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Research Paper

Nitrogen uptake kinetics and accumulation of soluble nitrogen fractions and soluble sugars in *Jatropha curcas L.*

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Abstract. *Jatropha curcas L.* presents high potential for biofuel production. Studies of its nutritional requirements are therefore highly relevant to agriculture.The aim of the present study was to investigate the Nitrogen (N) uptake in plants grown with either nitrate (NO_3^-) or ammonium (NH_4^+) as N source and to quantify nitrate-N, ammonium-N (nutrient solution), amino-N and soluble sugar (plant tissue) concentrations of the *J. curcas* variety CNPAS-170. Two different levels of NH_4^+ (0.4 or 4.0 mM) and two different levels of NO_3^- (1.0 or 4.0 mM) were tested. Plants were grown for 26 hours with the low N nutrient solutions and for 82 hours with the high N nutrient solutions. Uptake curves were measured for both of the N sources, and nitrate-N, ammonium-N (nutrient solution), amino-N and soluble sugar (plant tissues) concentrations were quantified. A randomized block experimental design was used, with a 2x2 factorial scheme and 8 replicates. The results indicate the presence of high-affinity uptake systems (low K_m and V_{max} values) in plants grown with 1 mM NO_3^- and preferential allocation of N-soluble fractions and soluble sugars to stems in *J. curcas* variety CNPAS-170.

Keywords: nitrogen metabolism, nitrate, ammonium, biofuel.

INTRODUCTION

The potential of *Jatropha curcas* L. for biofuel production has been extensively reported in the international literature, as it has the need of establishing agronomic parameters for this species, with an emphasis on its nutritional management (Laviola and dos Santos, 2008; Santos, 2014).

Studies with the aim of clarifying the nutritional needs of *J. curcas* are therefore highly relevant for agriculture (Carvalho *et al.*, 2013). Because nitrogen (N) is the nutrient required by plants in the greatest quantity and is often the most limiting for plant growth, it is one of the nutrients with greater influence on *J. curcas* management (Santos, 2014). Understanding the relation of N to *J. curcas* is essential to biofuel production.

Plants may take up inorganic N preferentially as either NO_3^- or NH_4^+, and the two N forms result in different plant growth and development responses. However, NH_4^+ tends to be preferentially taken up during early plant growth and NO_3^- in later stages of plant growth (Rocha *et al.*, 2014).

When studying NO_3^- and NH_4^+uptake dynamics, two parameters should be evaluated: the maximum uptake

velocity (V_{max}) and the Michaelis constant (K_m). A higher V_{max} indicates a higher nutrient uptake velocity, and a lower K_m indicates a higher transporter affinity for the nutrient taken (Souza and Fernandes, 2006; Rocha *et al.*, 2014).

Evaluation of the N uptake kinetics therefore aims at selecting high V_{max} and low K_m values because these levels indicate high nutrient uptake under conditions of low nutrient availability (Souza and Fernandes, 2006; Rocha *et al.*, 2014).

The quantification of soluble fractions is important because these fractions indicate changes to hormone synthesis and the presence of stresses, such as changes in cell pH and accumulation of soluble carbohydrates in plant tissues (Costa *et al.*, 2007; Taiz and Zeiger, 2013; Rocha *et al.*, 2014). Soluble carbohydrate concentrations may indicate plant regulatory mechanisms under conditions of excess NH_4^+ (Rocha *et al.*, 2014).

The aim of the present study was to evaluate N uptake in plants grown with either NO_3^- or NH_4^+ as the N source, with each supplied at two different doses, and to quantify nitrate-N, ammonium-N, amino-N and soluble sugar concentrations in *J. Curcas* variety CNPAS-170 grown under hydroponic conditions.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse, which was located in the experimental field of the Soil Department (Departamento de Solos) of the Institute of Agronomy (Instituto de Agronomia) of the Federal Rural University of Rio de Janeiro (Universidade Federal Rural do Rio de Janeiro- UFRRJ).The *J. curcas* variety CNPAS-170 was used. The seeds were disinfected in 2% sodium hypochlorite and 70% ethanol, and sown in washed sand. The seedlings were watered twice a day for 24 days, and the substrate was maintained at 80% of field capacity.

When seedlings presented two fully expanded leaves, uniform seedlings were selected and transferred into polyethylene pots containing 3 Lof nutrient solutions, with constant aeration. The seedlings were placed in Styrofoam lids used for support and fixed with synthetic foam. Two plants were grown per pot.

The plants were grown in half-strength (Hoagland and Arnon, 1950) nutrient solution during plant acclimatization, until 21 or 45 days after germination (DAG), and then in 1/8-strength nutrient solution (pH 6.5±0.2) from 45 to59 DAG. At 60 DAG, the plants were transferred to nutrient solution without N (pH 6.5±0.2) for 72 hours.

At 63 DAG, the plants were transferred to modified (Hoagland and Arnon, 1950)) nutrient solution with one of the following N sources: 0.4 mM NH_4^+ (low NH_4^+), 4 mM NH_4^+(high NH_4^+), 1mM NO_3^- (low NO_3^-) or 4 mM NO_3^- (high NO_3^-). NH_4^+ and NO_3^- were supplied as Ammonium Sulphate [$(NH_4)_2SO_4$] and Calcium Nitrate [$Ca(NO_3)_2$] respectively.

A randomized block experimental design was used, with a 2x2 factorial scheme and 8 replicates. N uptake curves were determined for all tested N sources. Nutrient solution samples (1 ml) were collected every 30 minutes for the first 14 hours and then every 3 hours for 12 hours. Samplings were performed for 26 hours for the low N supplies (0.4 mM NH_4^+ and 1 mM NO_3^-) and for 82 hours for the high N supplies (4 mM NH_4^+ or NO_3^-). The nutrient solution pH was measured at the moment of each sampling.

Following the last sampling for each treatment, the plant height and the stem diameter were measured. The plants were then separated into root, stem, petiole and leaves, and fresh weights of each plant part were determined. At weighing, 1 mg aliquots of each plant part were separated and used for the determination of nitrate-N (N-NO_3^-), ammonium-N (N-NH_4^+), amino-N and soluble sugars, according to Miranda *et al.* (2001), Felker (1977), Yemm *et al.* (1955) and Yemm and Willis (1954) respectively.

Averages for the soluble fractions were analyzed using a Tukey test, at $p<0.05$, using R. The kinetic parameters (V_{max} and K_m) were analyzed using the Kinetics software (Ruiz and Fernandes, 1992), according to Claassen and Barber (1974).

RESULTS AND DISCUSSION

Differences in pH were observed between the different NO_3^- and NH_4^+ treatments, due to differences in nitrogen uptake (Figure1). Following pH adjustment to 6.5, an increase in pH was observed with 4 mM NO_3^-. The observed decreases in pH with 1 mM NO_3^- were likely due to processes of charge balance maintenance following cation uptake (Taiz and Zeiger, 2004; Rocha *et al.*, 2014).

In contrast, a decrease in nutrient solution pH was observed with 4 mM NH_4^+. During NH_4^+ uptake, the electrochemical gradient is maintained by balancing NH_4^+ influx with proton efflux, resulting in acidification of the medium (Taiz and Zeiger, 2004). Rocha *et al.* (2014) investigated N kinetics in sunflower and observed similar pH variations to those observed in the present study.

For the low NO_3^- treatment (1 mM), the NO_3^- in the nutrient solution was fully depleted over the first 16 hours.The nutrient solution pH was correlated with the NO_3^- concentration in the nutrient solution, with both decreasing with increasing NO_3^- depletion. The pH was observed to be stable over the first 18.5 hours of the experiment and then increased starting at 22.5 hours (Figure 2).

With 4 mM NO_3^-, depletion of N-NO_3^- occurred over the

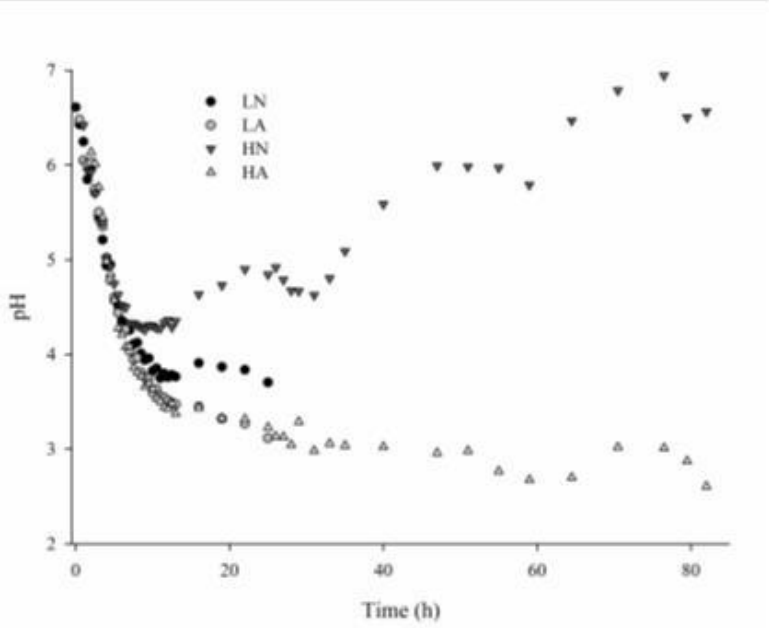

Figure. 1: Variation of the nutrient solution pH over time, for all treatments: high nitrate (4 mM; HN), low nitrate (1 mM; LN), high ammonium (4 mM; HA) and low ammonium (0.4 mM; LA).

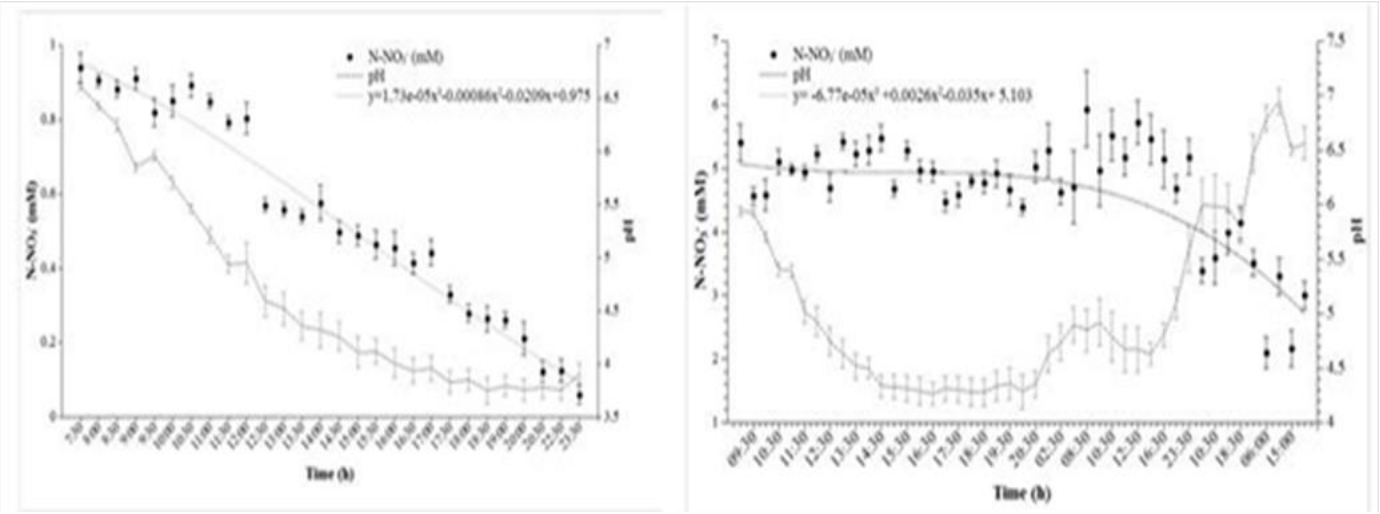

Figure. 2: Nutrient solutionpH and NO_3^- depletion over time for the treatments with (a) 1 mM NO_3^- or (b) 4 mM NO_3^- as the N source. Vertical bars indicate standard errors (n=8).

first 76.5 hours of the experiment. The pH significantly decreased in the first hours of the experiment, reaching minimum values of 3.8 during the periods with higher irradiance. The pH increased, and NO_3^- uptake decreased during the night. It should be noted that the pH decrease was similar for the two$N\text{-}NO_3^-$ supplies and that the tendency for pH increase observed at the end of the experiment for the low NO_3^- supply was confirmed for the high NO_3^-supply (Figure 2).

NO_3^- uptake may occur through secondary active transport of the symport type, by co-transport with H^+. Uptake of 1 mol NO_3^- requires 2 moles H^+ (Souza and Fernandes, 2006). This relation tends to increase pH at the apoplast when there is high-affinity transport (low K_m).

An exponential decrease of NH_4^+concentrations was not observed for either NH_4^+ treatment (Figure 3). For the high NH_4^+ treatment, the ammonium depletion results revealed no conclusive behavior for the effective NH_4^+

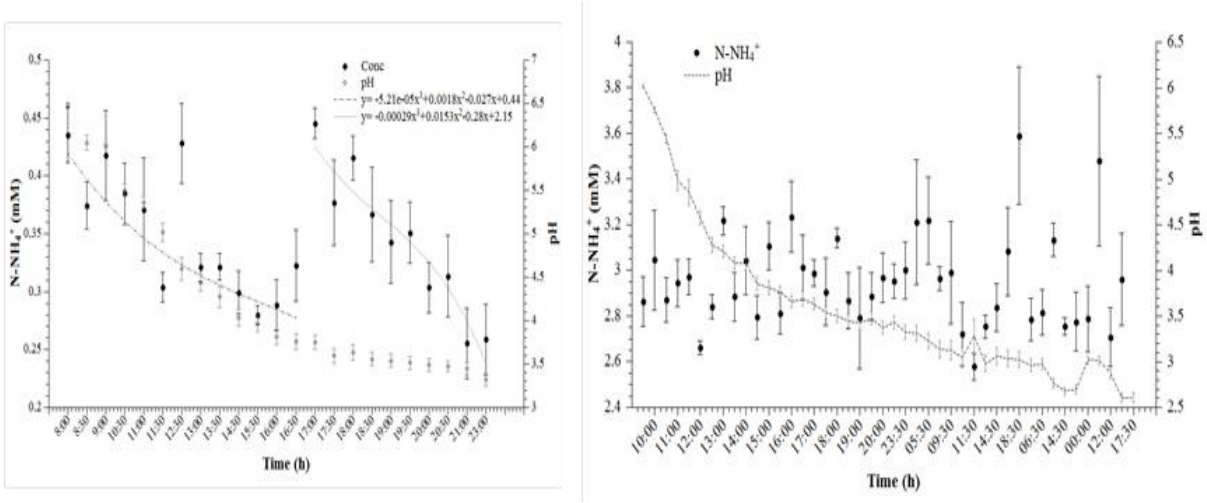

Figure. 3: Nutrient solution pH and NH_4^+ depletion over time for the treatments with (a) 0.4 mM NH_4^+, or (b) 4 mM NH_4^+ as N source. Vertical bars indicate standard errors (n=8).

Table 1: Uptake *Vmax* (µmol h^{-1} g^{-1} fresh weight) and Michaelis constant (K_m; µmol L^{-1}), for *J. curcas* plants grown with different nitrogen sources and concentrations (mM).

Nitrogen source	Concentration		*Vmax*	K_m
NO_3^-	1.0		0.09	0.05
	4.0		1.97	93.82
NH_4^+	0.4	Time 1	0.10	1.03
		Time 2	0.07	0.17
	4.0		-	-

uptake during the 72 hours of the experiment. In contrast, the high NH_4^+ concentration in the nutrient solution remained constant until the end of the experiment, and full NH_4^+ depletion was not observed. This result may have been due to cellular homeostasis, which maintains the electrochemical gradient.

For the low NH_4^+ treatment (0.4 mM), NH_4^+ efflux was observed over the first 15 hours of the experiment, with the NH_4^+ concentration increasing following an initial drop.

The analysis of the uptake kinetic parameters, the maximum velocity (V_{max}*Vmax*) and the Michaelis-Menten constant (K_m), revealed that the plants supplied with low NO_3^- concentrations presented low V_{max}(0.09) and K_m(0.05) values for NO_3^- uptake, indicating the presence of a high-affinity NO_3^- uptake system (Table 1).

For a more accurate analysis of the NH_4^+ uptake kinetic parameters with low NH_4^+ supply, the NH_4^+ uptake curve was divided into two periods (Table 1). The first period lasted from 8 to 16.5 hours and the second from 17 to 23 hours of the experiment. During the first period, there was NH_4^+ depletion from the nutrient solution. The NH_4^+ V_{max} was low (0.10), and the K_m was high, indicating that higher NH_4^+concentrations were needed to reach K_m.

For the second period, low $V_{max}x$ (0.07) and K_m (0.17) values were observed, indicating the presence of high-affinity transporters for NH_4^+ uptake. However, a trend line could not be fitted to NH_4^+ uptake for the high NH_4^+supply (4 mM) based on the calculated uptake kinetic parameters, according to Claassen and Barber (1974) and Ruiz and Fernandes, (1992).

No significant differences in amino-N concentrations were observed between treatments, either for the whole plant or for the different plant parts. However, the accumulation of soluble N fractions was higher in leaves and stems for all treatments (Figure 4).

This result indicates that amino-N was allocated to the stem, independently of the N source. It should be noted that, especially with NH_4^+ assimilation, approximately 80% of the total assimilated N is contained in asparagine and glutamine which are readily allocated to vacuoles, and subsequently enter various N metabolic pathways (Taiz and Zeiger, 2004; Rocha *et al.,* 2014).

Soluble sugars were mainly allocated to the stem in all the treatments (Figure 4). This pattern indicates that both the different N fractions and soluble sugars were mainly

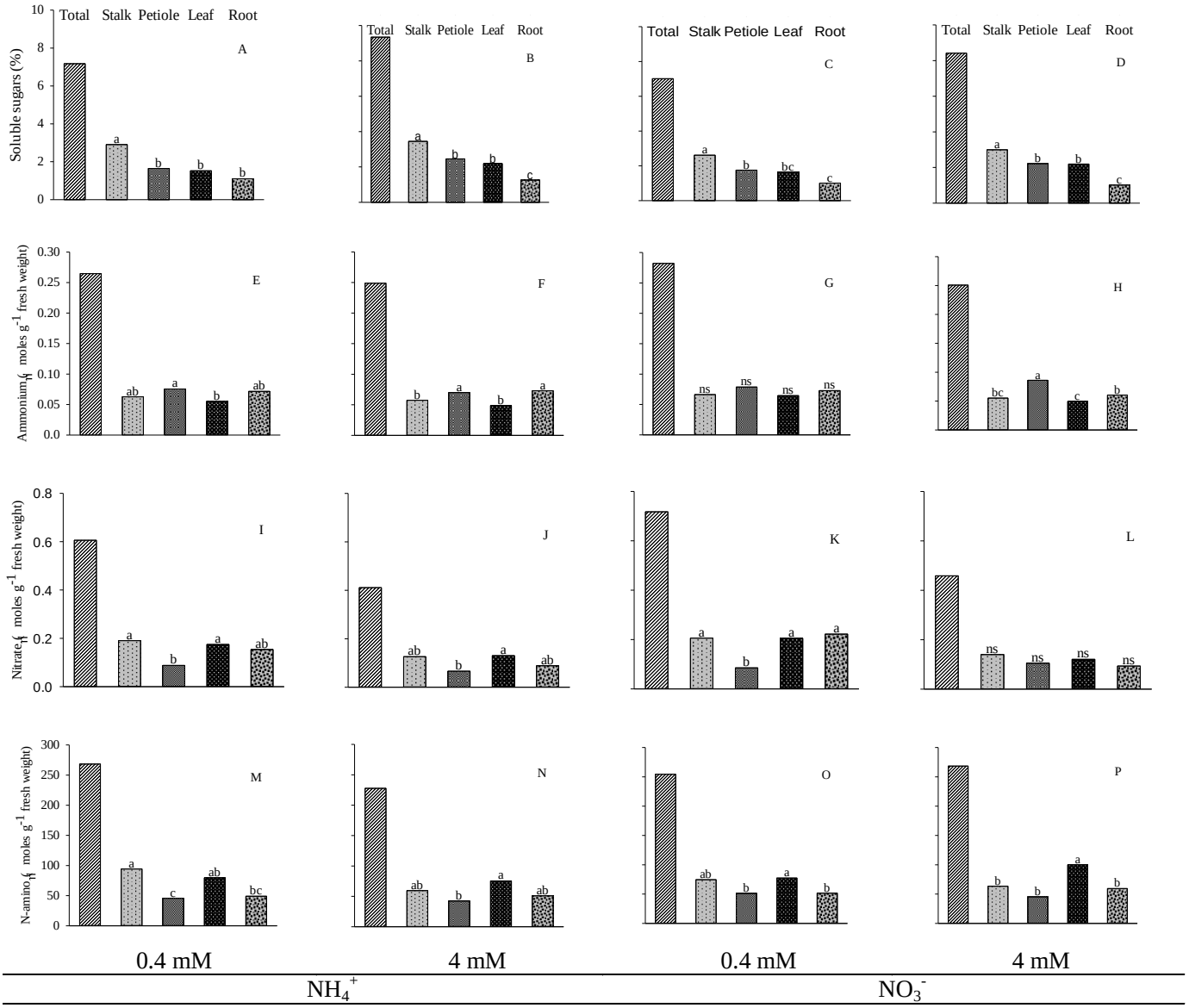

Figure. 4: N-NH_4^+, N-NO_3^-, amino-N and total soluble sugar (dag kg^{-1}; percentage) concentrations at the stem, petiole, leaves and roots of *J. curcas* variety CNPAS -170 grown in nutrient solution with 0.4 mM NH_4^+, 4 mM NH_4^+, 1 mM NO_3^- or 4 mM NO_3^- as the N source.

allocated to the stem.

Amino-N concentrations were positively correlated with N-NH_4^+concentrations in stems and with N-NO_3^- concentrations in leaves for the high NH_4^+ and high NO_3^- treatments, respectively (4 mM L^{-1}; Table 2). N-NH_4^+ concentrations in plant tissues are usually low due to the fast assimilation of NH_4^+ because high NH_4^+ concentrations in plant tissues can be toxic to the plant. Plants assimilate NH_4^+ close to its uptake or production sites and quickly store the excess NH_4^+ in vacuoles, to avoid toxic effects to cell membranes and cytosol (Taiz and Zeiger, 2013). High NH_4^+ supply favors N assimilation into asparagine and glutamine, which can account for more than 80% of the total free amino-N. This pattern results in increase of 10 to 20 times the amino-N/free amide-N ratio in response to NH_4^+ toxicity (Souza and Fernandes, 2006).

A negative correlation was observed between petiole sugar and root nitrate concentrations for the 0.4 mM NH_4^+ treatment. A similar negative correlation was observed between root sugar and leaf ammonium concentrations for the 4 mM NH_4^+ treatment. This pattern may be due to the need of plants to use carbon skeletons, i.e., carbohydrates that can be readily assimilated (soluble

Table 2: Pearson correlation matrix for soluble fractions at the stem, petiole, leaves and roots of *J. curcas* grown in nutrient solution with 0.4 mM NH_4^+, 4 mM NH_4^+, 1 mM NO_3^- or 4 mM NO_3^- as the N source.

								0-4mM								
	SsS	**SsP**	**SsL**	**SsR**	**NH_4^-S**	**NH_4^-P**	**NH_4^-L**	**NH_4^-R**	**NO_3^-s**	**NO_3^-P**	**NO_3^-L**	**NO_3^-R**	**N-a S**	**N-a P**	**N-a L**	**N-a R**
SsS	100															
SsP	051	1.0														
SsL	-0.11	0.23	1.00													
SsR	-0.52	-0.16	-0.08	100												
NH_4^-S	0.33	-0.05	-0.31	-0.47	1.00											
NH_4^-P	0.36	0.40	0.02	-0.44	0.39	1.00										
NH_4^-L	-0.04	-0.14	-0.02	0.11	0.00	0.16	1.00									
NH_4^-R	0.01	0.03	-0.30	-0.05	-0.26	-0.03	-0.01	1.00								
NO_3^-s	0.00	-0.27	-0.30	-0.21	0.48	0.14	0.00	-0.16	1.00							
NO_3^-P	-0.05	-0.09	-0.17	0.05	0.12	0.31	0.54	0.14	0.60	1.00						
NO_3^-L	0.00	-0.11	-0.45	-0.01	0.15	0.04	0.28	0.01	0.40	0.64	1.00					
NO_3^-R	-0.13	-0.58	0.04	-0.29	0.30	0.19	0.21	0.05	0.57	0.45	0.11	1.00				
N-a S	0.05	-0.24	-0.54	-0.08	0.40	0.34	0.24	-0.05	0.28	0.22	0.13	0.12	1.00			
N-a P	0.48	0.33	0.41	-0.29	-0.02	0.19	0.42	-0.02	0.08	0.34	-0.01	0.27	-0.15	1.00		
N-a L	-0.08	-0.02	0.15	0.10	0.21	0.43	-0.11	-0.41	0.18	0.02	-0.23	0.03	0.24	-0.40	1.00	
N-a R	0.09	-0.17	-0.06	0.17	-0.16	0.01	0.40	-0.05	0.05	0.31	0.31	0.26	-0.20	0.38	-0.31	1.00

Table 2. contd.

								0-4mM								
	SsS	SsP	SsL	SsR	NH_4^-S	NH_4^-P	NH_4^-L	NH_4^-R	NO_3^-s	NO_3^-P	NO_3^-L	NO_3^-R	N-a S	N-a P	N-a L	N-a R
SsS	100															
SsP	0.33	1.00														
SsL	-0.13	0.10	1.00													
SsR	0.01	0.25	-0.49	100												
NH_4^-S	0.36	0.49	0.12	-0.09	1.00											
NH_4^-P	0.06	0.06	-0.14	-0.42	0.46	1.00										
NH_4^-L	-0.08	-0.20	0.04	-0.63	-0.17	0.52	1.00									
NH_4^-R	0.55	0.09	-0.05	0.29	0.23	-0.08	-0.26	1.00								
NO_3^-s	0.04	-0.04	-0.38	-0.21	0.27	0.42	0.28	-0.11	1.00							
NO_3^-P	-0.18	-0.43	-0.17	-0.28	0.20	0.40	0.07	0.14	0.35	1.00						
NO_3^-L	-0.21	-0.35	-0.10	-0.34	-0.21	0.17	0.54	-0.60	0.23	0.06	1.00					
NO_3^-R	0.32	0.03	-0.12	0.06	0.34	0.36	0.22	0.12	0.26	0.11	0.03	1.00				
N-a S	0.20	0.27	-0.16	-0.22	0.52	0.50	0.45	-0.11	0.32	0.13	0.13	0.49	1.00			
N-a P	0.00	0.47	0.17	0.16	0.23	0.40	0.18	-0.10	0.31	-0.15	-0.05	0.33	0.29	1.00		
N-a L	-0.17	-0.14	0.44	-0.16	-0.20	0.25	0.43	-0.52	0.37	0.20	0.79	-0.09	0.17	0.15	1.00	
N-a R	0.22	0.00	0.10	0.15	-0.07	-0.04	-0.08	0.18	-0.22	-0.14	-0.15	0.50	-0.32	0.04	-0.17	1.00

sugars), originating at the shoot or roots, for ammonium assimilation to avoid ammonium accumulation to toxic levels to the plant. The energy used by plants for ammonium assimilation therefore did not contribute to root development. This suggestion was confirmed by the significant positive correlation observed between root ammonium and soluble sugar concentrations for the 4mmol L^{-1} NH_4^+ treatment.

CONCLUSION

1. The low K_m and V_{max} values observed with 1 mM NO_3^- as the N source indicated the presence of high-affinity uptake mechanisms in *J. curcas* variety CNPAS-170.
2. Amino-N and soluble sugars were preferentially allocated to the stem in *J. curcas* variety CNPAS-170.

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