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Growth of *Jatropha curcas* plants submitted to water deficit and increasing nitrogen doses

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Abstract

The objective of the present study was to assess the severity of water deficit in *Jatropha curcas* plants under different nitrogen doses. The experiment was set up in a randomized complete block design with a 5 x 2 factorial [five doses of urea with 45% nitrogen (0; 300; 600; 900; 1200 mg per pot)] and two water supply levels, irrigated and water deficit, and four replications. The *Jatropha curcas* seeds were sown in 5 L pots with substrate consisting of oxisol, sand and manure at the ratio 3: 1: 0.5, respectively. The nitrogen was added as organic matter in the soil may have contributed to absence of interaction between the factors water deficit and nitrogen dose because it already supplied the minimum nitrogen for the plant metabolism. The nitrogen doses studied did not interfere in the water deficit severity in *Jatropha curcas* plants, but the plants presented tolerance to water deficit and used delayed dehydration as tolerance strategy. *Jatropha curcas* plant production in substrate containing red-yellow latossol, sand and manure at the ratio 3:1:0.5, respectively, and organic matter content similar or equal to 1.9% did not require nitrogen fertilization.

Keywords: *Jatropha curcas*; nutrition, drought.
Abbreviations: ETo_Reference evapotranspiration, ETc_ Evapotranspiration of culture, Kc_Crop coefficient, ETo_Reference evapotranspiration, PH_Plant height, LA_ Leaf area, RWC_Relative water content, *E*_Transpiration, BIO_biomass, SD_stem diameter, NL_number of leaves, LA_leaf area, Evap_evapotranspiration, WD_ Water deficit, N_Nitrogen, RMR_Root matter ratio, LMR_Leaf matter ratio, SMR_Stem matter ratio, Chl_ Leaf concentration of chlorophyll, CAR_ Carotenoids, *E*_Transpiration, Aw_Wet tropical climate.

Introduction

The species Jatropha curcas L. is an oleaginous plant from Central America, considered hardy and adapted to diverse edaphoclimatic conditions, from very dry to wet tropical regions, tolerating [pluviometric precipitation] 600- 1500 mm year-1 rainfall (Freitas et al., 2011; Duarte et al., 2015). The plant has high phenotypic plasticity of morpho-physiological traits, and can be cultivated in saline and/or low fertility soils (Oliveira et al., 2016). Jatropha curcas has deciduous leaves and is very sensitive to low temperatures, growth is paralyzed in cooler periods with temperatures lower than 10 ºC (Matos et al., 2012).

The Jatropha curcas plant has high physical-chemical quality oil that can be used for biofuel and paraffin production (Durães et al., 2011). According to Drumond et al. (2010) the possibility of using Jatropha curcas oil for biofuel production opens wide perspectives for increasing areas cultivated with this crop in arid and semiarid regions.

The frequent drought periods associated, or not, with global warming in many regions of the planet have reduced agricultural productivity. Water restriction is the most important limitation to agricultural activity. Approximately 35% of the Earth's surface is considered arid or semi-arid and does not receive precipitation for adequate water supply for most cultivated species (Diaz-López et al., 2012a). As water resources become scarce, commercial exploitation of drought tolerant plants becomes priority to obtain high yields. Tolerance to drought results from many morpho-physiological adaptations that depend on the water deficit severity, plant age, soil type and depth, and edaphoclimatic conditions (Silveira et al., 2016).

Under natural conditions, abiotic stresses can occur simultaneously that sometimes cause irreversible damage to plants. It is known that the nitrogen metabolism requires a lot of energy and is affected at the initial stages of water deficit because of the sensitivity of the nitrate redutase enzyme (Matos et al., 2012), but studies assessing the possibility of minimizing the effects of water deficit in function of nitrogen availability are still scarce for Jatropha curcas plants. Well-performed fertilization makes significant productivity gains possible in most cultivated plants. Although Jatropha curcas is a rustic plant, higher seed productivity is obtained with use of fertilizers and soil pH correction. Nitrogen is the nutrient required in the bigget quantity by the Jatropha curcas plant, and it is essential at specific phenotypic stages, such as growth and grain swelling (Laviola and Dias, 2008; Matos et al., 2014). It is considered as the main nutrient for plant development, as it is directly involved in growth and photosynthetic activity (Freitas et al., 2012). There are relatively few commercial Jatropha curcas plantations in Brazil due to little scientific knowledge because it is a wild species, that is, it has not been bred, although there is some agronomic information, mainly about water deficit and nutritional stress. The objective of the present study was to assess the water deficit severity in Jatropha curcas plants under different nitrogen doses.

Results and Discussion

The means test, summary of the analysis of variance for water deficit and nitrogen dose (regression of the variables: height plant, stem diameter, number of leaves, leaf area, relative water content and transpiration) are shown in (Table 1). There was no significant interaction between the nitrogen doses and water deficit for the variables analyzed. The nitrogen doses did not fit any regression model (linear or quadratic), but the water deficit interfered decisively in the variables analyzed. The plants with adequate water supply presented increases of 15% in height, 17% in stem diameter, 42% in number of leaves, 56% in leaf area and 66% in transpiration compared to the plants under water deficit. The relative water content presented no significant difference in function of water supply.

The means test, summary of the analysis of variance for water supply and nitrogen dose of the variables: biomass, root, stem and leaf matter ratio, leaf carotenoid and total chlorophyll concentrations are shown in Table 2. There was no significant interaction between nitrogen doses and water deficit for the variables analyzed. The nitrogen doses did not fit any regression model (linear or quadratic). The root, stem, leaf matter ratios were not significantly different in function of water supply. The biomass was 45% less in the plants under water deficit compared to those irrigated daily. When the same comparison was made for carotenoid concentration, it was observed that there was 20% less of this pigment in the plants irrigated daily.

The multiple regression analysis assessing the influence of the variables analyzed on the total biomass of the plant is shown in Table 3. The model explained 96% of the variance in the biomass of the Jatropha curcas plants. In this regression model transpiration, leaf area and the root, stem and leaf matter ratios were the variables that contributed most to the biomass. The principal components analysis (PCA) shows that axis 1 explains 41% of the variation in the data and axis 2, 19%. The plants in the irrigated treatments with more vigorous vegetative growth formed a group located to the left of axis 1 while those under water deficit were concentrated to the right of axis 1. The determining variables to obtain the different groups were: biomass, stem diameter, leaf area and number of leaves, as shown in Figure 2. The distribution of the groups was significant at 1% probability by the PERMANOVA analysis.

Under natural conditions, simultaneous abiotic stresses can cause irreversible damage to plants. Drought tolerance can represent expansion of the agricultural frontier by obtaining economic return in areas unsuitable before. High adaptability to climate and soil variations, along with growth maintenance under stress conditions (Matos et al., 2011), may have contributed to the Jatropha curcas drought tolerance, as discussed below.

The plants under water deficit and irrigated plants were similar for relative water content. The succulent stem functions as a water buffer associated to reduction in transpiration rate, and contributed to maintaining high water content in the leaf. Reduction in water loss through transpiration and the stem succulence provide high tolerance to water deficit, because they maintain the leaves hydrated under conditions of low water availability in the soil. The results corroborated with findings by Matos et al. (2014) and Diaz-López et al. (2012b) who reported variation in transpiration and tissue hydration maintenance in Jatropha curcas plants after reducing water availability.

The beginning and development of the first leaf depend on the water status of the plant. Water deficit results in less water available for plant metabolism and new leaf formation and development. Reduction in the number of leaves and leaf area are important tolerance strategies against water deficit because they reduce the transpiration surface. Delay in dehydration allows immediate survival. The adjustment of the total leaf area and number of leaves is necessary for the plant to support the stress imposed and to acclimatize to the new condition. The smaller height, stem diameter, number of leaves, and total biomass indicate that Jatropha curcas growth is little affected by water deficit. Multiple regression analysis highlights the importance of transpiration, leaf area and root, leaf and stems matter ratios for biomass accumulation in Jatropha curcas plants and indicates that smaller transpiration and leaf area to minimize losses lead to lower of CO_2 inflow because of reduced stomata opening. This results in less carbon assimilation and less root, stem leaf and total biomass growth. About two thirds of the size of the plants is due to cell lengthening that depends exclusively on water (Taiz e Zeiger, 2012). Shoot growth limitation precedes reduction in photosynthesis and in this circumstance a bigger participation of biomass in the root system is common (Fini et al., 2013). This response to water deficit was not observed in the present study, probably because it dealt with variations that require a longer period of time under water deficit.

Biomass reduction under a water deficit condition is a common event in Jatropha curcas plants (Diaz-López et al., 2012b). Reduction in stomata conductance to minimize water loss through transpiration affects the net carbon assimilation rate, resulting in a smaller biomass accumulation. In addition, it has been reported that under water deficit the plants have less leaf area that in the last analysis results in less potential for assimilate and dry matter production. The differences in the variables analyzed between plants that suffered water deficit (re-irrigated) and those irrigated daily point to a tendency to recovery over a longer period of time, especially because they are young plants and therefore vulnerable to stress. Under reduced carbon assimilation due to stomata limitation, oxidative stress is common, in plants under water deficit and in these circumstances bigger carotenoid production becomes an important photo-protection mechanism to prevent photo-inhibition of photosynthesis.

Table 1. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), relative water content (RWC) and total transpiration (E) of *Jatropha curcas* plants submitted to water deficit and different nitrogen doses.

Mean squares							
Variation source	GL	PH(cm)	SD(cm)	NL	LA(cm^2)	RWC (%)	*E* (g dia^{-1})
WD	1	172.6**	143.5**	523.2**	$45x10^5$**	81.1^{ns}	$72x10^3$**
N	4	6.7^{ns}	5.0^{ns}	23.2^{ns}	$17x10^{4\ ns}$	116.5^{ns}	3986.6^{ns}
WD x N	4	0.3^{ns}	1.9^{ns}	6.9^{ns}	$17x10^{3ns}$	30.3^{ns}	2694.6^{ns}
Residue	30	9.0	4.2	12.7	$59x10^3$	151.9	343.1
CV (%)		11.5	9.73	25.4	28.6	15.9	21.2
Treatments		Averages					
Irrigated		28.1a	22.8a	17.7a	1189.3a	79.0a	130.0a
Water deficit		23.9b	19.0b	10.4b	517.0b	76.0a	44.5b
N		Level of significance by the F test					
$p \geq 0.05$: $_{Linear}$		0.24^{ns}	0.14^{ns}	0.22^{ns}	0.14^{ns}	0.60^{ns}	0.07^{ns}
$p \geq 0.05$: $_{Quadratic}$		0.49^{ns}	0.29^{ns}	0.49^{ns}	0.13^{ns}	0.77^{ns}	0.15^{ns}

*significant at 5% and at **1% probability; ns = not significant according to the F test. Means followed by the same lower case letter in the column do not differ at 5% probability according to the Newman-Keuls test.

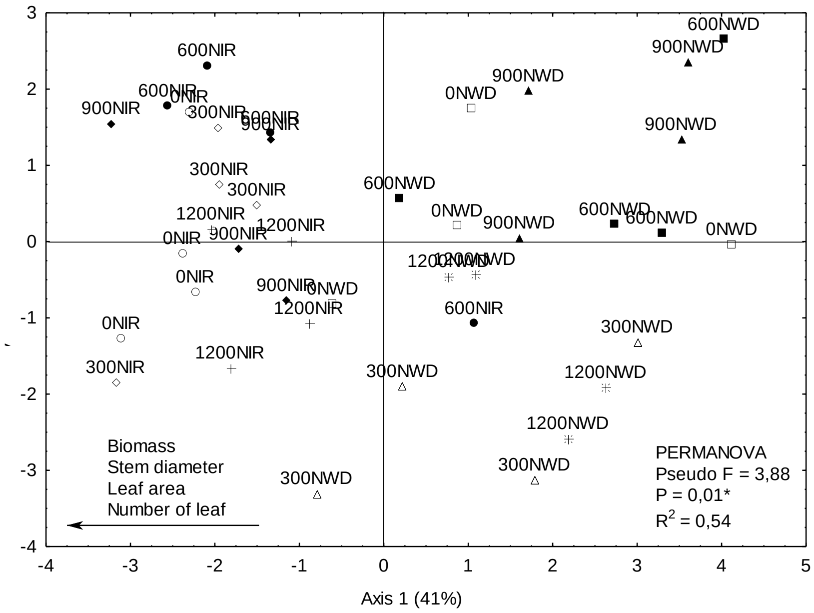

Fig 1. Principle components analysis for all variables analyzed of *Jatropha curcas* plants submitted to water deficit and different nitrogen doses. The numeral in front of the acronyms signifies the nitrogen dose applied while the acronyms IR mean irrigated and WD, water deficit.

Table 2. Summary of the analysis of variance for biomass (Bio), root matter ratio (RMR), stem matter ratio (SMR) and leaf matter ratio (LMS), leaf concentrations of carotenoids (Car) and total chlorophylls (Chl) of *Jatropha curcas* plants submitted to water deficit and different nitrogen doses of nitrogen.

Mean squares							
Variation source	GL	Bio (g)	RMR	RMC	RMF	Car (mg g^{-1})	Cl total (mg g^{-1})
WD	1	1532^{**}	$9x10^{-4ns}$	0.02^{ns}	0.014^{ns}	0.34^{**}	0.06^{ns}
N	4	35.0^{ns}	0.02^{ns}	0.01^{ns}	0.002^{ns}	0.27^{ns}	6.3^{ns}
WD x N	4	35.2^{ns}	$8.5x10^{-3ns}$	0.007^{ns}	0.002^{ns}	0.29^{ns}	7.1^{ns}
Residue	30	18.1	0.006	0.006	0.005	0.04	0.94
CV (%)		20.0	27.5	16.5	30.0	23.4	23.8
Treatments		Averages					
Irrigated		27.5a	0.30a	0.44a	0.26a	0.76b	4.0a
Water deficit		15.1b	0.29a	0.45a	0.21a	0.94a	4.1a
N		Level of significance by the F test					
$p \geq 0.05$: $_{Linear}$		0.72^{ns}	0.18^{ns}	0.38^{ns}	0.60^{ns}	0.21^{ns}	0.15^{ns}
$p \geq 0.05$: $_{Quadratic}$		0.32^{ns}	0.06^{ns}	0.06^{ns}	0.86^{ns}	0.46^{ns}	0.32^{ns}

*significant at 5% and at **1% probability; ns = not significant according to the F test. Means followed by the same lower case letter in the column do not differ at 5% probability according to the Newman-Keuls test.

Table 3. Multiple regression model to evaluate the effect of the treatments and variables analyzed [plant height (PH), stem diameter (SD), Number of leaves (NL), relative water content (RWC), carotenoids (Car), chlorophylls (Chl), transpiration (*E*), leaf area (LA), root matter ratio (RMR), shoot matter ratio (SMR), leaf matter ratio (LMR), leaf area ratio (LAR)] in the biomass of *Jatropha curcas* plants submitted to water deficit and different nitrogen doses.

	Explanation model	F	P			
Biomass	R^2= 0.96	$F(12.28)$=68.13	p<0.000			
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(28)	p-level
Intercept			-39.65	13.679	-2.898	0.007
PH	0.112	0.059	0.248	0.132	1.870	0.056
SD	0.147	0.074	0.417	0.210	1.985	0.663
NL	0.030	0.069	0.046	0.105	0.439	0.129
RWC	-0.059	0.038	-0.038	0.024	-1.561	0.787
Car	0.033	0.123	0.784	2.883	0.272	0.532
Chl	-0.077	0.121	-0.382	0.604	-0.632	0.192
E	0.059	0.044	0.008	0.006	1.334	0.000**
LA	1.014	0.121	0.017	0.002	8.373	0.000**
RMR	0.620	0.166	53.458	14.341	3.727	0.011*
SMR	0.501	0.185	41.079	15.221	2.698	0.001**
LMR	0.522	0.147	52.656	14.890	3.536	0.001**
LAR	-0.646	0.075	-37.723	4.417	-8.539	0.000**

*Significant at 1%; Explanation of model R^2=0.96.

The principal components analysis ranked the treatments into two groups in function of water availability and regardless of nitrogen dose. The growth variables were determinant for the group formation, especially biomass. The nitrogen made available by the organic matter in the soil may have contributed to lack of interaction between the factors water deficit and nitrogen doses because it already supplied the minimum nitrogen concentration for plant metabolism. The non-significance of the directly and indirectly nitrogen-dependent variables such as leaf area, chlorophyll concentration and growth indicates absence of nitrogen deficiency even in the treatments with small nitrogen doses.

Materials and Methods

Plant materials

The study was carried out in 5 L pots on benches in a greenhouse with a transparent roof and sombrite sides with 50% solar radiation interception at the state University of Goiás, Ipameri Campus, l (Lat. 170 43' 19'' S, Long. 48009' 35'' W, Alt. 773 m), Ipameri, Goiás, Brazil. The climate of the region is wet tropical (Aw) according to the Köppen classification, with a wet summer and dry winter. The experiment was set up in a randomized complete design in a 5 x 2 factorial arrangement (five doses of urea with 45% nitrogen: 0; 300; 600; 900; 1200 mg per pot and two water supply levels: irrigated and water deficit) and four replications. The experimental plot consisted of one plant per plot. The Jatropha curcas seeds were sown in 5 L pots with substrate consisting of oxisol, sand and manure at the ratio of 3: 1: 0.5, respectively. The substrate was prepared following recommendations by Do Carmo et al. (2014). Chemical analysis of the mixture showed the following values: pH 6.5; 19 g dm-3 M.O, 2.4 mg dm-3 P; 22 cmolc dm-3 K; 1.5 cmolc dm-3 H+Al; 3.2 cmolc dm-3 Ca; 1.6 cmolc dm-3 Mg; 27.7 mg dm-3 Zn; 77.20% SB and 6.58 CTC. The plants were fertilized ten days after germinating t with different nitrogen doses and 20 days after germination they were fertilized with phosphorus and potassium according to the recommendations by Laviola and Dias (2008). The plants were irrigated daily with a volume of water corresponding to the daily evapotranspiration. As the crop coefficient (kc) for Jatropha curcas has not yet been determined for the conditions of Ipameri, Goiás, kc equal to 1.00 was used following the estimate by the FAO 56 (Allen et al., 1998) for a group of crops at initial growth stage. The water volume supplied was estimated by determining the reference evapotranspiration and crop coefficient. The following equation was used to determine the crop evapotranspiration:

ETc = ETo x kc

Where:

ETc = crop evapotranspiration

kc = crop coefficient

ETo = reference evapotranspiration

The daily ETo was calculated by the Penman-Monteith method recommended by the FAO (Smith et al., 1991) using the daily data for maximum and minimum air temperature, relative air humidity, insolation and wind speed obtained from the National Institute of Meteorology, from a meteorological station located in the municipality of Ipameri, GO, at 1200 m from the experimental field.

At 60 days after germination, half of the plants were submitted to 10 days of water deficit and the other half was irrigated daily. On the 11th day without irrigation, the plants under water deficit were rehydrated for 10 days and then the following variables were analyzed: number of leaves, plant height, stem diameter, transpiration, leaf area, total chlorophylls and carotenoids, relative water content, root matter ratio (RMR), stem matter ratio (RMC), leaf matter ratio (RMF) and total biomass.

Growth and physiological variables

The number of leaves was obtained by counting. Plant height and stem diameter were measured and digital, pachymeter, respectively. The leaf area was assessed by using a graduated ruler to measure the width and length of all the leaves of each plant. The leaf area was calculated following recommendations by Severino et al. (2007).

The leaves, roots and stems were removed and placed to dry in a chamber at 72°C until constant dry matter was reached and then they were weighed separately. The matter data was used to calculate the leaf matter ratio (RMF), root matter ratio (RMR), stem matter ratio (RMC) and total biomass.

The total daily transpiration of the plant was measured by the difference in pot weight. First, each pot was placed inside a plastic bag that was fastened to the stem of the plant with a rubber band, leaving only the shoot (leaves and stem) outside the bag, then the pot with the plastic bag was weighed at 12:00 h (matter 01) and again 24 hours afterwards (matter 02). The total transpiration was estimated from the difference between matter 01 and matter 02. To obtain the relative water content, ten 14mm leaf discs were removed, weighed and placed in Petri dishes for 8 h to saturate in distilled water. They were then weighed and placed to dry at 70ºC for 72 horas.

To determine the total chlorophyll and carotenoids concentration, leaf discs were removed from the known area and placed in flasks containing dimethyl sulfoxide (DMSO). Later extraction was carried out in a water bath at 65 ºC for four hours. Aliquots were removed for spectrophotometry readings at 480. 649.1 and 665 nm. The chlorophyll a (Cl a) and chlorophyll b (Cl b) contents were determined following the equation by Wellburn (1994).

Statistical analysis

The variables were submitted to analysis of variance following a randomized complete block design in a 5 x 2 factorial arrangement. The means were compared using the Newman-Keuls test at 5% probability. Multivariate analyses used principal components techniques with a correlation matrix and the Broken stick axis selection criteria jointly with multivariate analysis of variance with permutation - PEMANOVA (Anderson, 2001). Biomass was assessed by multiple regression analysis using the forward stepwise model at 5% significance (Sokal and Rolf, 1969). R software was used to carry out these analyses R (R Core Team, 2016).

Conclusion

The nitrogen dose studied did not interfere in the water deficit severity in Jatropha curcas plants, but the plants presented tolerance to water deficit and used delayed dehydration as tolerance strategy.

Jatropha curcas plant production with substrate containing Red-yellow latossol, sand and manure at the ratio of 3:1:0.5, respectively, and organic matter contents similar or equal to 1.9%, dispense nitrogen fertilization.

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