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Physiological attributes of jatropha under different planting densities and nitrogen doses

Allan R. F. Campos¹, Rosiane L. S. de Lima², Carlos A. V. de Azevedo²,
Ronaldo do Nascimento² & Sonivagno S. Silva³

¹ Universidade Federal do Recôncavo da Bahia/Centro de Ciências Agrárias, Ambientais e Biológica/Programa de Pós-Graduação em Engenharia Agrícola. Cruz das Almas, BA. E-mail: allanradax@hotmail.com (Corresponding author)

² Universidade Federal de Campina Grande/Centro de Tecnologia e Recursos Naturais/Unidade Acadêmica de Engenharia Agrícola. Campina Grande, PB. E-mail: limarosiane@yahoo.com.br; cveiradeazevedo@gmail.com; ronaldo@deag.ufcg.edu.br

³ Universidade Federal de Campina Grande/Centro de Tecnologia e Recursos Naturais/Pós-Graduação em Engenharia Agrícola. Campina Grande, PB. E-mail: sonyvagno@yahoo.com.br

Key words:

Jatropha curcas
gas exchange
nitrogen fertilizer
water use efficiency

ABSTRACT

Nitrogen (N) fertilization associated with planting density is a practice that can improve the physiological aspects and consequently increase the yield of a crop. Thus, this study aimed to evaluate the effects of planting density and N levels on the physiological aspects of jatropha in the 'Agreste' region of Paraíba. Treatments consisted of four planting densities (833, 1,111, 1,666 and 2,500 plants ha⁻¹) representing the plots and five N doses (0, 40, 80, 120 and 180 kg ha⁻¹) corresponding the subplots. The effects of treatments on CO₂ assimilation rate, internal CO₂ concentration, transpiration, stomatal conductance, water use efficiency, instantaneous carboxylation efficiency and SPAD index were analysed. Except for stomatal conductance, the other variables were influenced by the interaction between planting densities and N levels. The association of the dose of 80 kg ha⁻¹ with a planting density of 2,200 plants ha⁻¹ provided adequate conditions for the production of plants with increased photosynthetic activity and efficient use of water.

Palavras-chave:

Jatropha curcas
trocas gasosas
fertilizante nitrogenado
eficiência do uso da água

Aspectos fisiológicos do pinhão-mansô sob diferentes densidades de plantio e doses de nitrogênio

RESUMO

A adubação nitrogenada associada à densidade de plantio é uma prática que pode melhorar os aspectos fisiológicos e, conseqüentemente, aumentar os rendimentos de uma cultura. Deste modo o presente trabalho teve, como objetivo, avaliar os efeitos da densidade de plantio e de doses de nitrogênio nos aspectos fisiológicos do pinhão-mansô, na região do Agreste Paraibano. Os tratamentos consistiram de quatro densidades de plantio de 833, 1.111, 1.666 e 2.500 plantas ha⁻¹, representando as parcelas e cinco doses de nitrogênio de 0, 40, 80, 120 e 180 kg ha⁻¹, representando as subparcelas. Foram analisados os efeitos sobre a taxa de assimilação de CO₂, concentração interna de CO₂, transpiração, condutância estomática, eficiência no uso da água, eficiência intrínseca da carboxilação e índice SPAD. Exceto a condutância estomática, as demais variáveis foram influenciadas pela interação entre as densidades de plantio e as doses de nitrogênio; a associação da dose de 80 kg ha⁻¹ com a densidade de plantio de 2.200 plantas ha⁻¹ proporcionou condições adequadas para a obtenção de plantas com maior atividade fotossintética e uso eficiente da água.



INTRODUCTION

Jatropha (*Jatropha curcas* L.) has been indicated as a promising alternative for the supply of oil aiming at the production of biodiesel in various countries, such as Mexico, Nicaragua, Thailand and parts of India, besides Brazil (Bangzhen & Zengfu, 2011; Laviola et al., 2012). Studies involving the strategy of absorption of mineral nutrients by plants deserve special attention in the case of nitrogen (N), whose assimilation is essential for plant metabolism, as a whole, since this nutrient participates in chlorophyll molecules, nitrogenous bases, amino acids and is directly associated with leaf expansion and better responses in the photosynthetic rates (Sousa et al., 2012; Brito et al., 2014). Some studies have been conducted regarding the fertilization of jatropha (Akbarian et al., 2010; Mohapatra & Panda, 2011; Simões et al., 2014; Freiberger et al., 2015).

For the N fertilization of jatropha, Mohapatra & Panda (2011) recommend the application of 60 g of N plant⁻¹ in plantations more than six years old. On the other hand, Akbarian et al. (2010) suggest the application of 70 kg ha⁻¹ of N in adult jatropha plantations distributed at spacing of 2 x 2 m. The available spacing recommendations for the crop are almost exclusively based on those established for other crops, which may cause the system to show low production. According to the literature, denser plantations increase the yield of jatropha plants (Akbarian et al., 2010; Horschutz et al., 2012; Sharma, 2012).

Despite the various indications of fertilization for the jatropha crop under field and greenhouse conditions, field data that serve as a support are still scarce, especially associated with different planting densities. In this context, this study aimed to evaluate the physiological aspects of jatropha as a function of the different planting configurations and N doses, in the 'Agreste' region of the Paraíba state.

MATERIAL AND METHODS

The experiment was carried out from June to December 2014, at the field, under rainfed regime, at the experimental

station of the Agricultural Research Company of the state of Paraíba (EMEPA), located in the municipality of Lagoa Seca-PB, 'Agreste' region of Paraíba, Brazil (7° 9' 28" S; 35° 52' 24" W; 630 m). According to Köppen's classification, the region has rainy tropical climate with dry season in the summer (Figure 1).

The experiment was installed in randomized blocks with four replicates using a 4 x 5 factorial scheme in split plots, corresponding to the planting densities of 833, 1,111, 1,666 and 2,500 plants ha⁻¹ as the plots and N doses of 0, 40, 80, 120 and 180 kg ha⁻¹ as the subplots, which were applied in the form of ammonium sulfate, according to the spacings.

Each plot had dimensions of 16 x 16 m varied from 4 rows with 5 plants to 7 rows with 7 plants. The densest plots were formed by 49 plants and the least dense ones by 20 plants each. Evaluations were performed considering an area with 5 plants of each plot. The N doses per plant were calculated based on the planting density, distributed as top-dressing fertilization, split into levels of 40, 30 and 30% of the dose. The first one was applied at 15 months of age after pruning and the second and third ones, respectively, at 30 and 60 days after the first fertilization. The phosphorus and potassium (PK) fertilization consisted in the doses of 90 and 60 kg ha⁻¹ of triple superphosphate and potassium chloride, 100% as basal fertilization in the area under the canopy projection at the depth of 20 cm (Brito et al., 2014).

The experiment was installed and conducted during the second cycle of the jatropha crop at 15 months of age in a soil with sandy loam texture (Table 1).

In order to start the experiment and study the effects of N doses and planting densities in the month of June, a drastic pruning was performed, which consisted in the total removal of the shoots at a height of 80 cm, to standardize the experimental area. The cultivation practices relative to the control of plants that compete for water and nutrients, and pests and diseases were performed according to the necessity.

The physiological variables were analyzed at the beginning of the reproductive stage (flowering). Gas exchanges were

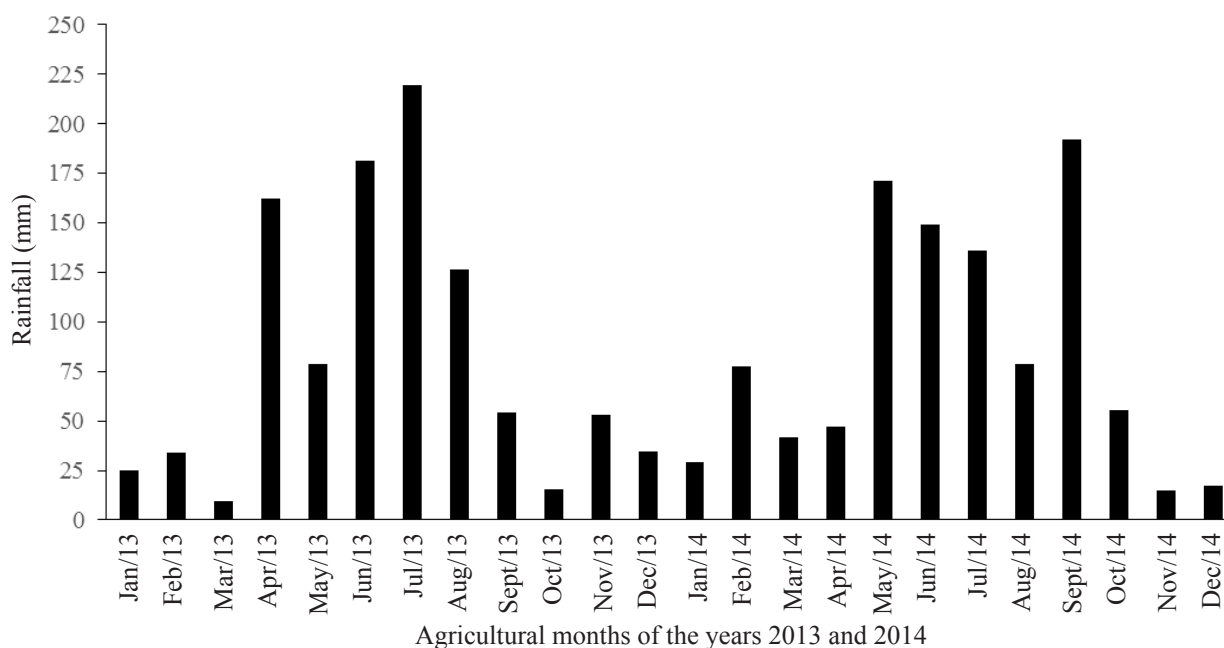


Figure 1. Rainfall in the experimental area in the agricultural years of 2013 and 2014

Table 1. Chemical characteristics of soil in the cultivated area

pH _{SP}	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	S	H + Al	Al ³⁺	T	V	P	OM
1:2.5	Exchange complex (mmol _c dm ⁻³)										
5.3	5.6	3.5	3.8	1.8	14.7	16.3	2.0	31.4	48.8	15.4	1.17

S – Sum of bases; T – Cation exchange capacity; V – Base saturation; OM – Organic matter; Methods of determination: OM – Walkley-Black wet digestion; Ca²⁺ and Mg²⁺ extracted with 1 mol L⁻¹ KCl at pH 7.0; Na⁺ and K⁺ extracted using 1 mol L⁻¹ NH₄OAc at pH 7.0; pH_{sp} – pH of the saturation paste

evaluated in fully expanded and totally formed leaves of two plants per plot from 6 to 10 a.m. The selection of the times was based on the recommendations of Oliveira et al. (2005).

Data were collected on the fourth fully expanded leaf from the apex of the plant, below the inflorescence, with the IRGA – LCPro + (Analytical Development, Kings Lynn, UK), measuring the following parameters: CO₂ assimilation rate (A) (μmol of CO₂ m⁻² s⁻¹); internal CO₂ concentration (Ci) (mmol of CO₂ m⁻²); transpiration (E) (mmol of H₂O m⁻² s⁻¹) and stomatal conductance (gs) (mol of H₂O m⁻² s⁻¹). The water use efficiency (WUE) [(μmol m⁻² s⁻¹) (mol H₂O m⁻² s⁻¹)⁻¹] was obtained by the ratio between CO₂ assimilation rate and transpiration (A/E) and the calculation of the A/Ci ratio was used to obtain the instantaneous carboxylation efficiency (EICi) [(μmol m⁻² s⁻¹) (mmol of CO₂ m⁻²)⁻¹] (Suassuna et al., 2014).

The SPAD index, which quantitatively evaluates the intensity of green color by measuring the transmissions of light from 650 to 940 nm, was determined using the portable device SPAD- 502 (Soil Plant Analysis Development - Minolta Camera Co.), following the procedures adopted by Guimarães et al. (1999). The data were subjected to analysis of variance by F test and regression using the statistical program SISVAR-UFLA.

RESULTS AND DISCUSSION

Except for stomatal conductance (gs), which did not respond to the effects of any treatment, the interaction between planting densities and N doses caused significant effect on all physiological variables shown in Table 2. The ideal is to search in the literature for studies of this nature or similar to

compare the statistical behavior of the data. As to stomatal conductance, it would be more important to compare it first with data of non-irrigated cultivation and then with data of irrigation with saline water.

The CO₂ assimilation rate data, except those referring to the dose of 40 kg ha⁻¹ of N -NF1 (Figure 2A), showed linear tendency, with increment of 0.0009 μmol of CO₂ m⁻² s⁻¹ for every increase in the number of plants per hectare. Among the N doses, the most efficient in CO₂ assimilation rate was 80 kg ha⁻¹ of N (NF3) associated with the maximum planting density of 2,250 plants ha⁻¹, which led to the value of 4.45 μmol of CO₂ m⁻² s⁻¹. This result exceeds in 85.4 and 4%, respectively, the lowest (833 plants ha⁻¹) and highest (2500 plants ha⁻¹) planting densities for this same N dose.

The results are lower than the value of 5.37 mmol m⁻² s⁻¹, found by Simões et al. (2014) evaluating the biometric and physiological parameters in the initial stage of jatropa. Freitas et al. (2012) suggest that the higher photosynthetic activity in the plants results from a more efficient photosynthetic apparatus, since N is a constituent of the chlorophyll and the RuBisCO enzyme, molecules that are essential for photosynthetic activity. The photosynthetic capacity of plants cultivated under adequate N supply is higher than that of plants cultivated under deficiency of this macronutrient (Pompelli et al., 2010).

According to the regression equations for the internal CO₂ concentration (Ci) during the flowering period (Figure 2B) as a function of planting densities and N doses, the linear model showed the best fit. In the treatments with N fertilization NF2, NF3, NF4 and NF5, there were reductions in the results of 0.0142, 0.0174, 0.0032 and 0.0229 mmol of CO₂ m⁻², respectively, as the

Table 2. Summary of the analysis of variance and polynomial regression components for CO₂ assimilation rate (A), internal CO₂ concentration (Ci), transpiration (E), stomatal conductance (Gs), water use efficiency (WUE), instantaneous carboxylation efficiency (EICi) and SPAD index in jatropa plants cultivated at different planting densities and subjected to different nitrogen (N) fertilization doses

Source of variation	DF	Mean square						
		A	Ci	E	Gs	WUE	EICi	SPAD index
Block	3	0.781 ^{ns}	200.47 ^{ns}	0.320 ^{ns}	0.028 ^{ns}	0.078 ^{ns}	0.000002 ^{ns}	0.640 ^{ns}
Planting density (PD)	3	5.826 ^{**}	641.94 ^{ns}	0.187 ^{ns}	0.010 ^{ns}	1.495 ^{**}	0.000048 ^{**}	6.944 [*]
Residual (a)	9	0.275	220.22	0.303	0.013	0.139	0.000005	1.561
N fertilization (NF)	4	7.071 ^{**}	419.70 ^{ns}	0.697 ^{**}	0.0012 ^{ns}	2.728 ^{**}	0.000071 ^{**}	155.075 ^{**}
PD x NF	12	2.153 ^{**}	579.24 ^{**}	0.602 ^{**}	0.010 ^{ns}	0.690 ^{**}	0.000026 ^{**}	8.557 ^{**}
Residual (b)	48	0.296	205.57	0.094	0.008	0.172	0.000004	0.953
CV (%) a	-	14.12	4.47	29.87	62.25	21.42	22.93	2.71
CV (%) b	-	14.75	4.32	18.68	49.19	23.84	20.94	2.11
PD/NF1	LR	5.509 ^{**}	897.139 ^{**}	0.0199 ^{ns}	0.016 ^{ns}	1.282 ^{**}	0.000144 ^{**}	1.075 ^{**}
	QR	0.161 ^{ns}	82.82 ^{ns}	0.0008 ^{ns}	0.010 ^{ns}	0.239 ^{ns}	0.000103 [*]	1.485 ^{ns}
PD/NF2	LR	9.036 [*]	1315.85 [*]	0.1054 ^{ns}	0.023 ^{ns}	0.725 ^{ns}	0.000124 ^{**}	8.475 ^{**}
	QR	6.038 [*]	43.41 ^{ns}	0.037 ^{ns}	0.017 ^{ns}	0.259 ^{ns}	0.000001 ^{ns}	4.197 ^{**}
PD/NF3	LR	16.635 ^{**}	1462.72 [*]	10.083 ^{ns}	0.004 ^{ns}	25.85 ^{**}	0.000131 ^{**}	4.010 [*]
	QR	3.52 [*]	10.083 ^{ns}	0.0102 ^{ns}	0.003 ^{ns}	9.93 [*]	0.000036 ^{ns}	5.766 [*]
PD/NF4	LR	5.113 ^{**}	29.84 ^{ns}	1.554 [*]	0.010 ^{ns}	0.0012 ^{ns}	0.000001 ^{ns}	10.534 ^{**}
	QR	2.870 [*]	5.95 ^{ns}	1.218 [*]	0.0003 ^{ns}	0.0181 ^{ns}	0.000014 ^{ns}	1.787 [*]
PD/NF5	LR	1.597 ^{**}	1692.44 ^{**}	0.596 [*]	0.036 ^{ns}	0.168 [*]	0.000000 ^{ns}	10.289 ^{**}
	QR	2.292 [*]	7.52 ^{ns}	0.407 [*]	0.010 ^{ns}	0.468 [*]	0.000000 ^{ns}	8.028 [*]

**Significant at 0.01; *Significant at 0.05; ^{ns}Not significant; LR – Linear regression; QR – Quadratic regression

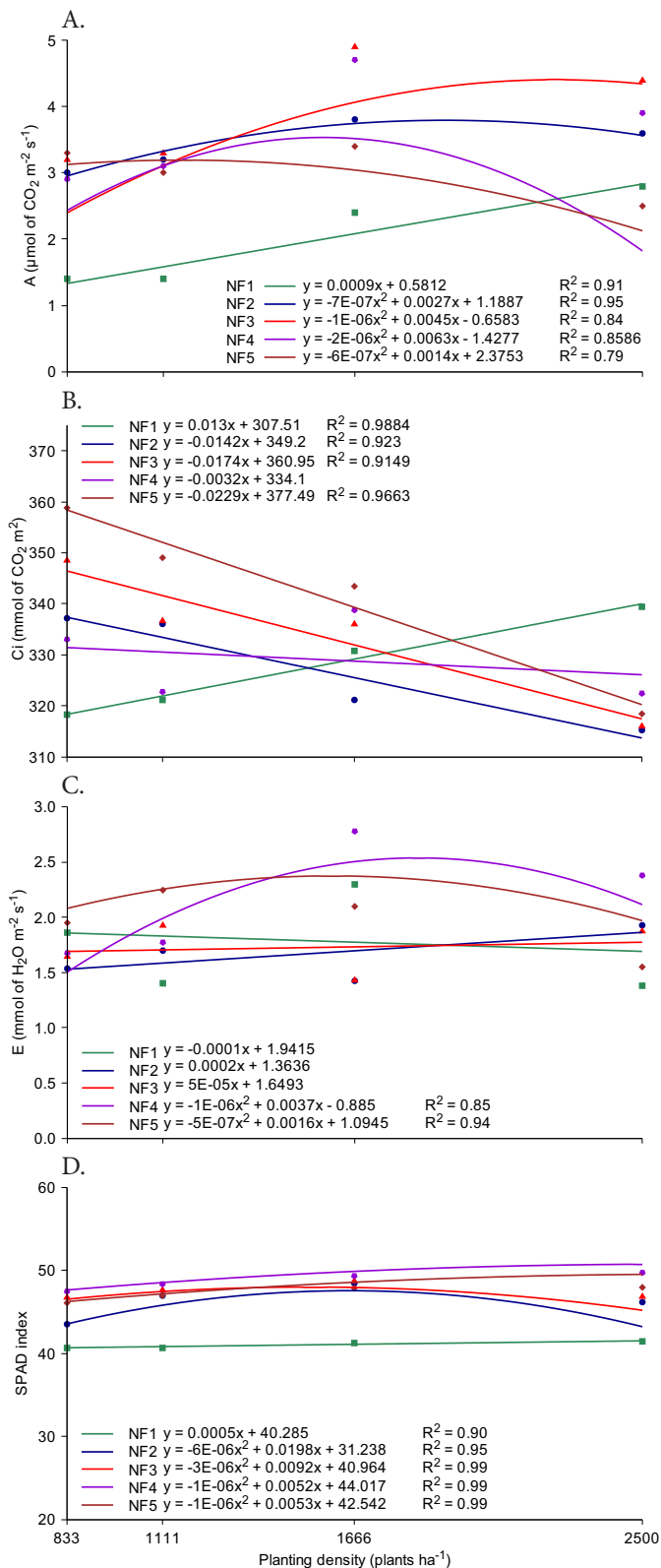


Figure 2. CO₂ assimilation rate-A (A), internal CO₂ concentration-Ci (B), transpiration-E (C) and SPAD index (D) of jatropha plants as a function of different planting densities and nitrogen fertilization doses

number of plants per hectare increased, with opposite response in relation to the treatment NF1. One of the explanations for this result is probably the increase in the competition between plants, since this treatment did not receive any N.

The observed reductions in internal CO₂ concentration reflect the decreases in CO₂ assimilation rate, justifying the

fact that, during the process of gas exchange, the absorption of CO₂ causes water loss and, in the opposite direction, it favors a reduction in the CO₂ assimilation, thus leading to lower internal CO₂ concentration (Ferraz et al., 2014). The internal CO₂ concentration depends on the climatic conditions and on the nutritional supply to the plants, both at planting and as top-dressing (Taiz & Zeiger, 2013). Another factor, such as the genetic load of the plant, favors differences regarding the level of response to a certain factor, particularly the internal C concentrations.

The CO₂ assimilation rate and transpiration are directly correlated, through the stomata, because, at the same time that the stomata offer resistance to water diffusion from the leaf to the atmosphere, they constitute a barrier for the acquisition of CO₂ (Ferraz et al., 2012). According to Figure 2C, the regression equations for jatropha transpiration fitted to a quadratic model for the treatments NF4 and NF5, at the adopted planting densities, and the highest transpiration rate was observed for the N dose of 120 kg ha⁻¹, with maximum transpiration of 2.537 mmol of H₂O m⁻² s⁻¹ for the density of 1850 plants ha⁻¹, surpassing in 62 and 20%, respectively, the lowest (833 plants ha⁻¹) and highest (2500 plants ha⁻¹) planting densities. These results are similar to the value of 2.48 mmol of H₂O m⁻² s⁻¹ found by Sousa et al. (2012) in jatropha plants irrigated with wastewater and saline water, during the fourth year of production.

The chlorophyll in jatropha leaves, based on the SPAD index (Figure 2D), evidences the existing relationship with the CO₂ assimilation rate and that, for the jatropha crop, the photosynthetic pigments are directly correlated. In this context, Engels & Marschener (1995) comment that the CO₂ assimilation rate must be proportional to the concentration of chlorophyll and that it is only valid for situations in which the concentration is below the optimal value for this process.

According to Figure 2D, the treatments with 40 (NF2), 80 (NF3) and 180 kg ha⁻¹ of N (NF5) promoted a maximum SPAD index of approximately 48, which is only 4% inferior to that of the treatment with 120 kg ha⁻¹ of N (NF4), which was equal to 50. The greater difference between the treatments indicates much more the influence of planting density than that of N fertilization, because the best combination was observed for the condition of 120 kg ha⁻¹ of N (NF4) and 2,450 plants ha⁻¹.

The obtained results are within the range indicated by Freiberger et al. (2015), who mention that the SPAD index of 46, measured in the first fully developed leaf from the apex, can be used as a nutritional reference of N in the initial development of the jatropha crop. The results are also in agreement with Possas et al. (2014), who obtained SPAD index of 47.7 in jatropha plants at 150 days after transplanting, under organic and phosphate fertilization.

The water use efficiency, expressed by the relationship between the CO₂ assimilation rate and the transpiration, is associated with the amount of carbon that the plant fixes per unit of water transpired. According to the data of Figure 3A, the N dose of 80 (NF3) resulted in the highest WUE of 3.56 [(μmol m⁻² s⁻¹) (mol H₂O m⁻² s⁻¹)⁻¹] at the planting density of 2,050 plants ha⁻¹. Comparatively, this result surpasses in 7 and 71.7%, respectively, those obtained in plants at the highest and lowest planting density.

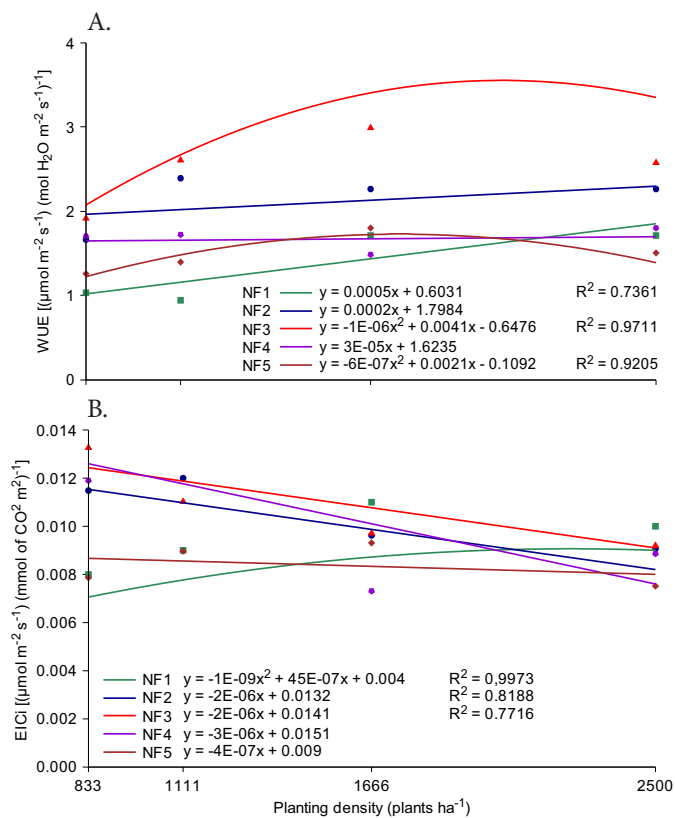


Figure 3. Water use efficiency-WUE (A), instantaneous carboxylation efficiency-EICi (B) of jatropha plants as a function of the interaction between different planting densities and nitrogen fertilization doses

The instantaneous carboxylation efficiency (EICi) is the relationship used to study the non-stomatal factors that interfere with the photosynthetic rate and is related to the net photosynthetic rate and CO₂ concentration inside the substomatal chamber (Suassuna et al., 2014). According to Figure 3B, the treatments NF2 and NF3 showed similar values, as well as a similar decrease in their values, a reduction of 0.000002 [(µmol m⁻² s⁻¹) (mmol of CO₂ m²)⁻¹] for every increase in planting density. The derivation of the equation of the treatment with 0 kg ha⁻¹ of N (NF1) theoretically results in an EICi of 0.0096 [(µmol m⁻² s⁻¹) (mmol of CO₂ m²)⁻¹] for a maximum population of 2,250 plants ha⁻¹. According to the results for the highest planting densities, the values of all treatments tend to approximately 0.010 [(µmol m⁻² s⁻¹) (mmol of CO₂ m²)⁻¹]. For Soares et al. (2012), the instantaneous carboxylation efficiency is mainly due to the increments in internal CO₂ concentration and the gains in the CO₂ assimilation rate, which was also observed in the behavior of the data for jatropha in the present study.

CONCLUSIONS

1. The use of different doses of nitrogen fertilization and planting densities directly influences the physiological aspects of jatropha, with a maximum point for CO₂ assimilation rate, internal CO₂ concentration, transpiration, water use efficiency, instantaneous carboxylation efficiency and SPAD index.

2. The use of nitrogen fertilization for the jatropha crop at the dose of 80 kg ha⁻¹ of N, associated with the density of

2,200 plants ha⁻¹, promotes higher CO₂ assimilation rate and water use efficiency.

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