

Performance of Genotypes of Physic Nut Conditioned by Water Availability

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Abstract: The present study aimed to investigate the selection of genotypes tolerant to drought are greatly important, especially considering the actual panorama of climate change, in which the temperature rises and the period of water restrictions may extend, greatly impacting the agriculture. In breeding programs, it is necessary to compare the performance of different genotypes when subjected to water stress to make possible to identify sources to improvement toward the drought tolerance and water use efficiency. This experiment aimed to evaluate the early growth of genotypes of physic nut (*Jatropha curcas* L.) cultivated with different levels of available water in the soil. The experiment followed a 5x4 factorial scheme, submitting five genotypes of physic nut (CNPAE 128, CNPAE 170, CNPAE 171, CNPAE 183 e CNPAE 198) to four levels of water availability in the soil (10%, 30%, 50% and 100%), in a completely randomized design, in order to evaluate their initial growth. Biometric variables and biomass production and allocation were measured. The results show that, to a certain degree, plants of physic nut are able to tolerate water deficit, keeping growth and biomass production in different levels of available water in the soil. It can be inferred that plants of physic nut are able to develop efficient strategies to tolerate these conditions, such as reduction of leaf area and preservation of stem juiciness. Plants of physic nut are able to tolerate drought without compromising the total production of biomass in the initial stages of development. It is possible to identify differences among genotypes of physic nut regarding the response to the drought.

Key words: *Jatropha curcas* • Biodiesel • Drought • Tolerance

INTRODUCTION

The search for alternative energy sources to fossil fuels has become more necessary and urgent, requiring a search for renewable sources, which are less impacting to the environment. Between the options, biodiesel is one of the most promising alternatives to generate clean energy [1] and physic nut (*Jatropha curcas* L.) is gaining prominence and economic importance due its potential for biodiesel production, which has contributed to the increase of commercial exploitation of this species.

Jatropha curcas L. is an oleaginous plant, originally from Central America, considered rustic and adapted to different climate conditions, able to survive in regions considered marginal for cultivation due to adverse soil conditions [2, 3]. It is characterized as a deciduous

species, with falling leaves in the dry season or at low temperatures, which reappear soon after the first rains and when the temperatures rises above 10C. It is also considered a xerophytic species, with strong drought resistance [4,5].

Recent reports of impacts, adaptation and vulnerability to climate change made by IPCC [6] keep warning about the water deficit and the evolution of growing areas to semi-arid conditions. These reports also indicate the vulnerability of areas from Central and South America to intense climate change effecting the amount and distribution of rainfall. The water deficit is a main factor which restricts the agricultural productivity. When subjected to drought, plants develop a range of different responses, such as reducing the water content in the tissues, limiting leaf water potential and turgor loss,

stomatal closure and decreasing cell growth [7]. Furthermore, the water stress, induced by osmotic effect, characterizing the physiological drought, can cause morphological and anatomical changes in plants, to the point of unbalancing the water uptake and transpiration rates [1]. Among the morphological changes, the reductions in size and number of leaves are the most significant [8].

Studies aiming at the selection of genotypes tolerant to drought are greatly important, especially considering the actual panorama of climate change, in which the temperature rises and the period of water restrictions may extend, greatly impacting the agriculture [9-11]. In breeding programs, it is necessary to compare the performance of different genotypes when subjected to water stress to make possible to identify sources to improvement toward the drought tolerance and water use efficiency.

The physic nut can survive with 200 mm of annual rainfall and, despite losing their leaves, the plant survives using the stored water in the juiciness of their stems [4]. This factor reinforces the importance of developing studies comparing genotypes under water deficit, to be able to identify the expression of the drought tolerance and classify genotypes regarding both the tolerance to low availability of water and to responsiveness to irrigation, allowing a proper recommendation of cultivars for different cultivation systems.

This experiment aimed to evaluate the early growth of genotypes of physic nut (*Jatropha curcas* L.) cultivated with different levels of available water in the soil.

MATERIALS AND METHODS

The experiment was conducted in greenhouse, located in the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), in the municipality of Alegre-ES, at an altitude of 136 m, with geographic coordinates 20°45'S and 41°30'W, between the months of June and September 2013.

The soil used was classified as dystrophic red-yellow oxisol, with clayed texture [12] and was collected to a depth of 10 to 30 cm. A sample of this soil was sent to the laboratory for analysis and the fertility was corrected following the recommendations for essays in controlled environments [13]. The soil was separated into samples of 10 dm³ and placed in plastic pots with a capacity of 14 dm³.

The experiment followed a 5x4 factorial scheme, submitting five genotypes of physic nut (CNPAC 128, CNPAC 170, CNPAC 171, CNPAC 183 e CNPAC 198) to

four levels of water availability in the soil (10%, 30%, 50% and 100%), in a completely randomized design, with four replications, in order to evaluate their initial growth. Six seeds were sown per pot and, after emergence, thinning was performed to allow the growth of only one plant per pot. The seeds of each genotype were provided by the Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agroenergia, which is responsible for the main physic nut breeding program in Brazil.

For the levels of water availability, the level of 100% of available water (AW) was used as standard irrigation for physic nut. The irrigation was managed to allow the AW to be depleted to 10%, 30%, 50% and 100% of the total AW, as levels of water availability. The irrigation was performed when the soil moisture reached the levels corresponding to each treatment, returning the water level back to the reference level.

The plants were kept in these conditions from 10 to 80 days after emergence, through daily monitoring of the moisture in the pots. The procedures to determinate the irrigation levels followed the methodology described by Bernard *et al.* [14], which required the hydro-physical analysis of the soil to obtain the required parameters. The hydro-physical analysis was performed according to the methodology proposed by Embrapa [15] (Table 1).

The nitrogen fertilization was split in three applications (20, 40 and 60 days after sowing) and the phosphorus and potassium fertilization was done in a single application at sowing, with fertilization following the recommendation for controlled environments [13].

The plants were cultivated for 80 days in order to gather information on biometric variables and biomass production and allocation. Data about plant height, leaf area, stem diameter, total dry matter, root: shoot ratio, leaf area ratio, leaf mass ratio, stem mass ratio and root mass ratio were obtained. The plant height was obtained using a ruler, graduated in 0.1 centimeters, measuring the length from soil level to apical meristem; the leaf area was measured using an leaf area integrator (Area Meter, model LiCor LI3100C, precision: 0.01 cm²); the stem diameter was evaluated with digital caliper (precision: 0.01 mm).

The plants were collected after the evaluation, separating stem, leaves and roots. These sections were placed separately in a paper bag and put into laboratory oven, with forced air circulation, at a temperature of 65°C, until their weight became constant, determining the dry matter values. Ratios between the variables were used to establish area and mass relations between the plant organs.

Table 1: Hydro-physical attributes of the soil

Granulometric analyses				FC ⁽²⁾	PWP ⁽³⁾
Sand	Silt	Clay	Ds ⁽¹⁾ kg dm ⁻³	-----%	-----
68.70	1.74	29.56	1.25	14.55	9.67

⁽¹⁾Ds = soil density; ⁽²⁾Proportion of soil humidity in the field capacity (tension: 0.01 Mpa); ⁽³⁾Proportion of soil humidity in the permanent wilting point (tension: 1.50 Mpa)

The collected data were subjected to analysis of variance and, according to the presence of significant differences, the means of the genotypes were studied using the Scott-Knott test (5% of probability) and the means of the levels of available water were studied using regression analysis (5% of probability). The analyses were performed using the statistical software GENES [16].

RESULTS AND DISCUSSION

The vegetative growth of the genotypes of physic nut was not affected, at least in part, by the interaction between the genotypes and the level of available water; more precisely, there was no significant interaction between the sources of variation for leaf area, stem diameter, plant height, ratio root: mass or mass ratios. In contrast, the accumulation of dry matter and leaf area ratio were influenced by this effect and, therefore, the interactions were unfolded in their study.

It is noticed different behavior among the genotypes of physic nut regarding leaf area. The genotype CNPAE 198 showed foliage with larger area, while the genotypes CNPAE 128, CNPAE 170 and CNPAE 171 presented smaller areas. The genotypes CNPAE 198 and CNPAE 128 had greater thickening of their stems, while the genotype CNPAE 170 presented thinner stem. For plant height, only the genotype CNPAE 170 presented differentiation from the others, with the expression of smaller plants (Table 2).

This different behavior among genotypes of physic nut for variables of vegetative growth have been reported by Laviola *et al.* [17], Laviola *et al.* [18], Amaral *et al.* [19] and Colodetti *et al.* [20] and the expression of different characteristics for some genotypes is an important tool to select promising genetic material to be explored in the breeding programs or to compose cultivars for cultivation in certain conditions.

The relationship between biomass allocation in the roots and shoots indicates a prioritization of the formation of the root system by the plants of the genotypes CNPAE

183 and CNPAE 170 instead of their aerial part, suggesting greater investment of assimilates in roots and a larger accumulation of biomass of these organs (Table 2).

Lower leaf mass ratio was observed in plants of the genotype CNPAE 128, indicating a lesser allocation of biomass in the leaves for this genotype. A larger proportion of the biomass of the genotype CNPAE 128 were accumulated in the stems, while the genotypes CNPAE 170 and CNPAE 183 had a smaller percentage of their biomass allocated in stems. These same two genotypes presented the larger allocation of biomass in the root system.

Increased investment in the formation of roots is a characteristic normally correlated with survival mechanisms to adapt or tolerate water deficit, since it enables a further exploration of the soil, favoring the search for water and nutrients. This information can be contextualized with the strategies that plants use to grow in environments with water restriction [21].

Unfolding the significant interaction between the effect of genotypes and levels of available water, it is possible to observe that in the level of 10% of AW, there was no differentiation between genotypes. However, at levels of 30%, 50% and 100% of AW, the genotypes CNPAE 128 and CNPAE 198 stood out for having greater production of biomass (Table 2).

There was also a significant interaction for the leaf area ratio, where differentiation between genotypes was only not observed at the level of 10% of AW. Providing 30% of water availability, the genotypes CNPAE 170, CNPAE 171 and CNPAE 183 presented greater ability to form leaf area per unit of biomass produced. At 50% of AW, higher means were observed for plants of the genotypes CNPAE 171, CNPAE 183 and CNPAE 198. The level of 100% of water availability promoted the leaf area for the genotype CNPAE 170, which lead to a higher mean of leaf area ratio (Table 2).

Great variability of responses of genotypes of physic nut has been reported to various environmental factors, such as aluminum stress, variations in the photons flow rate and levels of nutrient availability in the soil [22-24]. The expression of variability in different conditions is important to this species, since the high genetic variability in Brazilian genotypes of physic nut is still being explored to develop cultivars.

The leaf area of *Jatropha curcas* L. was increased linearly in response to the higher levels of AW, which can be observed by the regression analyses

Table 2: Means of leaf area, stem diameter, plant height, dry matter, root: shoot ratio, leaf area ratio, lead mass ratio, stem mass ratio and root mass ratio of genotypes of physic nut cultivated with different levels of water availability

	Available Water (%)				
Genotypes	10	30	50	100	Means
	Leaf area (cm ²)				
CNPAE 128	1411.58	1739.63	1795.70	2042.01	1747.23 c
CNPAE 170	1428.93	1520.40	1606.81	2261.90	1704.51 c
CNPAE 171	1558.70	1732.00	1780.03	2035.61	1776.58 c
CNPAE 183	1715.73	1671.35	1863.11	2196.50	1861.67 b
CNPAE 198	1750.98	2005.48	2135.67	2435.10	2081.80 a
	Stem diameter (mm)				
CNPAE 128	28.68	27.91	28.84	29.36	28.70 a
CNPAE 170	24.12	25.03	24.64	24.49	24.57 c
CNPAE 171	26.79	24.80	25.18	27.30	26.02 b
CNPAE 183	28.02	27.51	25.81	26.73	27.02 b
CNPAE 198	29.01	28.81	28.58	29.19	28.90 a
	Plant height (cm)				
CNPAE 128	30.05	34.38	35.53	40.48	35.11 a
CNPAE 170	29.73	30.73	33.13	34.25	31.96 b
CNPAE 171	33.40	36.95	39.23	38.08	36.91 a
CNPAE 183	31.48	34.70	36.93	40.13	35.81 a
CNPAE 198	34.38	36.20	36.38	37.90	36.21 a
	Dry matter (g)				
CNPAE 128	33.75 a	41.51 a	38.97 a	41.76 a	39.00
CNPAE 170	30.08 a	28.19 c	31.01 b	29.68 c	29.74
CNPAE 171	33.04 a	33.13 b	31.62 b	34.78 b	33.14
CNPAE 183	34.88 a	33.20 b	32.41 b	36.31 b	34.20
CNPAE 198	35.38 a	42.01 a	36.56 a	41.70 a	38.91
	Roots: Shoots mass ratio (g g ⁻¹)				
CNPAE 128	0.34	0.32	0.29	0.29	0.31 b
CNPAE 170	0.40	0.44	0.36	0.35	0.39 a
CNPAE 171	0.26	0.40	0.37	0.34	0.34 b
CNPAE 183	0.41	0.45	0.41	0.41	0.42 a
CNPAE 198	0.33	0.33	0.36	0.32	0.33 b
	Leaf area ratio (cm ² g ⁻¹)				
CNPAE 128	41.98 a	41.99 b	46.33 b	49.08 c	44.84
CNPAE 170	47.52 a	54.57 a	52.08 b	76.60 a	57.69
CNPAE 171	47.39 a	52.60 a	56.40 a	58.61 b	53.75
CNPAE 183	49.64 a	51.19 a	57.81 a	60.62 b	54.82
CNPAE 198	49.44 a	47.75 b	58.53 a	59.14 b	53.71
	Leaf mass ratio (g g ⁻¹)				
CNPAE 128	0.19	0.18	0.20	0.22	0.20 b
CNPAE 170	0.22	0.24	0.23	0.27	0.24 a
CNPAE 171	0.21	0.22	0.23	0.24	0.22 a
CNPAE 183	0.21	0.21	0.24	0.25	0.23 a
CNPAE 198	0.23	0.22	0.24	0.26	0.24 a
	Stem mass ratio (g g ⁻¹)				
CNPAE 128	0.56	0.58	0.57	0.56	0.57 a
CNPAE 170	0.50	0.46	0.51	0.47	0.48 c
CNPAE 171	0.58	0.50	0.50	0.51	0.52 b
CNPAE 183	0.50	0.48	0.48	0.46	0.48 c
CNPAE 198	0.53	0.53	0.50	0.50	0.51 b
	Root mass ratio (g g ⁻¹)				
CNPAE 128	0.25	0.24	0.23	0.22	0.24 b
CNPAE 170	0.28	0.30	0.26	0.26	0.28 a
CNPAE 171	0.21	0.28	0.27	0.25	0.25 b
CNPAE 183	0.29	0.31	0.29	0.29	0.29 a
CNPAE 198	0.24	0.25	0.26	0.24	0.25 b

Means followed by the same letter on the column, for each variable, do not differ by the Scott-Knott test ($p \leq 0.05$)

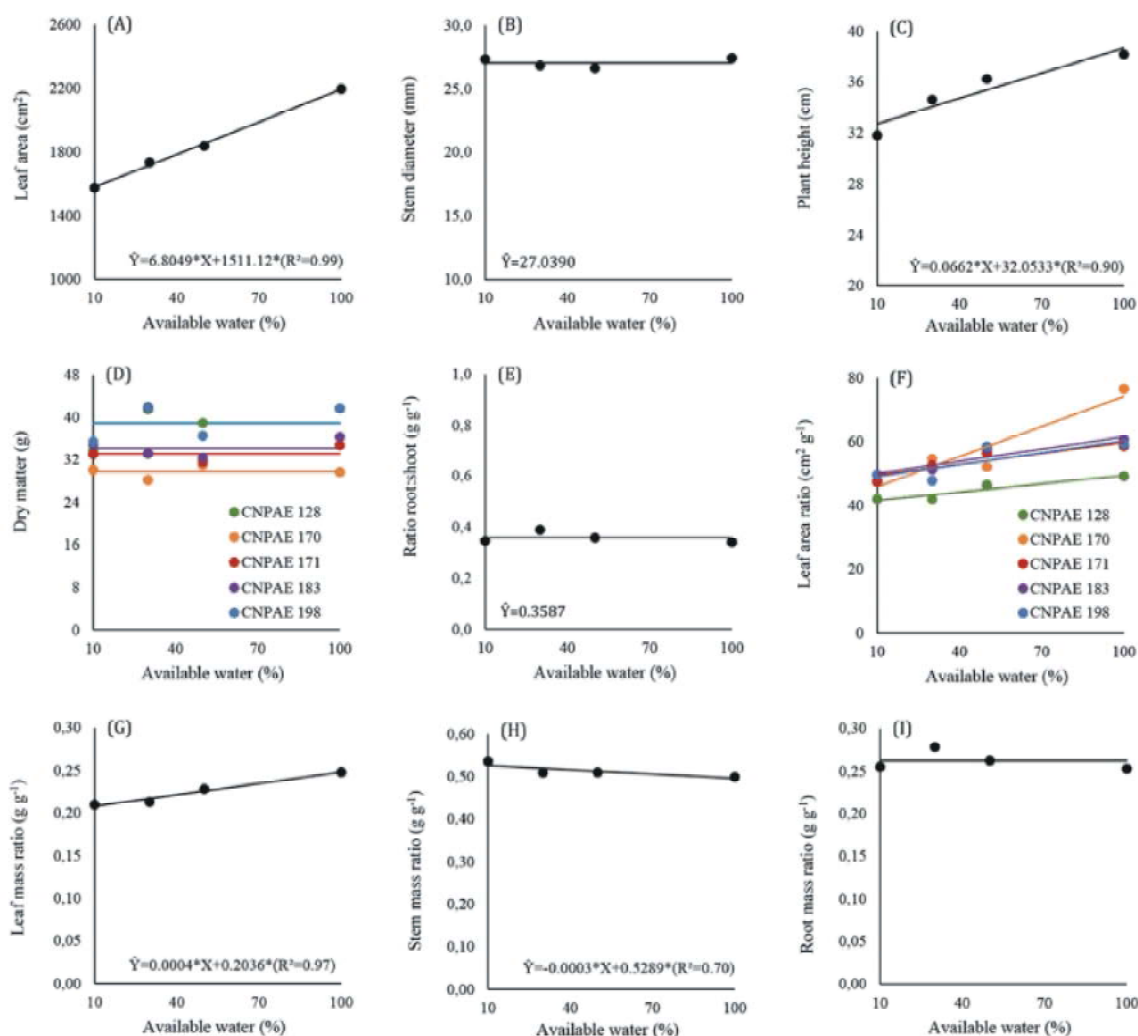


Fig. 1: Means of leaf area (A), stem diameter (B), plant height (C), dry matter (D), root: shoot ratio (E), leaf area ratio (F), leaf mass ratio (G), stem mass ratio (H) and root mass ratio (I) of genotypes of physic nut as function of the water availability

(linear of 1st degree) (Fig. 1A). Lower levels of water availability caused greater impairment in the development of leaf area. As the leaves are essential sources of photosynthetic assimilate to maintain the plant growth, this restriction may slow the growth of the plants of physic nut. According to Taiz and Zeiger [11], the reduction in leaf area is an early effect of water stress, as an adaptation in order to reduce water loss through transpiration.

Stem diameter, root: shoot ratio and root mass ratio were not affected by level of available water in the soil (Fig. 1B, 1E and 1I), while the plant height adjustment to a linear (1st degree) regression model, demonstrating a gain in plant height as the water availability in the soil increased (Fig. 1C).

The leaf mass ratio presented fit to a linear regression model (1st degree), demonstrating increase in this ratio when the availability of water increased. This indicates a larger amount of biomass being allocated in the leaves as the plant is subjected to a conditions of higher water availability

(Fig. 1G). The same adjustment was observed for the stem mass ratio, however with opposite behavior, since the proportion of biomass directed to the stem decreased as the AW increased (Fig. 1H).

For the production of dry matter and leaf area ratio, there was significant interaction between genotypes and levels of AW. For the production of dry matter, the genotypes present different patterns of production, but they were not changed by the levels of AW in the

soil. The genotypes CNPAE 128 ($Y = 38.9958$) and CNPAE 198 ($Y = 38.9122$) had, overall, higher dry matter production; while the genotypes CNPAE 171 ($Y = 33.1416$) and CNPAE 183 ($Y = 34.2035$) presented intermediate production of dry matter; and the genotype CNPAE 170 ($Y = 29.7404$) had the smaller amount of accumulated biomass (Fig. 1D).

For the leaf area ratio, different regressions were established for the genotypes as function of the levels of AW in the soil, but all adjusted to linear models (1st degree), showing higher leaf area ratios as the AW increased. The genotype CNPAE 170 ($Y = 0.3162^*X + 42.6717^*$, $R^2 = 0.89$) had the most expressive response in area per unit of biomass allocated in the leaves as function of the increased levels of AW, which is confirmed by the magnitude of the slope coefficient of the regression for this genotype being the highest. The lowest slope coefficient was obtained in the regression for the genotype CNPAE 128 ($Y = 0.0856^*X + 40.7741^*$, $R^2 = 0.89$), which represents a less intense response in leaf area ratio due to the increase of water availability in the soil. The other genotypes: CNPAE 171 ($Y = 0.1163^*X + 48.2226^*$, $R^2 = 0.83$), CNPAE 183 ($Y = 0.1273^*X + 48.7682^*$, $R^2 = 0.87$) and CNPAE 198 ($Y = 0.1254^*X + 47.7523^*$, $R^2 = 0.66$), showed similarity on the response regarding this variable (Fig. 1F).

The results show that, to a certain degree, plants of physic nut are able to tolerate water deficit, keeping growth and biomass production in different levels of available water in the soil. It can be inferred that plants of physic nut are able to develop efficient strategies to tolerate these conditions, such as reduction of leaf area and preservation of stem juiciness.

The juicy stems of these plants acts as mechanism of anticipation to drought, it contributes to the maintenance of the water content in the plant. In addition, physic nut has intermediate C3-CAM photosynthetic metabolism [25]. The stem succulence combined with the C3-CAM metabolism makes physic nut plants able to maintain the hydration of leaf tissues, increasing the water use efficiency and reducing the water loss through transpiration, which results in the tolerance to drought. Research results reported increase in water use efficiency in physic nut plants cultivated with limited water supply, which helps confirming this theory [26, 27].

Certain plants have different capacities to tolerate drought, exploring different strategies to maintain the metabolic activity, even when the condition of water restriction evolves [28]. Each species behaves in a manner

regarding the reduced availability of water, but often, the productivity of these plants is affected by the expression of those strategic characteristics to resist drought [29]. The study of these morpho-physiological mechanisms to tolerate drought is fundamentally necessary to allow the selection of genotypes in breeding programs.

CONCLUSIONS

Plants of physic nut are able to tolerate drought without compromising the total production of biomass in the initial stages of development. The adaptation to these conditions cause changes in plant height, leaf area and biomass allocation between the organs.

It is possible to identify differences among genotypes of physic nut regarding the response to the drought.

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