; P, 15.5 mg dm_{-3}; K, 54 mg dm^{-3}; Ca, 2.8 $cmol_c$ dm^{-3}; Mg, 0.5 $cmol_c$ dm^{-3}; Al, 0.4 $cmol_c$ dm^{-3}; Fe, 1,400 mg dm^{-3}; Cu, 0.3 mg dm^{-3}; Zn, 0.9 mg dm^{-3}; Mn, 27.0 mg dm^{-3}; CTC, 6.0 cmolc dm^{-3}; base saturation, 54%; organic matter, 1.4%; Clay, 15%, previously corrected according to soil analyses and based on the Brazilian Manual of Fertilization (CQFS, 2004). The cultivar "BMX Potência RR" belongs to the maturation group 6.7, presenting an indeterminate growth habit and a mass of one thousand seeds equal to 168 g (Brasmax, 2015). Irrigation was performed manually.

Experimental design

Sowing was performed on November 15, 2013, and the plants were cultivated during the 2013/14 growth period. The treatments were established at the Vn development stage of the plant, using urea nitrogen fertiliser (45% N). The urea doses applied were equivalent to 0 (control), 25, 50 and 75 kg of nitrogen per hectare. The experimental design was completely randomised with four replications in a 4 x 8 factorial scheme (four nitrogen doses and eight collection periods).

Measured variables

To obtain primary leaf area and dry mass growth data, successive collections were carried out at regular intervals of 14 days throughout the development cycle of the plants. At each collection, the plants were cut close to the ground, separated into organs (leaves, stems, roots and pods, if possible) and individually packaged in brown paper envelopes. Leaf area was determined using the Liquor brand area meter LI-3100 and leaf area index was calculated by the equation L = leaf area/soil surface. To obtain dry mass values, four replicates were used for each collection and treatment period and dried in the forced air circulation at 65 ° C until constant mass.

Total dry matter (W_t) primary data were adjusted by the simple logistic equation $W_t = W_m/(1 + A\ e^{-Bt})$, with "$W_m$" as the asymptotic maximum growth rate, "A" and "B" as adjustment constants, "e" as the natural basis of the Neperian logarithm and "t" time in days after emergence (Richards, 1969). The leaf area primary data were adjusted by orthogonal polynomials and the instantaneous values of the dry matter yield (C_t) obtained by the derived equations of total dry matter (W_t) and leaf area (A_f) in relation to time (Radford, 1967).

For determination of the instantaneous values of the relative growth rate (R_w), the equation $R_w = 1/W_t.d_w/d_t$ was used. The instantaneous values of the net assimilation rate (E_a), leaf area ratio (F_a), leaf mass ratio (F_w) and specific leaf area (S_a) were estimated by the equations $E_a = 1/A_f.d_W/dt$; $F_a = A_f/W_t$; $F_w = W_f/W_t$ and $S_a = A_f/W_f$, according to Radford (1967). The conversion efficiency of the solar energy (ξ) was determined by the equation $\xi\ (\%) = (100\ C_t.\delta)/R_a$, with R_a as the mean value of incident solar radiation (cal m^{-2} day $^{-1}$) before the corresponding C_t, recorded by pyrometer and δ as the calorific value of 4,460 cal g^{-1}, according to Silva Neto et al. (1991).

Dry matter partitioning along the different organs (roots, stem, leaves and pods) throughout the development of the plants was determined separately from the measurement of the mass in each plant structure, followed by the transformation of the dry mass allocation primary data in each organ to percentage allocated in each structure.

At the end of the development cycle, the seeds were collected and used for the seedling emergency test. This test was conducted using eight subsamples of 50 seeds of each treatment, arranged to germinate in black polyethylene trays containing the soil characterised above and moistened to meet the crop water demand under greenhouse conditions.

Twenty-one days after sowing, seedling emergence was evaluated and the emergence speed index was determined from the daily number of seedlings emerged from the substrate, as proposed by Nakagawa (1994). At the end of the emergence test, four subsamples of 20 plants were collected per treatment to determine leaf area; leaf, stem and root dry mass. Leaf area and dry matter were determined as previously described, with leaf area results expressed in square centimetres (cm²) and in grams (g).

Statistical analyses

Data regarding seedling emergence and emergence speed index, leaf area and organ mass were submitted to variance analysis and, since the F values were significant, Tukey´s test was applied at 5% probability. The primary data of dry mass, leaf area, leaf dry mass, roots, stem and pods were submitted to variance analysis; parameters related to growth were analysed by simple logistic equation (Lopes and Lima, 2015).

Conclusion

Nitrogen fertilisation applied in the Vn stage of soybean plants enhances growth, solar energy conversion and seed vigour. A nitrogen application dose of 50 kg ha^{-1} resulted in highest W_t values, whereas plants submitted to 75 kg ha^{-1} of N reached higher R_w and L values.

Nitrogen fertilisation with 50 kg ha^{-1} of N resulted in greatest seed vigour.

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