

Full Length Research Paper

## Differences between genotypes of *Jatropha curcas* L. are evidenced for absorption and use of nitrogen

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The objective of this study was to quantify the nutritional efficiency and responsiveness in relation to nitrogen of twelve genotypes of physic nut (*Jatropha curcas* L.) grown in soil without nitrogen supply and also with three levels of fertilization in a controlled environment. The experiment was conducted in factorial scheme 12 × 4 with four replications, the factors being: 12 genotypes of physic nut and four levels of nitrogen fertilization, studied in a randomized design. The plants were grown in pots, without restrictions, for 100 days and their dry matter and N contents were evaluated (for roots, leaves and total). Subsequently, the efficiency ratios for absorption, translocation, use and responsiveness in relation to the N supply were calculated. Overall, the nutritional efficiencies for nitrogen increase linearly with the increase of nitrogen supply in the soil. The genotypes CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 255-I and CNPAE 300-I were classified as efficient and responsive to nitrogen fertilization being highlighted for plant breeding programs aiming to improve this agronomic trait.

**Key words:** Physic nut, mineral nutrition, alpha parameter.

### INTRODUCTION

Given the accelerated industrial growth, the world population has increasingly explored non-renewable and mineral sources of energy, creating concerns about the depletion of these resources in long term. This scenario has intensified searches regarding renewable energy sources, like vegetal oils that can be used to produce biofuels. Between the options of oilseeds, physic nut (*Jatropha curcas* L.) has become known for presenting

desirable agronomic characteristics associated with high potential for oil production (Amaral et al., 2012).

The nutritional requirements of the species must be met to allow the expression of its productive potential. Among the nutrients, an adequate supply of nitrogen is essential considering the importance of this nutrient in the metabolism, growth and development of plants (Yong et al., 2010; Batista et al., 2014).

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The essentiality of nitrogen is undisputed for any vegetal species; and for physic nut, this is the most required nutrient (Laviola and Dias, 2008), therefore, studies involving nitrogen are important in order to increase the efficiency its use in the agriculture (Baligar et al., 2001; Yong et al., 2010).

To keep an adequate availability of N in the soil is one of the factors required to achieving high crop yields. Allied to the availability of the nutrient, the efficiency of uptake and use of the species need be studied and exploit to improve the rational use of fertilizers. The concepts of nutritional efficiency allow a better understanding of the internal capacity of production per unit of nutrient, with direct effects over the development and management of the culture (Fageria, 1998; Martins et al., 2013).

The acquisition of nutrients by plants can be influenced by several factors, such as the morphology of roots, its architecture and distribution in the soil profile. These features can undergo genetic changes to adapt to the environment, such as formation of deeper roots and greater lateral growth under conditions of water stress, or change in the roots distribution under low nutrient availability (Baligar et al., 2001; Chun et al., 2005; Hammer et al., 2009; Amaral et al., 2012; Martins et al., 2013).

This ability to adapt generates genetic variability in the species, which makes it possible to select genotypes with characteristics that predispose the plants to greater nutritional efficiency (Miranda et al., 2008; Souza et al., 2008; Vale et al., 2013; Kumar et al., 2014). The process of selection of genotypes is enhanced by the early discard of less promising genotypes, this can be done performing a preliminary selection between genotypes in young stages and using correlated traits.

Brazilian genotypes of *J. curcas* present large genetic divergence and the knowledge about the agronomic traits of those genotypes is still preliminary, therefore, studies to aid their characterization have great importance (Laviola et al., 2010; Laviola et al., 2011).

The objective of this study was to quantify the nutritional efficiency and responsiveness in relation to nitrogen of twelve genotypes of physic nut (*J. curcas* L.) grown in soil without nitrogen supply and also with three levels of fertilization in a controlled environment.

## MATERIALS AND METHODS

### Description of the study area and plant material

The experiment was conducted under greenhouse conditions in the experimental area of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), in Alegre, ES, Brazil with coordinates of 20°45' S latitude and 41°33' W longitude, and an average altitude of 277.41 m from December 2011 to March 2012.

The soil was collected at a depth of 10 to 40 cm, with the first 10 cm of the soil being discarded to reduce the effect of the organic matter present on the surface layer. A soil sample was sent to laboratory for chemistry and physic characterization, performing

according to the methodology described by Embrapa (2006). The soil was characterized as a clayey red-yellow latosol.

After the characterization, the soil was dried under shade and homogenized in a 2.0 mm mesh sieve. Subsequently, the soil was separated into samples of 10 dm<sup>3</sup>, standardized by weighing on a precision balance and placed in sealed plastic pots (with a capacity for fourteen liters).

### Experimental design and conduct of the study

The experiment was arranged in a factorial scheme 12 × 4, with fours replications, the factors being: 12 genotypes of physic nut (CNPAE 110-II, CNPAE 127-I, CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 191-I, CNPAE 192-I, CNPAE 210-II, CNPAE 255-I, CNPAE 275-I, CNPAE 300-I e CNPAE 302-I) and four levels of fertilization with nitrogen (0, 50, 100 and 150% of the level recommended by Novais et al. (1991); respectively 0.00, 1.07, 2.15 and 3.25 g of N per pot), following a completely randomized design. Four seeds were sown per pot, performing the thinning to one plant per pot on the 10<sup>th</sup> day after emergence. Therefore, the experimental plot consisted of one plant per pot.

The nitrogen fertilization was performed with NH<sub>2</sub>CONH<sub>2</sub> (pro analyze), diluted in distilled water and applied over the soil surface, distant 10 cm of the plant collar, following levels consistent with the treatments of 0, 50, 100 and 150% of nitrogen supply (respectively 0.00, 1.07, 2.15 and 3.25 g of N per pot). The fertilization was divided into four applications, performed at 20, 40, 60 and 80 days after sowing. The fertilization with phosphorus and potassium was provided to all parcels in a single application before sowing, using through the KH<sub>2</sub>PO<sub>4</sub> P. A. diluted in water and applied in the entire volume of soil, according to the recommendation for studies in controlled environment (Novais et al., 1991).

The physic nut seeds were provided by Embrapa Agroenergia (harvested in 2011), and processed by removing the immature and damaged seeds. The seeds were packaged and stored in the refrigerator (3°C) until use, with their water content being maintained between 10 to 12%.

### Evaluation of the study and calculated indices

After 100 days of cultivation, the determination of root dry matter (RDM), aerial part dry matter (ADM) and total dry matter (TDM) were performed, measured in grams per plant. For this determination, the plant materials (leaves, stems and roots) were collected and separated in paper bags, which were then dried in oven, with forced air circulation at 60 °C (STF SP-102/2000 CIR), until constant weight. After drying, the plant materials were weighed on analytical balance (SHIMADZU AUW-220D; precision: 0.00001 g) and triturated (CIENLAB EC-430, 8 blades, 1725 rpm, 20 mesh) to obtain a homogeneous powder.

To quantify the nitrogen content, 0.5 g (± 0.001 g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 x 200 mm) and submitted to the stages of digestion (H<sub>2</sub>SO<sub>4</sub>), distillation (NaOH 40%) and titration (NaOH 0.02 mol L<sup>-1</sup>) of nitrogen in "Kjeldahl" distillers (Marconi MA-036), according to the Kjeldahl method (Ma and Zuazaga, 1942).

The following indices were calculated: (AE) absorption efficiency = (total nutrient content in the plant)/(root dry matter), according to Swiader et al. (1994); (TE) translocation efficiency = [(nutrient content in the aerial part)/(total nutrient content in the plant)] × 100, according to Li et al. (1991); (UE) use efficiency = (total dry matter)<sup>2</sup>/(total nutrient content in the plant) according to Siddiqi and Glass (1981).

The response to fertilization with nitrogen was estimated by the criteria proposed by Ciat (1978), based on determination of the alpha parameter, according to the equation: Alpha parameter =

**Table 1.** Physical and chemical attributes of the soil used in the study.

Attribute	Values
Sand (g kg <sup>-1</sup> ) <sup>1</sup>	553.00
Silt (g kg <sup>-1</sup> ) <sup>1</sup>	43.60
Clay (g kg <sup>-1</sup> ) <sup>1</sup>	403.40
Soil density (kg dm <sup>-3</sup> ) <sup>2</sup>	1.21
pH <sup>3</sup>	6.00
P (mg dm <sup>-3</sup> ) <sup>4</sup>	3.00
K (mg dm <sup>-3</sup> ) <sup>5</sup>	59.00
Ca (cmol <sub>c</sub> dm <sup>-3</sup> ) <sup>6</sup>	1.40
Mg (cmol <sub>c</sub> dm <sup>-3</sup> ) <sup>6</sup>	1.00
Al (cmol <sub>c</sub> dm <sup>-3</sup> ) <sup>6</sup>	0.00
H+Al (cmol <sub>c</sub> dm <sup>-3</sup> ) <sup>6</sup>	1.70
Sum of bases (cmol <sub>c</sub> dm <sup>-3</sup> )	2.51
CTC potential (cmol <sub>c</sub> dm <sup>-3</sup> )	4.18
CTC effective (cmol <sub>c</sub> dm <sup>-3</sup> )	2.51
Base saturation (%)	60.10

<sup>1</sup>Pipette method (slow agitation); <sup>2</sup>Beaker method; <sup>3</sup>pH in water (1:2.5 ratio); <sup>4</sup>Extracted by Mehlich 1 and determined by colorimetry; <sup>5</sup>Extracted by Mehlich 1 and determined by flame photometry; <sup>6</sup>Extracted with potassium chloride 1 mol L<sup>-1</sup> and determined by titration.

(ADM N<sub>3</sub> - ADM N<sub>1</sub>) / (N<sub>3</sub> - N<sub>1</sub>), where: ADM is the dry mass of aerial part in the N<sub>1</sub> level, corresponding to the control without the addition of nitrogen; and N<sub>3</sub> level, corresponding to 100% of nitrogen fertilization.

The data for dry matter production of the aerial part in the level without nitrogen fertilization and the alpha parameter for each genotype were displayed in quadrants, allowing classifying the genotypes into four groups: ER – efficient and responsive genotypes; ENR – efficient and non-responsive genotypes; NER – non efficient and responsive genotypes; and NENR – non efficient and non-responsive genotypes.

### Statistical analysis

The data were subjected to analysis of variance ( $p \leq 0.05$ ) when the significant of the Scott-Knott test ( $p \leq 0.05$ ) was performed for qualitative factors and regression analysis for quantitative factors. The regression models were chosen based on the significance of regression coefficients, using the Student's t-test at 5% of probability, and on the coefficient of determination ( $R^2$ ). The analyses were performed using the statistical software "GENES" (Cruz, 2013).

## RESULTS

### Production of dry matter and nitrogen content

The analysis of variance indicated the existence of a significant interaction between the effects of genotype and nitrogen levels. Considering the dry matter and nitrogen content accumulated in different plant parts, the differential behavior among genotypes of physic nut was evident, verified by the formation of distinct groups and

by their different arrangement for each level of fertilization with N (Table 2).

Overall, the genotype CNPAE 180-I showed higher dry matter accumulation than all the others, independently of nitrogen level. In contrast, the genotype CNPAE 302-I had lower production of dry matter production having less accumulated biomass in all the plant parts.

For nitrogen contents, the genotype CNPAE 110-I showed higher capacity to accumulate N; while the genotypes CNPAE I-275 and I-180 CNPAE showed lower capacity to accumulate N in the roots, as well as the genotypes CNPAE I-191 and I-192 CNPAE for aerial part and for total content (Table 2).

The values of dry matter (RDM, ADM and TDM) and N content (RNC, ANC and TNC) of the genotypes of physic nut increased linearly with the increased availability of nitrogen in the soil (Table 3).

According with these results, the increase in the level of fertilization N up to the level of 150% promotes the accumulation of dry matter for most genotypes, indicating the possibility to obtain gains in physic nut with the review of the standard levels established by the studied recommendation.

### Nutritional efficiency

The study of nutritional efficiency parameters (AE, TE and UE) revealed the existence of significant interaction between the sources of variation. Therefore, differential behavior between genotypes of physic nut occurred, allowing the identification of different homogeneous

**Table 2.** Means for root dry matter (RDM), aerial part dry matter (ADM), total dry matter (TDM), root nitrogen content (RNC), aerial part nitrogen content (ANC) and total nitrogen content (TNC) of genotypes of physic nut cultivated with different levels of fertilization with nitrogen in the soil.

Genotype	Level of fertilization with nitrogen in relation to the recommendation (%)															
	0				50				100				150			
	0	50	100	150	0	50	100	150	0	50	100	150	0	50	100	150
	<b>RDM (g)</b>				<b>ADM (g)</b>				<b>TDM (g)</b>							
110-II	6.63 <sup>b</sup>	6.82 <sup>e</sup>	7.54 <sup>b</sup>	8.52 <sup>b</sup>	23.05 <sup>d</sup>	28.56 <sup>e</sup>	34.26 <sup>g</sup>	42.96 <sup>f</sup>	29.78 <sup>d</sup>	36.10 <sup>f</sup>	41.08 <sup>g</sup>	51.48 <sup>c</sup>				
127-I	5.48 <sup>c</sup>	6.43 <sup>e</sup>	7.35 <sup>b</sup>	7.13 <sup>e</sup>	24.07 <sup>c</sup>	27.07 <sup>f</sup>	33.17 <sup>h</sup>	37.56 <sup>h</sup>	31.58 <sup>b</sup>	35.59 <sup>g</sup>	41.58 <sup>g</sup>	46.48 <sup>d</sup>				
161-II	6.01 <sup>b</sup>	6.28 <sup>f</sup>	6.71 <sup>c</sup>	6.97 <sup>f</sup>	24.53 <sup>b</sup>	37.43 <sup>a</sup>	40.91 <sup>c</sup>	44.27 <sup>e</sup>	30.54 <sup>c</sup>	43.14 <sup>b</sup>	46.79 <sup>d</sup>	51.14 <sup>c</sup>				
167-II	5.67 <sup>c</sup>	7.17 <sup>d</sup>	6.36 <sup>d</sup>	7.62 <sup>d</sup>	25.08 <sup>b</sup>	36.03 <sup>b</sup>	42.09 <sup>b</sup>	40.14 <sup>g</sup>	31.75 <sup>b</sup>	42.19 <sup>c</sup>	49.16 <sup>b</sup>	47.76 <sup>d</sup>				
180-I	8.10 <sup>a</sup>	8.41 <sup>a</sup>	8.52 <sup>a</sup>	8.92 <sup>a</sup>	26.10 <sup>a</sup>	36.20 <sup>b</sup>	43.36 <sup>a</sup>	52.13 <sup>a</sup>	33.17 <sup>a</sup>	44.25 <sup>a</sup>	51.19 <sup>a</sup>	59.26 <sup>a</sup>				
191-I	5.14 <sup>d</sup>	6.20 <sup>f</sup>	6.50 <sup>d</sup>	7.17 <sup>e</sup>	21.05 <sup>e</sup>	36.24 <sup>b</sup>	40.36 <sup>d</sup>	45.57 <sup>d</sup>	26.19 <sup>f</sup>	43.74 <sup>b</sup>	46.29 <sup>e</sup>	52.74 <sup>b</sup>				
192-I	4.74 <sup>e</sup>	7.28 <sup>c</sup>	7.45 <sup>b</sup>	8.17 <sup>b</sup>	24.99 <sup>b</sup>	32.11 <sup>d</sup>	37.82 <sup>f</sup>	44.30 <sup>e</sup>	29.73 <sup>d</sup>	39.56 <sup>e</sup>	45.10 <sup>f</sup>	52.47 <sup>b</sup>				
210-II	5.30 <sup>c</sup>	8.00 <sup>b</sup>	6.33 <sup>d</sup>	7.61 <sup>d</sup>	19.05 <sup>g</sup>	35.30 <sup>c</sup>	41.42 <sup>b</sup>	45.73 <sup>d</sup>	23.62 <sup>h</sup>	41.63 <sup>d</sup>	49.57 <sup>b</sup>	53.34 <sup>b</sup>				
255-I	4.71 <sup>e</sup>	7.15 <sup>d</sup>	6.44 <sup>d</sup>	8.08 <sup>c</sup>	24.91 <sup>b</sup>	35.04 <sup>c</sup>	41.07 <sup>b</sup>	46.25 <sup>c</sup>	29.62 <sup>d</sup>	41.48 <sup>d</sup>	48.22 <sup>c</sup>	54.33 <sup>b</sup>				
275-I	6.33 <sup>b</sup>	7.04 <sup>d</sup>	7.58 <sup>b</sup>	7.63 <sup>d</sup>	22.73 <sup>d</sup>	26.02 <sup>f</sup>	33.55 <sup>h</sup>	34.73 <sup>i</sup>	29.06 <sup>e</sup>	33.80 <sup>h</sup>	37.59 <sup>h</sup>	42.36 <sup>e</sup>				
300-I	5.07 <sup>d</sup>	7.19 <sup>d</sup>	6.73 <sup>c</sup>	8.20 <sup>b</sup>	24.62 <sup>b</sup>	35.92 <sup>b</sup>	39.17 <sup>e</sup>	47.58 <sup>b</sup>	29.69 <sup>d</sup>	42.65 <sup>c</sup>	46.36 <sup>e</sup>	55.78 <sup>b</sup>				
302-I	4.57 <sup>f</sup>	5.54 <sup>g</sup>	5.10 <sup>e</sup>	6.32 <sup>g</sup>	20.77 <sup>f</sup>	25.10 <sup>g</sup>	25.04 <sup>i</sup>	29.07 <sup>j</sup>	25.07 <sup>g</sup>	30.90 <sup>i</sup>	31.24 <sup>i</sup>	35.59 <sup>f</sup>				
	<b>RNC (G)</b>				<b>ANC (G)</b>				<b>TNC (G)</b>							
110-II	5.50 <sup>a</sup>	9.55 <sup>a</sup>	9.95 <sup>c</sup>	14.88 <sup>a</sup>	22.73 <sup>a</sup>	41.61 <sup>a</sup>	53.41 <sup>a</sup>	54.76 <sup>a</sup>	27.92 <sup>a</sup>	50.13 <sup>a</sup>	60.06 <sup>d</sup>	67.87 <sup>a</sup>				
127-I	4.37 <sup>c</sup>	8.45 <sup>b</sup>	9.50 <sup>d</sup>	12.42 <sup>c</sup>	17.51 <sup>f</sup>	40.53 <sup>b</sup>	45.46 <sup>e</sup>	51.00 <sup>d</sup>	23.01 <sup>e</sup>	48.99 <sup>b</sup>	55.66 <sup>e</sup>	63.37 <sup>e</sup>				
161-II	4.52 <sup>b</sup>	7.45 <sup>d</sup>	10.33 <sup>c</sup>	12.22 <sup>c</sup>	19.85 <sup>c</sup>	35.27 <sup>d</sup>	46.73 <sup>d</sup>	52.17 <sup>c</sup>	24.37 <sup>d</sup>	42.72 <sup>e</sup>	57.07 <sup>d</sup>	64.39 <sup>d</sup>				
167-II	4.48 <sup>c</sup>	7.30 <sup>e</sup>	10.15 <sup>c</sup>	12.12 <sup>c</sup>	19.43 <sup>d</sup>	35.49 <sup>d</sup>	45.80 <sup>e</sup>	53.67 <sup>b</sup>	24.31 <sup>d</sup>	42.79 <sup>e</sup>	55.95 <sup>e</sup>	65.80 <sup>c</sup>				
180-I	4.55 <sup>b</sup>	8.16 <sup>c</sup>	9.06 <sup>e</sup>	11.18 <sup>e</sup>	21.60 <sup>b</sup>	35.10 <sup>d</sup>	48.40 <sup>b</sup>	53.24 <sup>b</sup>	26.15 <sup>b</sup>	43.06 <sup>e</sup>	58.35 <sup>c</sup>	65.22 <sup>c</sup>				
191-I	4.68 <sup>b</sup>	7.47 <sup>d</sup>	10.09 <sup>c</sup>	11.87 <sup>d</sup>	13.68 <sup>h</sup>	32.54 <sup>f</sup>	46.84 <sup>d</sup>	53.44 <sup>b</sup>	18.37 <sup>h</sup>	40.01 <sup>f</sup>	56.94 <sup>d</sup>	65.41 <sup>c</sup>				
192-I	4.11 <sup>c</sup>	7.73 <sup>d</sup>	10.69 <sup>b</sup>	11.80 <sup>d</sup>	16.34 <sup>g</sup>	34.62 <sup>e</sup>	44.18 <sup>f</sup>	50.15 <sup>e</sup>	21.45 <sup>g</sup>	41.35 <sup>f</sup>	54.10 <sup>f</sup>	62.10 <sup>f</sup>				
210-II	4.40 <sup>c</sup>	8.12 <sup>c</sup>	11.11 <sup>b</sup>	12.13 <sup>c</sup>	15.87 <sup>g</sup>	34.76 <sup>e</sup>	48.00 <sup>b</sup>	53.56 <sup>b</sup>	19.78 <sup>g</sup>	42.88 <sup>e</sup>	55.50 <sup>e</sup>	66.89 <sup>b</sup>				
255-I	4.19 <sup>c</sup>	8.52 <sup>b</sup>	10.08 <sup>c</sup>	13.13 <sup>b</sup>	21.43 <sup>b</sup>	35.91 <sup>c</sup>	48.96 <sup>b</sup>	52.98 <sup>c</sup>	26.81 <sup>b</sup>	45.47 <sup>d</sup>	59.04 <sup>b</sup>	65.63 <sup>c</sup>				
275-I	3.11 <sup>d</sup>	6.40 <sup>f</sup>	9.57 <sup>d</sup>	12.25 <sup>c</sup>	21.60 <sup>b</sup>	38.60 <sup>c</sup>	46.71 <sup>d</sup>	52.50 <sup>c</sup>	25.40 <sup>c</sup>	45.44 <sup>d</sup>	55.88 <sup>e</sup>	66.71 <sup>b</sup>				
300-I	4.18 <sup>c</sup>	8.54 <sup>b</sup>	12.32 <sup>a</sup>	13.48 <sup>b</sup>	17.97 <sup>e</sup>	34.87 <sup>e</sup>	45.32 <sup>e</sup>	53.21 <sup>b</sup>	22.02 <sup>f</sup>	43.41 <sup>d</sup>	57.65 <sup>c</sup>	66.69 <sup>b</sup>				
302-I	4.20 <sup>c</sup>	8.61 <sup>b</sup>	10.80 <sup>b</sup>	11.80 <sup>d</sup>	18.70 <sup>e</sup>	38.12 <sup>c</sup>	47.50 <sup>c</sup>	53.60 <sup>b</sup>	23.70 <sup>d</sup>	46.73 <sup>c</sup>	58.30 <sup>c</sup>	65.51 <sup>c</sup>				

Means followed by the same letter do not differ by the Scott-Knott test ( $p \leq 0.05$ ).

groups for each level nitrogen fertilization. The genotype CNPAE 255-I showed high absorption efficiency when growth without the addition of nitrogen in the soil and with 50% of the recommended level. This tendency was modified

with the increase in the level of available N, for it appears that the genotype CNPAE 161-II has higher absorption efficiency at 100 and 150% of the recommended level. Overall, the genotype CNPAE 127-I had significantly lower efficiency to

acquire nitrogen than the others (Table 4). The efficiency of absorption of the genotypes of physic nut increased linearly with the increase of the nitrogen supply in the soil (Table 4). When compared to others, the genotype CNPAE 180-I

**Table 3.** Regression equations for root dry matter (RDM), aerial part dry matter (ADM), total dry matter (TDM), root nitrogen content (RNC), aerial part nitrogen content (ANC) and total nitrogen content (TNC) of genotypes of physic nut cultivated with different levels of fertilization with nitrogen in the soil.

Genotype	Regression equation for RDM and RNC	R <sup>2</sup>	Regression equation for ADM and ANC	R <sup>2</sup>	Regression equation for TDM and TNC	R <sup>2</sup>
110-II	RDM = 0.012*N + 6.419* RNC = 0.006*N + 5.996*	0.92 0.99	ADM = 0.012*N + 6.419 ANC = 43.12	0.92 -	TDM = 0.011*N + 5.717* TNC = 0.005*N + 8.102*	0.81 0.95
127-I	RDM = 0.012*N + 5.294* RNC = 0.018*N + 5.185*	0.95 0.72	ADM = 0.020*N + 5.341 ANC = 0.008*N + 6.479	0.81 0.89	TDM = 38.80 TNC = 0.017*N + 5.458*	- 0.78
161-II	RDM = 0.009*N + 4.661* RNC = 0.125*N + 27.38*	0.70 0.87	ADM = 0.130*N + 22.39 ANC = 0.102*N + 28.14	0.98 0.75	TDM = 0.093*N + 23.48* TNC = 0.170*N + 26.66*	0.98 0.99
167-II	TDM = 0.172*N + 22.45* RNC = 0.140*N + 26.31*	0.90 0.97	ADM = 0.127*N + 25.25 ANC = 0.087*N + 22.72	0.99 0.93	RDM = 0.155*N + 24.15* TNC = 0.144*N + 26.00*	0.90 0.96
180-I	RDM = 0.049*N + 21.26* RNC = 0.130*N + 33.08*	0.89 0.90	ADM = 0.140*N + 29.09 ANC = 0.11*N + 34.46	0.97 0.80	TDM = 0.101*N + 31.20* TNC = 0.170*N + 34.18*	0.99 0.99
191-I	RDM = 0.164*N + 29.91* RNC = 0.161*N + 31.28*	0.87 0.97	ADM = 0.147*N + 30.65 ANC = 0.087*N + 29.14	0.98 0.99	TDM = 0.194*N + 27.47* TNC = 0.164*N + 31.32*	0.89 0.95
192-I	RDM = 0.063*N + 25.91* RNC = 0.052*N + 4.733*	0.90 0.99	ADM = 0.057*N + 5.689 ANC = 0.051*N + 4.647	0.92 0.99	TDM = 0.050*N + 4.905* TNC = 0.041*N + 5.119*	0.95 0.94
210-II	RDM = 0.048*N + 4.899* RNC = 0.056*N + 4.723*	0.99 0.96	ADM = 0.052*N + 4.678 ANC = 0.061*N + 3.244	0.95 0.99	TDM = 0.052*N + 5.013* TNC = 0.063*N + 4.878*	0.94 0.94
255-I	RDM = 0.05*N + 5.104* RNC = 0.216*N + 22.24*	0.91 0.95	ADM = 0.215*N + 26.94 ANC = 0.226*N + 21.64	0.88 0.97	TDM = 0.210*N + 22.81* TNC = 0.216*N + 23.35*	0.85 0.96
275-I	RDM = 0.267*N + 16.58* RNC = 0.215*N + 23.66*	0.95 0.95	ADM = 0.222*N + 19.67 ANC = 0.201*N + 24.73	0.94 0.93	TDM = 0.252*N + 19.10* TNC = 0.232*N + 20.41*	0.94 0.96
300-I	RDM = 0.228*N + 22.36* RNC = 0.268*N + 26.97*	0.93 0.96	ADM = 0.259*N + 32.02 ANC = 0.275*N + 26.56	0.93 0.98	TDM = 0.255*N + 28.59* TNC = 0.265*N + 28.32*	0.88 0.96
302-I	RDM = 0.316*N + 21.47* RNC = 0.260*N + 29.73*	0.96 0.95	ADM = 0.269*N + 24.54 ANC = 0.268*N + 28.20	0.96 0.97	TDM = 0.307*N + 23.17* TNC = 0.296*N + 25.20*	0.96 0.96

\* Significant by the t test ( $p \leq 0.05$ ).

**Table 4.** Means and regression equations for the nitrogen absorption efficiency (AE –  $\text{mgg}^{-1}$ ) of genotypes of physic nut cultivated with different levels of fertilization with nitrogen in the soil.

Genotype	0% of N	50% of N	100% of N	150% of N	Regression equation	R <sup>2</sup>
110-II	120.44 <sup>e</sup>	242.37 <sup>e</sup>	350.82 <sup>f</sup>	396.02 <sup>g</sup>	AE = 1.870* N + 137.1*	0.96
127-I	91.167 <sup>g</sup>	204.47 <sup>g</sup>	267.24 <sup>h</sup>	334.23 <sup>j</sup>	AE = 1.583* N + 105.4*	0.97
161-II	122.41 <sup>e</sup>	274.79 <sup>b</sup>	501.43 <sup>a</sup>	541.96 <sup>a</sup>	AE = 2.599* N + 149.7*	0.86
167-II	135.63 <sup>c</sup>	292.64 <sup>a</sup>	394.61 <sup>d</sup>	413.06 <sup>e</sup>	AE = 1.868* N + 168.8*	0.90
180-I	150.68 <sup>b</sup>	260.34 <sup>d</sup>	434.00 <sup>c</sup>	480.08 <sup>b</sup>	AE = 2.695* N + 144.6*	0.99
191-I	103.52 <sup>f</sup>	268.86 <sup>c</sup>	446.39 <sup>b</sup>	479.08 <sup>b</sup>	AE = 2.668* N + 121.8*	0.93
192-I	136.85 <sup>c</sup>	211.78 <sup>f</sup>	380.62 <sup>e</sup>	403.99 <sup>f</sup>	AE = 1.984* N + 139.9*	0.89
210-II	106.28 <sup>f</sup>	281.72 <sup>b</sup>	366.69 <sup>e</sup>	469.84 <sup>c</sup>	AE = 2.351* N + 129.7*	0.97
255-I	172.52 <sup>a</sup>	297.34 <sup>a</sup>	397.52 <sup>d</sup>	456.77 <sup>d</sup>	AE = 1.905* N + 188.1*	0.97
275-I	112.93 <sup>f</sup>	201.81 <sup>g</sup>	298.86 <sup>g</sup>	366.38 <sup>h</sup>	AE = 1.714* N + 116.3*	0.99
300-I	129.12 <sup>d</sup>	275.18 <sup>b</sup>	371.18 <sup>e</sup>	452.05 <sup>d</sup>	AE = 2.129* N + 147.1*	0.98
302-I	126.87 <sup>d</sup>	245.96 <sup>e</sup>	294.76 <sup>g</sup>	353.00 <sup>i</sup>	AE = 1.454* N + 146.0*	0.95

Means followed by the same letter do not differ by the Scott-Knott test ( $p \leq 0.05$ ). \* Significant by the t test ( $p \leq 0.05$ ).

had higher efficiency to translocate nitrogen in the level of 0 and 150% of N, having potential to be used in environments with limitation of nitrogen and also in conditions where the fertilization exceed the recommended level. At levels of 50 and 100% of N, the genotype CNPAE 161-II had higher mean for TE. The lowest translocation efficiency of N was observed for the genotype CNPAE I-127 in all levels of N supply (Table 5).

The genotypes of physic nut present TE increasing linearly in function of the levels of nitrogen applied in the soil, except for the genotype CNPAE 167-II which showed TE set to a quadratic model, characterized by a curve with a maximum point near the level of 50% of the nitrogen recommendation. It was not possible to set a standard regression model for the genotypes CNPAE 110-II, CNPAE 255-I and CNPAE 300-I (Table 5).

Regarding the use efficiency, it can be verified that the genotype CNPAE 191-I showed higher

means for all levels of N applied to the soil, except in the level of 150%, for which the genotype CNPAE 180-I presented higher efficiency than the others. Overall, considering the control level and the different supplies of nitrogen, the genotype CNPAE 302-I presented the lowest means for use efficiency. Additionally, the genotype CNPAE I-302 is not just inefficient in use of N, but also inefficient in absorb and transport it. Despite occasionally not being classified into the inferior group, this genotype is always allocated in the lower groups for most of the efficiency parameters (Table 6).

The genotypes of physic nut had adjust to different regression models for UE (Table 6). The genotypes CNPAE 302-I, CNPAE 275-I, CNPAE 255-I, CNPAE 191-I, CNPAE 210-II, CNPAE 161-II and CNPAE 167-II have use efficiency decreasing linearly with the increasing level of N applied in the soil, however the genotypes CNPAE 127-I, CNPAE 110-II, CNPAE 192-I,

CNPAE 180-I and CNPAE 300-I had adjust to quadratic models, with inflection point close to the level of 50% of N recommended.

### Efficiency and responsiveness

Figure 1 shows the graphic analyses of efficiency and responsiveness of the genotypes in relation to the level of 0 and 100% of the recommended level of nitrogen, according to the model analyzed proposed by Ciat (1978). The genotypes CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 255-I and CNPAE 300-I behaved as efficient and responsive (ER), which means they have high capacity to produce dry matter in conditions of limited N supply and respond well to the fertilization (Figure 1). The genotypes CNPAE 191-I and CNPAE 210-II were classified as non-efficient and responsive (NER) (Figure 1), demonstrating that although those genotypes

**Table 5.** Means and regression equations for the nitrogen translocation efficiency (TE – %) of genotypes of physic nut cultivated with different levels of fertilization with nitrogen in the soil.

Genotype	0% of N	50% of N	100% of N	150% of N	Regression equation	R <sup>2</sup>
110-II	65.01 <sup>d</sup>	64.99 <sup>g</sup>	68.76 <sup>e</sup>	66.78 <sup>f</sup>	TE = 66.38	-
127-I	57.03 <sup>i</sup>	62.99 <sup>h</sup>	67.10 <sup>g</sup>	64.81 <sup>g</sup>	TE = 0.054* N + 58.86*	0.67
161-II	65.12 <sup>d</sup>	71.51 <sup>a</sup>	72.52 <sup>a</sup>	70.03 <sup>c</sup>	TE = 2.599* N + 149.7*	0.86
167-II	65.85 <sup>c</sup>	70.87 <sup>b</sup>	70.21 <sup>d</sup>	68.73 <sup>d</sup>	TE = 69.09	0.92
180-I	68.26 <sup>a</sup>	67.65 <sup>e</sup>	69.94 <sup>d</sup>	72.75 <sup>a</sup>	TE = 0.031* N + 67.29*	0.79
191-I	59.87 <sup>h</sup>	69.35 <sup>c</sup>	71.41 <sup>b</sup>	71.35 <sup>b</sup>	TE = 0.073* N + 62.52*	0.73
192-I	62.96 <sup>f</sup>	66.33 <sup>f</sup>	68.90 <sup>e</sup>	68.43 <sup>d</sup>	TE = 0.038* N + 63.80*	0.82
210-II	62.12 <sup>g</sup>	68.79 <sup>d</sup>	67.86 <sup>f</sup>	69.56 <sup>c</sup>	TE = 0.042* N + 63.87*	0.66
255-I	67.42 <sup>b</sup>	66.39 <sup>f</sup>	70.71 <sup>c</sup>	66.37 <sup>f</sup>	TE = 67.85	-
275-I	65.53 <sup>c</sup>	66.14 <sup>f</sup>	67.78 <sup>f</sup>	68.12 <sup>e</sup>	TE = 0.018* N + 65.48*	0.93
300-I	67.46 <sup>b</sup>	67.62 <sup>e</sup>	66.51 <sup>h</sup>	68.26 <sup>e</sup>	TE = 67.46	-
302-I	63.63 <sup>e</sup>	66.24 <sup>f</sup>	67.62 <sup>f</sup>	68.20 <sup>e</sup>	TE = 0.023* N + 64.02*	0.75

Means followed by the same letter do not differ by the Scott-Knott test ( $p \leq 0.05$ ). \* Significant by the t test ( $p \leq 0.05$ ).

**Table 6.** Means and regression equations for the nitrogen use efficiency (UE – g<sup>2</sup> mg<sup>-1</sup>) of genotypes of physic nut cultivated with different levels of fertilization with nitrogen in the soil.

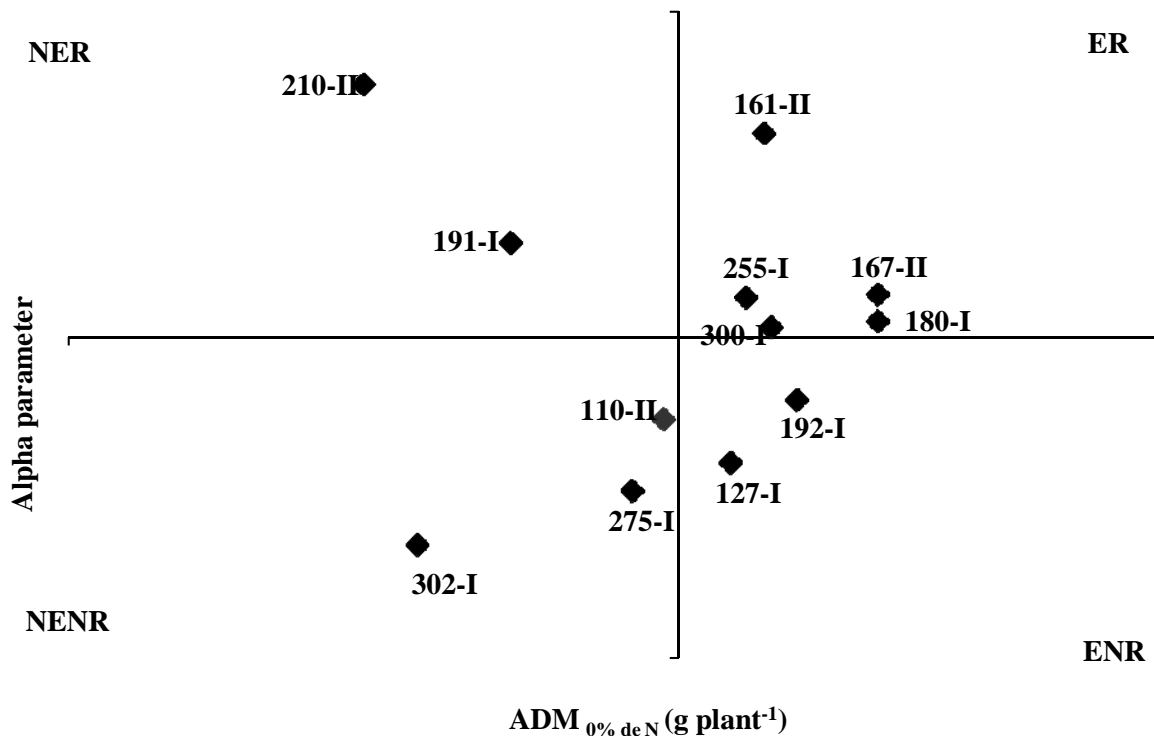
Genotype	0% of N	50% of N	100% of N	150% of N	Regression equation	R <sup>2</sup>
110-II	1111.61 <sup>i</sup>	727.23 <sup>i</sup>	734.63 <sup>f</sup>	783.38 <sup>d</sup>	UE = 0.043* N <sup>2</sup> - 8.451* N + 1094*	0.93
127-I	1396.01 <sup>c</sup>	725.92 <sup>i</sup>	752.24 <sup>e</sup>	735.36 <sup>e</sup>	UE = 0.029* N <sup>2</sup> - 7.322* N + 965.8*	0.79
161-II	1258.94 <sup>f</sup>	1011.51 <sup>c</sup>	897.74 <sup>b</sup>	795.40 <sup>c</sup>	UE = - 2.868* N + 1223*	0.93
167-II	1302.35 <sup>e</sup>	985.30 <sup>d</sup>	895.82 <sup>b</sup>	724.03 <sup>f</sup>	UE = - 3.648* N + 1250*	0.94
180-I	1207.18 <sup>h</sup>	1023.17 <sup>b</sup>	820.21 <sup>c</sup>	908.73 <sup>a</sup>	UE = 0.027* N <sup>2</sup> - 6.284* N + 1222*	0.94
191-I	1425.73 <sup>a</sup>	1091.77 <sup>a</sup>	970.88 <sup>a</sup>	803.68 <sup>c</sup>	UE = - 4.282* N + 1355*	0.89
192-I	1409.41 <sup>b</sup>	969.98 <sup>f</sup>	713.63 <sup>g</sup>	837.25 <sup>b</sup>	UE = 0.056* N <sup>2</sup> - 12.39* N + 1419*	0.99
210-II	1243.35 <sup>g</sup>	970.33 <sup>e</sup>	816.80 <sup>c</sup>	799.06 <sup>c</sup>	UE = - 2.664* N + 1195*	0.87
255-I	1042.57 <sup>i</sup>	926.38 <sup>g</sup>	815.79 <sup>c</sup>	801.50 <sup>c</sup>	UE = -1.667* N + 1021*	0.92
275-I	1108.03 <sup>i</sup>	741.08 <sup>h</sup>	674.18 <sup>h</sup>	621.72 <sup>g</sup>	UE = - 3.051* N + 1015*	0.80
300-I	1350.39 <sup>d</sup>	983.12 <sup>d</sup>	803.31 <sup>d</sup>	834.05 <sup>b</sup>	UE = 0.039* N <sup>2</sup> - 9.427* N + 1351*	0.99
302-I	969.47 <sup>j</sup>	661.21 <sup>j</sup>	534.77 <sup>i</sup>	536.74 <sup>h</sup>	UE = - 2.969* N + 893.2*	0.83

Means followed by the same letter do not differ by the Scott-Knott test ( $p \leq 0.05$ ). \* Significant by the t test ( $p \leq 0.05$ ).

are not efficient at the absorption and translocation of N, they are efficient at converting

it in dry matter, this characteristic is highly correlated with UE, for which the genotype

CNPAE I-191 was highlighted (Table 6). The genotypes CNPAE 192-I and CNPAE 127-I



**Figure 1.** Classification of genotypes of physic nut according to their efficiency and responsiveness regarding the nitrogen fertilization.

were allocated in the quadrant that ranks the efficient and non-responsive genotypes (ENR) (Figure 1), demonstrating their capacity to grow and accumulate dry matter in significantly low availability of N in the soil, but those do not respond in the same magnitude to the increase in the fertilization with N. The genotypes CNPAE 110-II, CNPAE 302-I and CNPAE 275-I were classified as non-efficient and non-responsive (Figure 1), which means they have low dry matter production, even with the increase of the fertilization, this classification as NENR is justified as these genotypes were allocated in lower groups for all nutritional indices (Tables 4, 5 and 6).

## DISCUSSION

### Nutritional efficiency of N in physic nut

Overall, the genotypes 161-II, 167-I and 255-I (Table 4), although not allocated in groups of superior means for TNC (Table 2), showed higher efficiency for absorption of N in each specific level of fertilization. This fact is associated to their greater root development as evidenced by the values of TDM (Table 2) with lower use of units of TNC. Those genotypes are capable of producing more dry matter root employing lesser amounts of N.

The different means of AE among genotypes presented

in this study can be explained by the great genetic variability existing in *J. curcas* L., as evidenced in many other studies (Laviola et al., 2010; Laviola et al., 2011; Bhering et al., 2013; Martins et al., 2013).

It is commonly observed (Lee et al., 2005; Miranda et al., 2008; Souza et al., 2008; Vale et al., 2013) that efficiencies of absorption, translocation and use of N are controlled by genotypic factors. In plants of physic nut, there is a high genetic variability among the genotypes (Laviola et al., 2010), inducing a wide phenotypic variability (Laviola et al., 2011), which reinforces the results found in this study. These results indicate the possibility to explore the nutritional efficiency of N in breeding programs of physic nut, selecting efficient genotypes in uptake, translocation and utilization of this nutrient.

Another determining factor for those differences is that physic nut does not have a standardized cultivar in Brazil yet, so morphological characteristics present high variability between Brazilian genotypes. The differences in the root morphology (root specific surface, area, length, size and volume) promote different effects over the ability of the plant to acquire and absorb nutrients from the soil (Baligar, 2001; Chun et al., 2005; Hammer et al., 2009).

Overall, the genotypes CNPAE 180-I and CNPAE 161-II showed high translocation efficiency at the doses studied.



This fact is evidence that some genotypes of physic nut may present more efficient transport systems, what may be related to morphological differences in the configuration of their vascular elements.

The translocation efficiency is related to the capacity of the xylem vessels to conduct the nutrient for the green leaf tissue. However, even if the transport of N is effective, the availability of this nutrient is also associated to energetic efficiency and metabolism of the genotype, so plants with high TE have higher concentration of nitrogen compounds (e.g. amino acids and proteins) in the aerial part at expense of roots, also presenting higher energetic efficiency (Baligar et al., 2001).

The genotypes CNPAE 180-I and CNPAE 191-I showed high UE (Table 6), since they produce higher rates of dry matter using lower rates of N from the TCN (Table 2). These results are supported by the evidence of genetic variability among the genotypes. Several studies indicate that the genetic variability of the species is crucial to differentiate rates of utilization of nutrients, including N (Fageria et al., 1998; Baligar et al., 2001; Reis et al., 2005; Vale et al., 2013).

Besides the large genetic variability found in populations of physic nut (Laviola et al., 2010; Alves et al., 2013; Bhering et al., 2013), these results can also be explained by physiological and biochemical differences inherent of each genotype.

Genotypes with high UE possibly invest relatively more in the formation of cell walls and/or secondary compounds, which may increase the additional investment in photosynthetic performance and metabolic machinery, as described for other conditions of stresses in physic nut (Yong et al., 2010; Rajaona et al., 2013; Sapeta et al., 2013). Differences in the use of N between organs or in the investment of N in different types of compounds may strongly modulate the efficiency of the plant regarding the nitrogen use (Fageria, 1998; Baligar et al., 2001).

### **Increase of the N content in green leaf tissues of physic nut**

The increase of the level of N applied in the soil induced a linear growth in N content, physic nut plants and consequently increases dry matter of the plants (Table 3). However, this significant gain was higher in function of the application of N even in the higher levels, which does not necessarily indicate responsiveness of the genotypes.

Primarily, in environments with low N supply, the RDM/ADM ratio is higher. But as the level of N available in the soil raises, this relationship changes, verifying inverse behavior with greater development of the aerial part at the expense of roots (Taiz and Zeiger, 2006). This relationship is verified in this study (Table 3). Studies with similar results can be found in published papers,

describing a tendency of linear increase of N content in relation to the increase of N available in the soil (Presterl et al., 2003; Yong et al., 2010; Batista et al., 2014). Results show that a high nutrition with N can increase the vegetable oil yield, the total number of fruits and seeds produced per plant (Yong et al., 2010) and relate this results to the capacity of the plants in absorb and utilize N. This behavior may be linked to the fact that, at low N supply, plants tend to use the nutrient near the sites of absorption (root cells) in order to develop a larger root volume, increasing the explored area. Therefore, with the increase of the supply, the nutrient is taken to other parts of the plant (Taiz and Zeiger, 2006), justifying the results found in this study (Tables 3, 4, 5 and 6).

### **Efficiency and responsiveness of physic nut to N**

The genotypes CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 255-I and CNPAE 300-I were classified as ER (Figure 1), because those had high capacity to accumulate dry matter in low N supply, and responded to the N supply with an significant increase in this capacity. This statement can be verified by the fact that the genotypes CNPAE 161-II, CNPAE 167-II e CNPAE 180-I presented 24 to 31% of increase in TDM in low N supply (control) when compared to other genotypes (Table 2).

In addition to being responsive in an environment with low N supply, an efficient and responsive genotype should possess favorable characteristics, such as being able to translocate enough N from roots to growing tissues, remobilize nutrients from senescent leaves before their abscission and present a reproductive development suitable for the largest amount of N applied (Marschner, 1995; Amaral et al., 2012). It is noteworthy that the parameters to study nutritional efficiency and response to nutrients, involving different genotypes proposed by Ciat (1978) have been successfully applied in other studies (Amaral et al., 2012; Martins et al., 2013) being considered an excellent tool for selection.

### **Conclusions**

The genotypes of physic nut have different behavior in relation to the nutritional efficiency of nitrogen, in different levels of fertilization. Overall, the efficiencies of absorption, translocation and use of nitrogen of genotypes of physic nut increase linearly with the increase of the level of nitrogen available in the soil. The genotypes CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 255-I and CNPAE 300-I are efficient and responsive to nitrogen fertilization.

### **Conflict of interests**

The authors have not declared any conflict of interests.

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